Published in final edited form as:

J Control Release. 2013 December 10; 172(2): . doi:10.1016/j.jconrel.2013.04.010.

Recent Advances in Delivery of Drug-Nucleic Acid Combinations for Cancer Treatment

Jing Li^a, Yan Wang^a, Yu Zhu^a, and David Oupický^{a,*}

^aDepartment of Pharmaceutical Sciences and Center for Drug Delivery and Nanomedicine, University of Nebraska Medical Center, Omaha, NE 68198-5830

Abstract

Cancer treatment that uses a combination of approaches with the ability to affect multiple disease pathways has been proven highly effective in the treatment of many cancers. Combination therapy can include multiple chemotherapeutics or combinations of chemotherapeutics with other treatment modalities like surgery or radiation. However, despite the widespread clinical use of combination therapies, relatively little attention has been given to the potential of modern nanocarrier delivery methods, like liposomes, micelles, and nanoparticles, to enhance the efficacy of combination treatments. This lack of knowledge is particularly notable in the limited success of vectors for the delivery of combinations of nucleic acids with traditional small molecule drugs. The delivery of drug-nucleic acid combinations is particularly challenging due to differences in the physicochemical properties of the two types of agents. This review discusses recent advances in the development of delivery methods using combinations of small molecule drugs and nucleic acid therapeutics to treat cancer. This review primarily focuses on the rationale used for selecting appropriate drug-nucleic acid combinations as well as progress in the development of nanocarriers suitable for simultaneous delivery of drug-nucleic acid combinations.

Keywords

Gene delivery; cancer treatment; drug delivery; combination therapy; multifunctional nanocarriers; siRNA delivery

1. Introduction

Cancer is a disease caused by multiple gene mutations. The progression of cancer involves dynamic changes in the genome and a complex network of interactions among cancer cells with multiple, distinct cell types that form tumors. The complexity of the signaling web, combined with multiple mechanisms to evade programmed cell death, makes curing cancer a formidable challenge [1]. As a result, approaches using combination therapy have been explored and successfully applied in the treatment of many types of cancer. Whether based on combinations of multiple chemotherapeutics or combinations of chemotherapeutics with other treatment modalities like radiation, combination treatments have been proven highly effective due to their ability to affect multiple disease pathways [2].

© 2013 Elsevier B.V. All rights reserved.

*Corresponding Author: david.oupicky@unmc.edu; Tel.: +1-402-559-9363.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

In recent years, nucleic acid therapeutics such as small interfering RNA (**siRNA**), antisense oligonucleotides (**AON**), small hairpin RNA (**shRNA**), and plasmid DNA (**pDNA**) have shown promise in cancer treatment [3–5]. The majority of the research in this area has focused on the delivery of a single gene, siRNA, or AON against a single gene target. While this is an effective approach to treat monogenic diseases, it has become clear that in the treatment of cancer, nucleic acids face the same problems with cancer heterogeneity and adaptive resistance as traditional small molecule drugs. The elaborate signaling pathways in cancer cells and various compensatory mechanisms involved predict that, consistent with the current cancer treatment protocols, successful nucleic acid therapies will need to be based on combination approaches. These may include combinations of multiple nucleic acids, nucleic acids with small molecule drugs, or other treatment modalities.

Even with the widespread clinical use of combination therapies, relatively little attention has been paid to the potential of modern nanocarriers, such as polyplexes, nanoparticles, liposomes, and micelles, to enhance the efficacy of combination therapies. This lack of attention is particularly obvious in the limited selection of vectors for the simultaneous delivery of combinations of traditional small molecule drugs with nucleic acids. Delivery of drug-nucleic acid combinations is a challenging task because of the stark differences in the physicochemical properties of the two types of agents. Moreover, differences in molecular weight, hydrophobicity, and metabolic stability of small molecule drugs and nucleic acids greatly affect their biodistribution and pharmacokinetics. This results in challenges from often disparate delivery and can lead to very different drug concentrations at the target location. Recent advances in the development of nanocarriers suggest that these systems can be designed to carry and protect drugs of many different types and sizes, ranging from small molecules to macromolecules and from hydrophilic to hydrophobic agents. The preparation of versatile nanocarriers that are capable of delivering drug-nucleic acid combinations, especially combinations where the timing and sequence of delivery of individual agents can be controlled independently, will provide a more-potent, controlled, and less-toxic means of cancer treatment.

2. Approaches for Selecting Drug-Nucleic Acid Combinations in Cancer Treatment

Due to the heterogeneity of cancer and the complexity of signaling networks that control tumor growth, progression, and metastasis, selecting the correct drug-nucleic acid combination is vital for achieving the best therapeutic outcomes. In this review article, we focus primarily on approaches in which the main target for both the drug and the nucleic acid is the cancer cell, as these approaches are expected to benefit most from simultaneous delivery. Other possible approaches where the individual agents target different cell types (i.e., one targets tumor endothelium while the other targets cancer cells) will be discussed only briefly. Several mechanism-based strategies utilizing the current understanding of cancer have been explored in drug-nucleic acid combinations, and they are discussed herein. In the absence of broadly applicable, rational algorithms for selecting effective drug-nucleic acid combinations, large-scale library screening for different drug-nucleic acid combinations is also a viable strategy in discovering new and effective combinations [6, 7]. Examples of proven drug-nucleic acid combinations are summarized in Table 1.

2.1 Combining Chemotherapy with Nucleic Acids to Overcome Multidrug Resistance (MDR)

Drug resistance is a major and persistent obstacle to the successful treatment of local and metastatic cancers. At some point during treatment, patients receiving chemotherapy often develop resistance to drugs that were previously effective, thus making the cancer treatment

even more difficult. Resistance to chemotherapeutic agents (i.e., chemoresistance) is a major cause of treatment failure and relapse or recurrence of many tumors. To help overcome chemoresistance, chemotherapy drugs are typically administered in combination. However, in many cases, after a particular type of cancer has developed resistance to a given chemotherapeutic drug, the cancer cells develop resistance to other drugs as well. Fortunately, the molecular mechanism and genes involved in multidrug resistance (**MDR**) are relatively well understood. Approaches based on combinations of chemotherapeutics with gene silencing features to suppress the expression of efflux transporters are a logical solution and among the most widely investigated examples of drug-nucleic acid combinations.

In many cases, MDR is highly associated with P-glycoprotein (**Pgp**), also known as multidrug resistance protein 1 (**MDR1**). Pgp is a glycoprotein responsible for transporting a wide variety of substances across extra- and intracellular membranes. Importantly, Pgp is found to be overexpressed in various malignant tumor tissues, where it is actively involved in pumping chemotherapy drugs out of the cells. This active role in removal of the chemotherapeutic makes Pgp a prime target in combination approaches to overcome MDR. For example, numerous studies have shown that successful inhibition of Pgp expression by gene silencing with siRNA or AON dramatically increases the accumulation of chemotherapy drugs in tumors and results in improved anti-tumor efficiency [35–37, 51]. However, efflux proteins like Pgp also play a crucial role in physiological regulation of endogenous substrates in healthy tissues throughout the body. It is important to control the silencing of Pgp expression specifically only in the tumor cells to avoid side effects caused by undesirable pharmacological activity in healthy organs and tissues [61]. For example, some Pgp modulators reduce clearance of chemotherapeutic drugs but, at the same time, prolong the exposure of healthy tissues to the harmful effects of the drugs [62]. Specifically, blocking Pgp also interferes with cytochrome P450 3A (**CYP3A**)-mediated intestinal and liver metabolism due to considerable substrate overlap, causing undesirable effects [63].

Other MDR proteins such as MRP1, MRP2, BCRP and certain cell signaling pathways also contribute to chemoresistance. For example, in ovarian cancer, the Notch signaling pathway is a key regulator of tumor resistance to the treatment agent platinum [41]. Markedly, both *in vivo* and *in vitro* evidence shows that Notch 3 overexpression results in expansion of cancer stem cells and increased chemoresistance to platinum. On the other hand, using either a Notch-pathway inhibitor or a Notch 3-siRNA knockdown strategy sensitizes tumors to platinum therapy, making the Notch pathway a therapeutic target for treating patients with platinum-resistant relapses by using a combination approach.

Proper sequencing and timing of the delivery and activity of combination treatments is vital for their efficacy. Unlike many other combination treatments in which optimum sequencing and timing are not well understood, treatments that rely on inhibition of the drug efflux mechanism are fairly straightforward. Ideally, using a combination treatment approach, the inhibition of efflux mechanism will precede the chemotherapy treatment. This notion is supported by the available evidence in drug-resistant SKOV3 cancer cells that suggests that a 24-hour time lapse between treatment using the Notch pathway inhibitor Pgp siRNA followed by treatment with the mitotic-inhibitor chemotherapy drug paclitaxel (**PTX**) is the most beneficial for improving the therapeutic outcome [34]. It is also important to note that other studies have shown that simultaneous treatment can work as well in combination approaches involving MDR inhibition [35].

2.2 Combining Chemotherapy with Nucleic Acids that Promote Apoptosis

One hallmark of cancer is its ability to escape programmed cell death, or apoptosis [1]. Pre-cancerous cells undergoing fatal mutations are usually eliminated from the body through

apoptosis, which is a natural self-destruct mechanism. A complex, balanced network of pro-apoptotic and anti-apoptotic genes regulates apoptosis. However, this often becomes dysfunctional during tumor progression as a result of multiple gene mutations. Thus, delivery of gene therapy to reverse the apoptotic functionality of the mutated cells together with apoptosis-inducing chemotherapy is a possible strategy to avoid tumor recurrence and relapse.

Many drugs used in chemotherapy function by directly inducing apoptosis of cancer cells. Without functional cellular networks to signal apoptosis, this mechanism of action is impossible. For example, defects in the apoptotic networks of cancer cells greatly reduce the efficacy of chemotherapy drugs. Fortunately, a combination of agents that restore the apoptotic mechanism with other pro-apoptosis drugs shows promise as an effective strategy for cancer treatment [64]. These approaches rely on expression of tumor suppressor genes like p53, retinoblastoma protein or MDA-7, introduction of apoptosis-inducing proteins like tumor necrosis factor-related apoptosis-inducing ligand (**TRAIL**) or tumor necrosis factor-alpha (**TNF- α**). Other potential approaches rely on silencing of anti-apoptotic genes like survivin, Bcl-2 or BAX.

2.2.1 Tumor Suppressor Genes—The tumor suppressor p53 gene is a key regulator in cell cycle and functions in conserving genome stability. Many cancer cells with a p53 deficiency fail to activate apoptosis and cell-cycle arrest because of gene mutations and DNA damage, resulting in failure to halt the progression of the tumor. Additionally, p53 loss-of-function reduces the therapeutic activity and effectiveness of many chemotherapy drugs. Through using gene therapy *in vivo*, restoring the apoptotic function by introducing functional p53 genes has been shown to sensitize various cancers (e.g., prostate, liver, and breast) to chemotherapeutics like doxorubicin (**DOX**) and to lead to inhibition of tumor growth and enhanced survival. For example, Malkin et al. (2006) demonstrated that treatment with wild-type p53 delivered via an adenovirus significantly increased the sensitivity of various osteosarcoma cell lines to the chemotherapeutics cisplatin and DOX [45]. In a different example, Pack et al. (2012) reported that simultaneous delivery of the p53-encoding plasmid and DOX using double-walled microspheres also improved drug activity [44].

2.2.2 Pro-Apoptotic Genes—In the treatment of head and neck squamous cell carcinoma (**HNSCC**), Zheng et al. (2011) demonstrated that local delivery of the TRAIL gene by adeno-associated virus-2 (**AAV-2**) synergized with cisplatin chemotherapy [54]. Furthermore, cisplatin pre-treatment significantly increased TRAIL-induced apoptosis *in vitro*. Using an HNSCC xenograft tumor model in nude mice, a combination of AAV-2/TRAIL and cisplatin not only exhibited a synergistic anti-cancer effect by remarkably suppressing tumor growth, but also significantly reduced cisplatin-induced bodyweight loss. In another example, a combination of the TRAIL gene therapy and DOX chemotherapy in a model of U87 human glioblastoma was successfully co-delivered using non-viral dendrimer-based delivery system [57]. As shown in Figure 1, successful delivery of the DOX/TRAIL gene combination using the dendrimer system achieved a significantly improved therapeutic effect, as demonstrated by delayed tumor progression and prolonged survival time of the tumor-bearing mice compared with monotherapy of DOX or TRAIL alone.

In addition to TRAIL gene therapy being used in combination with chemotherapy drugs to successfully treat cancer, Wagner and Ogris et al. (2012) described the application of a combined treatment using TNF- α gene and DOX [53]. TNF- α is a cytokine involved in direct killing, of tumor cells and promoting tumor angiogenesis, or the formation of new blood vessels. In both murine and human tumor models, Wagner's group successfully used polyplexes targeted at the epidermal growth factor receptor (**EGFR**), which is

overexpressed in tumor cells, to systemically deliver the TNF- α gene. This treatment strategy synergistically enhanced DOX activity by increasing the accumulation of liposomal DOX. Specifically, multiple injections *in vivo* of combined TNF- α gene and DOX therapy significantly delayed tumor growth in subcutaneous murine neuroblastoma as well as liver metastases of human LS174T colon carcinoma [53].

2.2.3 Silencing Anti-Apoptotic Genes—A combination approach for using anti-apoptotic genes to treat human colorectal cancer has been described by Kiwada et al. (2011) using a mouse xenograft model. This strategy employed a double-modulation strategy of chemotherapy drug 5-fluorouracil (**5-FU**) and Bcl-2 siRNA that were formulated in PEGylated lipoplexes [9]. As 5-FU resistance in colorectal cancer is usually caused by overexpression of anti-apoptotic proteins like Bcl-2, knocking down Bcl-2 is expected to chemosensitize resistant tumors and improve treatment outcomes. As expected, in the mouse xenograft model, the combination showed significantly improved cell growth inhibition and enhanced tumor suppression when compared with either monotherapy.

Another anti-apoptotic gene that has been widely investigated as a target in combination with traditional chemotherapy is survivin. For instance, Bisen et al. (2012) investigated the effect of cisplatin in combination with survivin siRNA on apoptosis in HNSCC. The results confirmed that halting the function of the survivin gene through lentivirus-mediated RNAi silencing therapy significantly increased the sensitivity of cancer cells to cisplatin-mediated apoptosis [47]. Furthermore, Chu et al. (2012) reported the successful application of survivin silencing in combination with PTX for the treatment of ovarian cancer in mice [49]. The siRNA/drug combination was delivered by non-viral delivery platform consisting of β -cyclodextrin (**-CD**) and polyethylenimine (**PEI**) and resulted in effective induction of apoptosis and suppression of tumor growth both *in vitro* and *in vivo* [49].

2.3 Combining Drugs with Nucleic Acids for Immunotherapy

In addition to chemosensitizing tumor cells using combinations of silencing agents and chemotherapeutics, another widely explored strategy is the combination of anti-cancer drugs and immunotherapy agents, including plasmids that code for various cytokines, with a synergistic effect. For example, in resistant 4T1 and sensitive EMT-6 syngeneic mouse breast tumor models Kim et al. (2004) described the combined local interleukin-12 (**IL-12**) pDNA delivery by a non-viral vector (cholesterol-modified PEI) with systemic PTX chemotherapy [25]. This combination therapy showed improved inhibition of both the growth of the primary tumor as well as lung metastases compared with untreated and monotherapy-treated controls. This study provides evidence that local cytokine gene delivery can augment systemic chemotherapy without exposing the host to severe immunotoxicity (Figure 2). In another example, to treat human glioma xenografts, Kaliberov et al. (2009) investigated the use of a combination of interleukin-24 (**IL-24**) gene therapy mediated by adenoviral vectors and chemotherapy using the alkylating chemotherapy agent temozolomide (**TMZ**) [26]. The results suggested that the combination not only increased cell killing *in vitro*, but it also inhibited tumor progression and prolonged survival *in vivo* compared with monotherapy alone. Importantly, such combination treatments rely on the dual action of the chemotherapeutic that triggers apoptosis and immunotherapy by expressing tumor-suppressing cytokine IL-24 to control cell survival and proliferation.

2.4 Combining Drugs with Nucleic Acids with Anti-Angiogenic Functions

Tumor progression and growth requires an adequate supply of blood to transport oxygen and nutrients. Inhibiting angiogenesis is widely regarded as a promising anti-cancer treatment strategy. Specifically, vascular endothelial growth factor (**VEGF**) is one of the best-known regulators to promote angiogenesis. Successful downregulation of VEGF using various

methods like the siRNA or anti-VEGF antibody drug bevacizumab together with chemotherapy has been shown to control tumor growth and prolong survival [65]. Furthermore, Kompella et al. (2009) successfully co-administered a deslorelin-docetaxel conjugate and an anti-VEGF intraceptor Flt23k plasmid using Arg-Gly-Asp (RGD)-conjugated nanoparticles in mice bearing lung tumor xenografts [60]. The results from this study showed additive effects in tumor growth inhibition and improvements in the apoptotic index. Additionally, in a mouse model of human hepatocarcinoma, co-delivery of VEGF siRNA and DOX, using micelles composed of PEI grafted with stearic acid, showed a promising effect on anti-tumor growth [59]. Similarly, Zhong et al. (2010) applied biodegradable cationic micelles to co-delivery of VEGF siRNA and PTX [58]. By in large, the successful results from these studies applying combination strategies to inhibit angiogenesis reinforce the anti-cancer potential in downregulating VEGF in combination with chemotherapeutic drugs.

3. Delivery Strategies for Drug-Nucleic Acid Combinations

When selecting a strategy for the delivery of drug-nucleic acid combinations, multiple factors need to be considered. For example, evaluating issues related to the kinetics of action of the individual agents, the need for sequential or simultaneous exposure, and mutual chemical compatibility helps determine the most-suitable approach. In cases where there is no need for simultaneous delivery, often the best choice is simply combining standard chemotherapy with an existing delivery vector for nucleic acids. One feasible option that does not require the development of novel systems is combining existing nanocarriers for drug delivery with nanocarriers for delivery of nucleic acids. In other cases where the drug-nucleic acid combination benefits from simultaneous delivery, there is a potential need for the development of new delivery methods because existing systems have been developed specifically for a certain class of active agents, and these systems are generally not suitable for the dual delivery of both drugs and nucleic acids.

3.1 Delivery Strategies Based on Viral Vectors

Most types of viral delivery vectors under current investigation in gene therapy have been tested and used in combination treatments with chemotherapeutics. The main focus of these studies has been on lentiviruses, adenoviruses, and adeno-associated viruses. Similar to their use in cancer gene-targeting monotherapies, the main reason for the use of viral delivery vectors is the high efficiency of their transgene expression. To exploit this feature, many studies have used local or regional virus-mediated gene delivery in combination with systemically administered chemotherapy to achieve high levels of the transgene product at the tumor site, while minimizing the risks of systemic exposure to the virus [47, 54]. While the most-common delivery approach using viral vectors is to combine them with sequential administration of standard chemotherapy, a few studies have examined delivering the chemotherapy drug in liposomes in order to improve pharmacokinetics and drug safety. Even though there are a number of reports on chemical modification of viruses with polymers and targeting ligands, direct conjugation of drugs to the virus (either directly or through a polymer) to achieve simultaneous delivery has not been explored. This is most likely because the low viral doses required to achieve therapeutic effect would not allow for a sufficient amount of drug to be delivered to cancer cells when conjugated to the virus.

3.2 Delivery Strategies Based on Non-Viral Vectors

A large variety of synthetic lipid- and polymer-based nanocarriers have been proposed and tested as delivery platforms for drug-nucleic acid combinations. These nanocarriers offer great versatility because of their easy chemical modification and functionalization, which allows for accommodating both small molecule drugs and nucleic acids. In many cases, they

also appear promising in controlling the rate and sequence of release of the individual agents, such as systems based layer-by-layer assembly [66, 67]. Another advantage of non-viral vectors often cited by the field is the favorable safety profile compared to viral vectors.

3.2.1. Co-Encapsulation of Nucleic Acids and Drugs in Biodegradable Polyester Particles

—A simple approach to the delivery of drug-nucleic acid combinations relies on the direct encapsulation of both types of agents in biodegradable polyester particles. For instance, Panyam et al. (2010) described the simultaneous delivery of PTX and Pgp siRNA using poly(D,L-lactide-*co*-glycolide) (**PLGA**) nanoparticles [35]. The nanoparticles were formulated using a double emulsion-solvent evaporation technique to encapsulate both agents. PEI was introduced into the PLGA matrix in order to improve the siRNA loading. To achieve targeted delivery and improved therapeutic outcome both *in vitro* and *in vivo*, these dual-agent nanoparticles were further functionalized using biotin on the surface [35].

Another co-encapsulation techniques was explored by Pack et al. (2012) and investigated the use of double-walled PLGA microspheres consisting of a PLGA core surrounded by a poly(lactic acid) shell to deliver a combination of the anti-cancer drug DOX along with a plasmid that encodes the p53 tumor suppressor protein [44]. This delivery method utilized the double-walled structure of the microspheres to encapsulate chitosan-DNA nanoparticles, while simultaneously loading DOX into the core of the particles. Importantly, the microparticles allowed for the DNA nanoparticles to be released first, followed by subsequent and simultaneous release of DNA nanoparticles and DOX at zero-order rate.

3.2.2. Delivery Systems Based on Complexes of Nucleic Acids with Cationic Lipids and Polymers

—Also widely investigated in nucleic acid monotherapies and in multiple nucleic acid combination therapies are cationic polymers that form complexes with nucleic acids (i.e., polyplexes) and lipids that form complexes with nucleic acids (i.e., lipoplexes). However, unlike polyester particles, these complexes do not allow for easy co-encapsulation of small molecule drugs. The approaches for incorporating drugs into polyplexes and lipoplexes rely on either enhancing the hydrophobicity of the nucleic acid complexes in order to improve drug loading, incorporating the drug into the complex in the form of inclusion complexes, or directly conjugating the drug to the polymeric vector via a biodegradable linker [32, 57]. For example, a co-delivery system based on polyamidoamine (**PAMAM**) dendrimers modified with hyaluronic acid (**HA**) has been shown to efficiently co-deliver DOX and siRNA. The nanoparticles were formulated by embedding DOX in the hydrophobic interior of the synthesized PAMAM-HA conjugate, followed by electrostatically binding siRNA to the positively charged surface. The utility of the system was demonstrated using a knock-down of the major vault protein (**MVP**) [40]. MVP is associated with drug resistance in DOX-resistant human breast cancer cells MCF-7/ADR because of MVP's ability to transport DOX out of the cell nucleus. Effective co-delivery of a combination of DOX and MVP siRNA has been shown to alter the intracellular distribution of DOX and help contain the drug in the nucleus, thus helping to overcome drug resistance [40].

An alternative to directly embedding drugs into the lipid or polymer carrier is the incorporation of β -CD into the structure of the polycation and forming inclusion complexes with drugs. In particular, to overcome MDR, Mao et al. (2012) developed a series of quantum dots (**QDs**) that were functionalized with β -CD for the simultaneous delivery of MDR1 siRNA and DOX [33]. In this co-delivery system, CdSe and ZnSe QDs were first modified with β -CD coupled to an amino acid (i.e., L-Arg or L-His). DOX was then encapsulated into the hydrophobic cavity of β -CD via electrostatic interactions before complexation with MDR1 siRNA (Figure 3). Using a similar strategy, Chu et al. (2012) also

reported a successful application of survivin silencing using shRNA in combination with PTX in the treatment of ovarian cancer [49]. The combination was delivered using polymeric nanoparticles composed of PEI conjugated with β -CD. The hydrophobic cavities of β -CD in the PEI-CD conjugates were loaded with PTX that was conjugated with 2-amino-adamantane and used to form polyplexes with survivin shRNA. This co-delivery system effectively induced apoptosis and suppressed tumor growth *in vivo*. The same system has also been shown to have synergistic anti-tumor activity when used to co-deliver DOX and the TRAIL plasmid [55].

Hybrid systems consisting of polycations modified with lipids have been shown to be beneficial in the delivery of drug-nucleic acid combinations. This is due to their improved release properties, decreased toxicity, and better encapsulation of hydrophobic drugs than simple polycation-based systems. For instance, lipids such as dioleoylphosphatidylethanolamine (DOPE) and cholesterol have been incorporated into polymeric delivery systems to facilitate drug solubilization and membrane fusion of the resultant nanocarriers. Specifically, DOPE has been conjugated to PEI and used to deliver a combination of Pgp siRNA and DOX to overcome drug resistance in human breast cancer cells *in vitro* [37]. This mode of simultaneous delivery significantly sensitized drug-resistant MCF-7 cells to DOX. Another alternative strategy reported by Torchilin et al. (2012) explored a micelle system consisting of a mixture of DOPE-PEG with a DOPE-modified PAMAM dendrimer. The resulting mixed micelles showed increased loading capacity for both active agents (i.e., DOX and siRNA) and promising activity in the treatment of MDR tumors [68].

Considering that polyplexes and lipoplexes form independently of a sequence of the nucleic acids, combining multiple nucleic acids in a single carrier is a particularly attractive option for these complexes. For example, Minko et al. (2008) described a strategy for simultaneous co-delivery of DOX in a single cationic liposome using a mixture of the following two siRNAs: one targeted against MRP1 and one targeted against Bcl-2 [15]. The system exhibited enhanced anti-cancer efficacy through suppressing both the drug-efflux pump resistance and the non-pump anti-apoptotic resistance. Similarly, Li et al. (2012) explored the possibility of using combinations of multiple siRNAs to target both Pgp and survivin by reduction-sensitive cationic poly(β -amino esters), which revealed enhanced anti-cancer activity of DOX chemotherapy in a drug-resistant human breast cancer model [51].

3.2.3. Inorganic Nanoparticles—Inorganic nanoparticles offer an interesting alternative to polymer and lipid-based nanocarriers in delivering drug-nucleic acid combinations. In recent years, mesoporous silica nanoparticles (MSN) have emerged as a promising platform for delivering drug-nucleic acid combinations [13, 38, 69, 70]. The primary advantage of MSN over other delivery platforms is their ability to encapsulate drugs with a broad range of physicochemical properties (e.g., hydrophobic, hydrophilic, differing sizes). Furthermore, the well-established surface chemistry of MSN allows for easy surface modification with polycations, thus enabling the creation of hybrid organic-inorganic particles that can also serve as carriers for nucleic acids. Another advantage of MSN over other gene and siRNA delivery systems is that their porous structure allows nucleic acids to bind to the outer surface particles and large amounts of small molecules can be encapsulated within the particles. Unfortunately, while the large surface area and free volume of MSN allow for easy drug loading, the drugs are typically released rapidly from the complex, with limited ability for sustained release. In turn, additional delivery methods are required to control the release, particularly for soluble drugs that have a low affinity for silica. Numerous inventive strategies have been devised to control the rate of drug release from MSN, but many of these strategies are not robust enough for systemic use *in vivo* [71–75]. In a recent example by Nel et al. (2010), the MSN surface was functionalized with negatively charged phosphonate

groups, allowing both electrostatic binding of DOX to the porous interior and coating with PEI for electrostatic complexation with siRNA. The activity of the system was demonstrated *in vitro* by the co-delivery of DOX with Pgp siRNA in drug-resistant human cervical carcinoma KB-V1 cells [38]. The system was able to enhance intracellular drug concentration as a result of the Pgp silencing.

Gold nanoparticles are another example of inorganic nanocarriers that show promise in the delivery of drug-nucleic acid combinations. In particular, Gong et al. (2012) developed multifunctional gold nanorods capable of co-delivering DOX and siRNA against achaete-scute complex-like 1 (**ASCL1**). Using octreotide as the targeting ligand, the particles were delivered specifically to neuroendocrine cancer cells that overexpressed somatostatin receptors [8]. DOX was conjugated to the gold nanorods via a pH-sensitive hydrazone linker, and siRNA was electrostatically bound using polyarginine. The delivery system exhibited high efficiency in altering the cancer cell phenotype and strong anti-proliferative activity.

3.2.4. Multifunctional and Theranostic Delivery Systems—In the past several years chemotherapy research has demonstrated an increasing focus on the development of complex, multifunctional delivery systems with an emphasis on combining the primary delivery function with imaging capability (thera(g)nostics) [33]. Such systems are typically assembled with multiple components designed to simultaneously provide delivery of drugs, nucleic acids, specific targeting, and imaging. A typical example of such a multifunctional system has been reported by Bhujwalla et al. (2012) and consists of (i) siRNA to downregulate choline kinase, (ii) an enzyme to convert nontoxic prodrug 5-fluorocytosine into a cytotoxic 5-FU, (iii) a ligand to target the prostate-specific membrane antigen, and (iv) a probe for SPECT/CT imaging [17]. While intellectually attractive, it remains to be determined if the benefits of such multifunctional systems outweigh the challenges brought on by the complexity associated with their assembly and characterization.

3.3 Delivery Strategies Based on Dual-Function Vectors

An interesting alternative to the above delivery vectors are systems in which the carrier doubles as a nucleic acid delivery vector and a (pro)drug that can enhance the therapeutic outcome of nucleic acids. A growing number of examples of such carriers successfully combine the delivery function of vectors with pharmacologic activity. Pluronic block copolymers are probably the best-known and most-investigated example of such carriers. These copolymers chemosensitize MDR cancer cells through multiple mechanisms, including inhibition of the Pgp drug efflux system [76], depletion of cellular ATP pools [77], and dysfunction of mitochondria [78]. While most of the available reports address the use of Pluronics for delivery of chemotherapeutics, importantly, the copolymers can be also used to deliver nucleic acids. Namely, it has been demonstrated that Pluronics can activate the nuclear factor kappa-light-chain-enhancer of activated B cells (**NF- κ B**), which is a transcription factor that enhances gene expression [79]. By introducing Pluronics into existing polyplexes, the gene delivery efficiency can be significantly improved, without causing cytotoxicity, as a result of increased cell uptake and nuclear transport of pDNA.

In most cases, dual-function carriers are rationally designed for specific applications. For example, Huang et al. (2009) developed a novel cationic lipid named DSGLA that is capable of delivering siRNA while simultaneously down-regulating the phosphorylated extracellular signal-regulated kinase (**pERK**) to enhance anti-cancer activity of the delivered siRNA [22]. The lipid, which contains guanidinyll and lysyl headgroups, has been shown to efficiently down-regulate pERK in H460 human lung carcinoma cells and to induce their apoptosis. Furthermore, a significant enhancement in tumor growth inhibition was observed in a mouse

model of lung tumors after intravenous administration of DSGLA/siRNA lipoplexes containing EGFR siRNA.

In addition to the use of lipoplexes as a dual-function vectore, Rice et al. (2008) designed a type of novel cationic polypeptides capable of incorporating peptides with an intrinsic proteasome inhibitory function in order to improve transfection activity of plasmid DNA [80]. Proteasomes are important cellular enzymes responsible for the degradation of a variety of proteins. Furthermore, the inhibition of proteasomes has been shown to enhance transfection efficiency. Interestingly, in human hepatocellular carcinoma HepG2 cells, Rice et al. incorporated a tripeptide aldehyde proteasome inhibitor into the C-terminal end of a cationic gene delivery peptide (Cys-Trp-Lys₁₈), and the resulting carrier showed elevated gene expression. Rice et al. note that using peptides with a proteasome inhibitory function as the carrier for pDNA delivery is more beneficial than concurrent treatment with pDNA polyplexes and free proteasome inhibitors. Thus, introducing intrinsic proteasome inhibitory activity has shown potential to boost the efficiency of anti-cancer gene therapy.

We have recently reported synthesis of biodegradable polycations that can function dually as gene delivery vectors and as antagonists of the CXCR4 chemokine receptor [81]. As a result of CXCR4 inhibition, the synthesized polycations were able to block the invasion of cancer cells, while simultaneously mediating efficient transfection *in vitro* (Figure 4). Evidence shows that the chemokine receptor CXCR4 and its cognate ligand SDF-1 (CXCL12) play critical roles in tumor invasion and metastasis. Likewise, many clinical studies have revealed that CXCR4-positive tumors metastasize to distant sites with high levels of SDF-1. Blocking the CXCR4/SDF-1 axis either by CXCR4 antagonists or by siRNA silencing of the CXCR4 gene has been shown to be capable of preventing metastasis [82]. Such dual-function delivery vectors are expected to enhance anti-metastatic efficacy in a variety of cancer gene therapy methods.

Another example of a gene delivery carrier with dual functionality relies on a type of anti-cancer agent based on analogs of the natural polyamines spermine and spermidine [83, 84]. Frequently in cancer, the metabolism of natural polyamines is dysregulated. The anti-cancer agents based on the polyamine analogs exploit the self-regulatory nature of the metabolism of cellular polyamines and have multiple targets in the polyamine pathway [85]. This makes the polyamine analogues attractive building blocks for the synthesis of cationic lipids and polymers suitable for the delivery of therapeutic nucleic acids to tumors, where the polymers can augment the nucleic acid activity by exerting their own pharmacologic effect. Among the most successful of the polyamine analogues developed is N¹,N¹¹-bisethylnorspermine (BENSpm) [84, 86]. We have utilized the anti-cancer drug BENSpm to synthesize a lipid prodrug that can function dually as gene delivery vector and, after intracellular degradation, as an active anti-cancer agent that targets dysregulated polyamine metabolism [87]. This lipid prodrug uses a thiololytically sensitive dithiobenzyl carbamate linker to release BENSpm into the intracellular-reducing environment and to restore the anti-cancer activity of BENSpm. Furthermore, BENSpm showed a strong synergistic enhancement of cytotoxic activity of TRAIL in human breast cancer cells, suggesting suitability of this delivery vector for cancer gene therapy protocols utilizing TRAIL as the therapeutic gene.

4. Timing Considerations for Delivery of Drug-Nucleic Acid Combinations

Pharmacologic effects of various drug-nucleic acid combinations and the interplay between the signaling pathways involved are complicated and not necessarily well understood. Improved understanding of optimally timing the action of the two agents involved is critical for the design of delivery systems used in combination therapy. A recent study by Yaffe et al. (2012) elegantly demonstrates the importance of timing, duration, and the order of

exposure to individual drugs in combination cancer treatment [6]. This study by Yaffe et al. reveals that inhibition of EGFR significantly enhances sensitivity of triple-negative breast cancer cells to DNA-damaging chemotherapy *only* when the treatments were administered sequentially and in a specific order. These findings emphasize the need for improved understanding of the cell signaling network and the crosstalk between pathways involved in combination treatments in order to improve treatment strategies.

4.1 Co-Delivery vs. Separated Delivery

In addition to mechanistic considerations, co-delivery of chemotherapeutics and therapeutic nucleic acids in a single nanocarrier may offer benefits in terms of convenience, synchronized pharmacokinetics, and delivery of defined, relative amounts of both agents to the same population of cells. On the other hand, differences in optimal administration routes or toxicity profiles may require formulating the two agents individually for separate delivery in order to achieve the best therapeutic effects. For example, in the previously discussed case using a combination of PTX chemotherapy with IL-12 gene therapy [25], the IL-12 plasmid was administered locally in order to reduce the risk of systemic IL-12 toxicity. Because systemic administration of IL-12 causes severe immunotoxicity and fails to maintain adequate IL-12 levels in the tumor, the local delivery of IL-12 not only circumvents the unnecessary chemokine exposure of healthy tissues, but is also able to increase the concentration of the therapeutic agent at the targeted tissue site. Additionally, co-delivery of drug-nucleic acid combinations in a single nanocarrier may not be feasible when the two agents are chemically incompatible or when their combined presence affects the stability of the nanocarrier. For example, Wang et al. (2010) described an effective, separated delivery strategy to treat drug-resistant breast cancer using a mouse model. The treatment consisted of administering RGD-modified liposomes containing Pgp siRNA followed by DOX-containing liposomes [36]. Attempted co-encapsulation of both agents in the same liposome formulation caused aggregation of liposomes and DOX leakage from the liposomes, suggesting incompatibility of the two active agents in a single delivery system and the need for separate individual delivery vectors.

4.2 Simultaneous Release vs. Sequential Release

As briefly discussed earlier, many therapeutic combinations only work when the tumors are exposed to the drugs in a particular sequence. For example, many drug-nucleic acid combinations discussed in this review are based on first altering signaling pathways in cancer cells to sensitize them to the drug, thus providing the rationale for the sequential release. In general, in cases where co-delivery of drug-nucleic acid combinations is warranted, there is a compelling rationale for the development of delivery systems with a controlled rate and sequence of release of the individual agents. This is, in part, the result of a natural lag between administration of therapeutic nucleic acids (e.g., genes, siRNA) and their effect due to the need for transgene expression of the delivered gene or the time it takes for the amount of targeted protein to decline to desired levels in the case of gene silencing.

Development of delivery systems capable of independently controlling the release of individual agents in drug-nucleic acids remains a compelling pursuit, but the complexity and challenges faced are immense. Primarily, there is a need for an improved quantitative understanding of the relationship between release kinetics of the individual agents and their therapeutic activity in the combination treatments. Among such rare kinetic studies, Amiji et al. (2009) explored the effect of time lag between siRNA treatment and PTX treatment using biodegradable nanoparticles and found that the maximum combination effect was observed when PTX was administered 24 hours after siRNA treatment in order to allow for sufficient Pgp knockdown [34]. Torchilin et al. (2012), on the other hand, found that the combination activity is not always influenced by the timing of exposure to the two therapeutic agents.

The Torchilin study demonstrated that activity of DOX was significantly increased by siRNA-mediated Pgp silencing regardless of the time lag (0–48 h) between the two treatments [37], further emphasizing the need for better understanding the fundamental kinetic aspects of such combination treatments.

5. Conclusion and Future Directions

The benefits of drug-nucleic acid combinations for improved cancer therapies are clearly established. This review article provides an overview of emerging trends in using nanocarriers to deliver drug-nucleic acid combinations. Recent progress in the development of a wide variety of multifunctional nanocarriers promises to extend the success of traditional combination treatments to a new category of drug-nucleic acid combinations. Still, there are many scientific and technical challenges that can hinder progress (the benefits and limitations of each delivery mode discussed previously are summarized in Table 2). Also, there is the temptation of many of us in the field to create overly complex delivery systems with little chance of having a practical application, simply for the satisfaction of creating the complex structures. Nevertheless, in order to optimize the overall anti-cancer activity and minimize the toxicity of combination treatments, among the primary opportunities is the great need for an improved fundamental understanding of the relationship among the mechanism of action of individual agents, the kinetics of their pharmacologic activity, and the mode of delivery. This improved understanding can, in turn, guide the design of delivery vectors with appropriate timing and sequence of delivery of individual agents of the combination. Furthermore, with the commercial availability of genome-wide siRNA libraries suitable for high throughput testing, there is tremendous opportunity for the discovery of new drug-nucleic combinations that would extend beyond the current dominant focus on overcoming drug resistance and enhance the effectiveness and safety of drug-nucleic acid combinations.

Acknowledgments

The authors would like to thank Ms. Melody Montgomery for help in the professional editing of this manuscript and the NIH for support (EB014570).

Abbreviations

siRNA	small interfering RNA
AON	antisense oligonucleotides
shRNA	small hairpin RNA
pDNA	plasmid DNA
CYP3A	cytochrome P450 3A
MDR	multidrug resistance
MDR1	multidrug resistance protein 1
Pgp	P-glycoprotein
TRAIL	tumor necrosis factor-related apoptosis-inducing ligand
TNF-	tumor necrosis factor-alpha
DOX	doxorubicin
AAV-2	adeno-associated virus-2
HNSCC	head and neck squamous cell carcinoma

5-FU	5-fluorouracil
PEI	polyethylenimine
TMZ	temozolomide
IL-24	interleukin-24
IL-12	interleukin-12
VEGF	vascular endothelial growth factor
PTX	paclitaxel
PLGA	poly(D,L-lactide-co-glycolide)
PAMAM	polyamidoamine
HA	hyaluronic acid
-CD	-cyclodextrin
MVP	major vault protein
QDs	quantum dots
DOPE	dioleoylphosphatidylethanolamine
MSN	Mesoporous silica nanoparticles
ASCL1	achaete-scute complex-like 1
CXCL12	CXCR4 cognate ligand SDF-1
pERK	phosphorylated extracellular signal-regulated kinase
NF B	nuclear factor kappa-light-chain-enhancer of activated B cells
EGFR	epidermal growth factor receptor
BENSpm	N^1,N^{11} -bisethylmorphine

References

1. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell*. 2000; 100:57–70. [PubMed: 10647931]
2. Miles D, von Minckwitz G, Seidman AD. Combination versus sequential single-agent therapy in metastatic breast cancer. *Oncologist*. 2002; 7(Suppl 6):13–19. [PubMed: 12454315]
3. Dachs GU, Dougherty GJ, Stratford IJ, Chaplin DJ. Targeting gene therapy to cancer: a review. *Oncol Res*. 1997; 9:313–325. [PubMed: 9406237]
4. Izquierdo M. Short interfering RNAs as a tool for cancer gene therapy. *Cancer Gene Ther*. 2005; 12:217–227. [PubMed: 15550938]
5. Mercola D, Cohen JS. Antisense approaches to cancer gene therapy. *Cancer Gene Ther*. 1995; 2:47–59. [PubMed: 7621254]
6. Lee MJ, Ye AS, Gardino AK, Heijink AM, Sorger PK, MacBeath G, Yaffe MB. Sequential application of anticancer drugs enhances cell death by rewiring apoptotic signaling networks. *Cell*. 2012; 149:780–794. [PubMed: 22579283]
7. Bauer JA, Ye F, Marshall CB, Lehmann BD, Pendleton CS, Shyr Y, Arteaga CL, Pietenpol JA. RNA interference (RNAi) screening approach identifies agents that enhance paclitaxel activity in breast cancer cells. *Breast Cancer Res*. 2010; 12:R41. [PubMed: 20576088]
8. Xiao Y, Jaskula-Sztul R, Javadi A, Xu W, Eide J, Dammalapati A, Kunnimalaiyaan M, Chen H, Gong S. Co-delivery of doxorubicin and siRNA using octreotide-conjugated gold nanorods for targeted neuroendocrine cancer therapy. *Nanoscale*. 2012; 4:7185–7193. [PubMed: 23070403]

9. Nakamura K, Abu Lila AS, Matsunaga M, Doi Y, Ishida T, Kiwada H. A double-modulation strategy in cancer treatment with a chemotherapeutic agent and siRNA. *Mol Ther.* 2011; 19:2040–2047. [PubMed: 21878904]
10. Cao N, Cheng D, Zou S, Ai H, Gao J, Shuai X. The synergistic effect of hierarchical assemblies of siRNA and chemotherapeutic drugs co-delivered into hepatic cancer cells. *Biomaterials.* 2011; 32:2222–2232. [PubMed: 21186059]
11. Cheng D, Cao N, Chen J, Yu X, Shuai X. Multifunctional nanocarrier mediated co-delivery of doxorubicin and siRNA for synergistic enhancement of glioma apoptosis in rat. *Biomaterials.* 2012; 33:1170–1179. [PubMed: 22061491]
12. Koganti S, Jagani HV, Palanimuthu VR, Mathew JA, Rao MC, Rao JV. In vitro and in vivo evaluation of the efficacy of nanoformulation of siRNA as an adjuvant to improve the anticancer potential of cisplatin. *Exp Mol Pathol.* 2012
13. Chen AM, Zhang M, Wei D, Stueber D, Taratula O, Minko T, He H. Co-delivery of doxorubicin and Bcl-2 siRNA by mesoporous silica nanoparticles enhances the efficacy of chemotherapy in multidrug-resistant cancer cells. *Small.* 2009; 5:2673–2677. [PubMed: 19780069]
14. Wang Y, Gao S, Ye WH, Yoon HS, Yang YY. Co-delivery of drugs and DNA from cationic core-shell nanoparticles self-assembled from a biodegradable copolymer. *Nat Mater.* 2006; 5:791–796. [PubMed: 16998471]
15. Saad M, Garbuzenko OB, Minko T. Co-delivery of siRNA and an anticancer drug for treatment of multidrug-resistant cancer. *Nanomedicine (London, U K).* 2008; 3:761–776.
16. Mu P, Nagahara S, Makita N, Tarumi Y, Kadomatsu K, Takei Y. Systemic delivery of siRNA specific to tumor mediated by atelocollagen: combined therapy using siRNA targeting Bcl-xL and cisplatin against prostate cancer. *Int J Cancer.* 2009; 125:2978–2990. [PubMed: 19422046]
17. Chen Z, Penet MF, Nimmagadda S, Li C, Banerjee SR, Winnard PT Jr, Artemov D, Glunde K, Pomper MG, Bhujwalla ZM. PSMA-targeted theranostic nanoplex for prostate cancer therapy. *ACS Nano.* 2012; 6:7752–7762. [PubMed: 22866897]
18. Chen Y, Wu JJ, Huang L. Nanoparticles targeted with NGR motif deliver c-myc siRNA and doxorubicin for anticancer therapy. *Mol Ther.* 2010; 18:828–834. [PubMed: 20068551]
19. Chen Y, Bathula SR, Li J, Huang L. Multifunctional nanoparticles delivering small interfering RNA and doxorubicin overcome drug resistance in cancer. *J Biol Chem.* 2010; 285:22639–22650. [PubMed: 20460382]
20. Neschadim A, Wang JC, Lavie A, Medin JA. Bystander killing of malignant cells via the delivery of engineered thymidine-active deoxycytidine kinase for suicide gene therapy of cancer. *Cancer Gene Ther.* 2012; 19:320–327. [PubMed: 22388453]
21. Kim C, Shah BP, Subramaniam P, Lee KB. Synergistic induction of apoptosis in brain cancer cells by targeted codelivery of siRNA and anticancer drugs. *Mol Pharm.* 2011; 8:1955–1961. [PubMed: 21793576]
22. Chen Y, Sen J, Bathula SR, Yang Q, Fittipaldi R, Huang L. Novel cationic lipid that delivers siRNA and enhances therapeutic effect in lung cancer cells. *Mol Pharm.* 2009; 6:696–705. [PubMed: 19267451]
23. Sher YP, Chang CM, Juo CG, Chen CT, Hsu JL, Lin CY, Han Z, Shiah SG, Hung MC. Targeted endostatin-cytosine deaminase fusion gene therapy plus 5-fluorocytosine suppresses ovarian tumor growth. *Oncogene.* 2012
24. Dias JD, Liikanen I, Guse K, Foloppe J, Sloniecka M, Diaconu I, Rantanen V, Eriksson M, Hakkarainen T, Lusky M, Erbs P, Escutenaire S, Kanerva A, Pesonen S, Cerullo V, Hemminki A. Targeted chemotherapy for head and neck cancer with a chimeric oncolytic adenovirus coding for bifunctional suicide protein FCU1. *Clin Cancer Res.* 2010; 16:2540–2549. [PubMed: 20388844]
25. Janat-Amsbury MM, Yockman JW, Lee M, Kern S, Furgeson DY, Bikram M, Kim SW. Combination of local, nonviral IL12 gene therapy and systemic paclitaxel treatment in a metastatic breast cancer model. *Mol Ther.* 2004; 9:829–836. [PubMed: 15194049]
26. Kaliberova LN, Krendelchtchikova V, Harmon DK, Stockard CR, Petersen AS, Markert JM, Gillespie GY, Grizzle WE, Buchsbaum DJ, Kaliberov SA. CRAdRGDflt-IL24 virotherapy in combination with chemotherapy of experimental glioma. *Cancer Gene Ther.* 2009; 16:794–805. [PubMed: 19363468]

27. Posthumadeboer J, van Egmond PW, Helder MN, de Menezes RX, Cleton-Jansen AM, Belien JA, Verheul HM, van Royen BJ, Kaspers GJ, van Beusechem VW. Targeting JNK-interacting-protein-1 (JIP1) sensitises osteosarcoma to doxorubicin. *Oncotarget*. 2012; 3:1169–1181. [PubMed: 23045411]
28. Chang RS, Suh MS, Kim S, Shim G, Lee S, Han SS, Lee KE, Jeon H, Choi HG, Choi Y, Kim CW, Oh YK. Cationic drug-derived nanoparticles for multifunctional delivery of anticancer siRNA. *Biomaterials*. 2011; 32:9785–9795. [PubMed: 21937102]
29. Kang SH, Cho HJ, Shim G, Lee S, Kim SH, Choi HG, Kim CW, Oh YK. Cationic liposomal co-delivery of small interfering RNA and a MEK inhibitor for enhanced anticancer efficacy. *Pharm Res*. 2011; 28:3069–3078. [PubMed: 21879387]
30. Yu YH, Kim E, Park DE, Shim G, Lee S, Kim YB, Kim CW, Oh YK. Cationic solid lipid nanoparticles for co-delivery of paclitaxel and siRNA. *Eur J Pharm Biopharm*. 2012; 80:268–273. [PubMed: 22108492]
31. Shim G, Han SE, Yu YH, Lee S, Lee HY, Kim K, Kwon IC, Park TG, Kim YB, Choi YS, Kim CW, Oh YK. Trilysinoyl oleylamide-based cationic liposomes for systemic co-delivery of siRNA and an anticancer drug. *J Controlled Release*. 2011; 155:60–66.
32. Xiong XB, Lavasanifar A. Traceable multifunctional micellar nanocarriers for cancer-targeted co-delivery of MDR-1 siRNA and doxorubicin. *ACS Nano*. 2011; 5:5202–5213. [PubMed: 21627074]
33. Li JM, Wang YY, Zhao MX, Tan CP, Li YQ, Le XY, Ji LN, Mao ZW. Multifunctional QD-based co-delivery of siRNA and doxorubicin to HeLa cells for reversal of multidrug resistance and real-time tracking. *Biomaterials*. 2012; 33:2780–2790. [PubMed: 22243797]
34. Yadav S, van Vlerken LE, Little SR, Amiji MM. Evaluations of combination MDR-1 gene silencing and paclitaxel administration in biodegradable polymeric nanoparticle formulations to overcome multidrug resistance in cancer cells. *Cancer Chemother Pharmacol*. 2009; 63:711–722. [PubMed: 18618115]
35. Patil YB, Swaminathan SK, Sadhukha T, Ma L, Panyam J. The use of nanoparticle-mediated targeted gene silencing and drug delivery to overcome tumor drug resistance. *Biomaterials*. 2010; 31:358–365. [PubMed: 19800114]
36. Jiang J, Yang SJ, Wang JC, Yang LJ, Xu ZZ, Yang T, Liu XY, Zhang Q. Sequential treatment of drug-resistant tumors with RGD-modified liposomes containing siRNA or doxorubicin. *Eur J Pharm Biopharm*. 2010; 76:170–178. [PubMed: 20600887]
37. Navarro G, Sawant RR, Biswas S, Essex S, Tros de Ilarduya C, Torchilin VP. P-glycoprotein silencing with siRNA delivered by DOPE-modified PEI overcomes doxorubicin resistance in breast cancer cells. *Nanomedicine (London, U K)*. 2012; 7:65–78.
38. Meng H, Liong M, Xia T, Li Z, Ji Z, Zink JJ, Nel AE. Engineered design of mesoporous silica nanoparticles to deliver doxorubicin and P-glycoprotein siRNA to overcome drug resistance in a cancer cell line. *ACS Nano*. 2010; 4:4539–4550. [PubMed: 20731437]
39. Xu J, Mo Y, Wang X, Liu J, Zhang X, Wang J, Hu L, Yang C, Chen L, Wang Y. Conditionally replicative adenovirus-based mda-7/IL-24 expression enhances sensitivity of colon cancer cells to 5-fluorouracil and doxorubicin. *J Gastroenterol*. 2012
40. Han M, Lv Q, Tang XJ, Hu YL, Xu DH, Li FZ, Liang WQ, Gao JQ. Overcoming drug resistance of MCF-7/ADR cells by altering intracellular distribution of doxorubicin via MVP knockdown with a novel siRNA polyamidoamine-hyaluronic acid complex. *J Controlled Release*. 2012; 163:136–144.
41. McAuliffe SM, Morgan SL, Wyant GA, Tran LT, Muto KW, Chen YS, Chin KT, Partridge JC, Poole BB, Cheng KH, Daggett J Jr, Cullen K, Kantoff E, Hasselbatt K, Berkowitz J, Muto MG, Berkowitz RS, Aster JC, Matulonis UA, Dinulescu DM. Targeting Notch, a key pathway for ovarian cancer stem cells, sensitizes tumors to platinum therapy. *Proc Natl Acad Sci U S A*. 2012; 109:E2939–2948. [PubMed: 23019585]
42. Cun B, Song X, Jia R, Zhao X, Wang H, Ge S, Fan X. Combination of oncolytic adenovirus and dacarbazine attenuates antitumor ability against uveal melanoma cells via cell cycle block. *Cancer Biol Ther*. 2012; 13:77–84. [PubMed: 22336909]

43. Quirin C, Mainka A, Hesse A, Nettelbeck DM. Combining adenoviral oncolysis with temozolomide improves cell killing of melanoma cells. *Int J Cancer*. 2007; 121:2801–2807. [PubMed: 17724714]
44. Xu Q, Xia Y, Wang CH, Pack DW. Monodisperse double-walled microspheres loaded with chitosan-p53 nanoparticles and doxorubicin for combined gene therapy and chemotherapy. *J Controlled Release*. 2012; 163:130–135.
45. Ganjavi H, Gee M, Narendran A, Parkinson N, Krishnamoorthy M, Freedman MH, Malkin D. Adenovirus-mediated p53 gene therapy in osteosarcoma cell lines: sensitization to cisplatin and doxorubicin. *Cancer Gene Ther*. 2006; 13:415–419. [PubMed: 16211088]
46. Sun TM, Du JZ, Yao YD, Mao CQ, Dou S, Huang SY, Zhang PZ, Leong KW, Song EW, Wang J. Simultaneous delivery of siRNA and paclitaxel via a “two-in-one” micelleplex promotes synergistic tumor suppression. *ACS Nano*. 2011; 5:1483–1494. [PubMed: 21204585]
47. Khan Z, Tiwari RP, Khan N, Prasad G, Bisen PS. Induction of apoptosis and sensitization of head and neck squamous carcinoma cells to cisplatin by targeting survivin gene expression. *Curr Gene Ther*. 2012
48. Fetz V, Bier C, Habtemichael N, Schuon R, Schweitzer A, Kunkel M, Engels K, Kovacs AF, Schneider S, Mann W, Stauber RH, Knauer SK. Inducible NO synthase confers chemoresistance in head and neck cancer by modulating survivin. *Int J Cancer*. 2009; 124:2033–2041. [PubMed: 19130609]
49. Hu Q, Li W, Hu X, Hu Q, Shen J, Jin X, Zhou J, Tang G, Chu PK. Synergistic treatment of ovarian cancer by co-delivery of survivin shRNA and paclitaxel via supramolecular micellar assembly. *Biomaterials*. 2012; 33:6580–6591. [PubMed: 22717365]
50. Hossain MM, Banik NL, Ray SK. Survivin knockdown increased anti-cancer effects of (–)-epigallocatechin-3-gallate in human malignant neuroblastoma SK-N-BE2 and SH-SY5Y cells. *Exp Cell Res*. 2012; 318:1597–1610. [PubMed: 22507272]
51. Yin Q, Shen J, Chen L, Zhang Z, Gu W, Li Y. Overcoming multidrug resistance by co-delivery of Mdr-1 and survivin-targeting RNA with reduction-responsive cationic poly(beta-amino esters). *Biomaterials*. 2012; 33:6495–6506. [PubMed: 22704597]
52. Lee ES, Ko KK, Joe YA, Kang SG, Hong YK. Inhibition of STAT3 reverses drug resistance acquired in temozolomide-resistant human glioma cells. *Oncol Lett*. 2011; 2:115–121. [PubMed: 22870139]
53. Su B, Cengizeroglu A, Farkasova K, Viola JR, Anton M, Ellwart JW, Haase R, Wagner E, Ogris M. Systemic TNFalpha Gene Therapy Synergizes With Liposomal Doxorubicine in the Treatment of Metastatic Cancer. *Mol Ther*. 2012
54. Jiang M, Liu Z, Xiang Y, Ma H, Liu S, Liu Y, Zheng D. Synergistic antitumor effect of AAV-mediated TRAIL expression combined with cisplatin on head and neck squamous cell carcinoma. *BMC Cancer*. 2011; 11:54. [PubMed: 21291526]
55. Fan H, Hu QD, Xu FJ, Liang WQ, Tang GP, Yang WT. In vivo treatment of tumors using host-guest conjugated nanoparticles functionalized with doxorubicin and therapeutic gene pTRAIL. *Biomaterials*. 2012; 33:1428–1436. [PubMed: 22079004]
56. Han L, Huang R, Li J, Liu S, Huang S, Jiang C. Plasmid pORF-hTRAIL and doxorubicin co-delivery targeting to tumor using peptide-conjugated polyamidoamine dendrimer. *Biomaterials*. 2011; 32:1242–1252. [PubMed: 20971503]
57. Liu S, Guo Y, Huang R, Li J, Huang S, Kuang Y, Han L, Jiang C. Gene and doxorubicin co-delivery system for targeting therapy of glioma. *Biomaterials*. 2012; 33:4907–4916. [PubMed: 22484049]
58. Zhu C, Jung S, Luo S, Meng F, Zhu X, Park TG, Zhong Z. Co-delivery of siRNA and paclitaxel into cancer cells by biodegradable cationic micelles based on PDMAEMA-PCL-PDMAEMA triblock copolymers. *Biomaterials*. 2010; 31:2408–2416. [PubMed: 19963269]
59. Huang HY, Kuo WT, Chou MJ, Huang YY. Co-delivery of anti-vascular endothelial growth factor siRNA and doxorubicin by multifunctional polymeric micelle for tumor growth suppression. *J Biomed Mater Res A*. 2011; 97:330–338. [PubMed: 21465641]

60. Sundaram S, Trivedi R, Durairaj C, Ramesh R, Ambati BK, Kompella UB. Targeted drug and gene delivery systems for lung cancer therapy. *Clin Cancer Res.* 2009; 15:7299–7308. [PubMed: 19920099]
61. Szakacs G, Paterson JK, Ludwig JA, Booth-Genthe C, Gottesman MM. Targeting multidrug resistance in cancer. *Nat Rev Drug Discov.* 2006; 5:219–234. [PubMed: 16518375]
62. Relling MV. Are the major effects of P-glycoprotein modulators due to altered pharmacokinetics of anticancer drugs? *Ther Drug Monit.* 1996; 18:350–356. [PubMed: 8857549]
63. Benet LZ, Cummins CL, Wu CY. Unmasking the dynamic interplay between efflux transporters and metabolic enzymes. *Int J Pharm.* 2004; 277:3–9. [PubMed: 15158963]
64. Wendel HG, De Stanchina E, Fridman JS, Malina A, Ray S, Kogan S, Cordon-Cardo C, Pelletier J, Lowe SW. Survival signalling by Akt and eIF4E in oncogenesis and cancer therapy. *Nature.* 2004; 428:332–337. [PubMed: 15029198]
65. Glade-Bender J, Kandel JJ, Yamashiro DJ. VEGF blocking therapy in the treatment of cancer. *Expert Opin Biol Ther.* 2003; 3:263–276. [PubMed: 12662141]
66. Su X, Kim BS, Kim SR, Hammond PT, Irvine DJ. Layer-by-layer-assembled multilayer films for transcutaneous drug and vaccine delivery. *ACS Nano.* 2009; 3:3719–3729. [PubMed: 19824655]
67. Yan Y, Such GK, Johnston AP, Lomas H, Caruso F. Toward therapeutic delivery with layer-by-layer engineered particles. *ACS Nano.* 2011; 5:4252–4257. [PubMed: 21612259]
68. Biswas S, Deshpande PP, Navarro G, Dodwadkar NS, Torchilin VP. Lipid modified triblock PAMAM-based nanocarriers for siRNA drug co-delivery. *Biomaterials.* 2012
69. Bhattarai SR, Muthuswamy E, Wani A, Brichacek M, Castaneda AL, Brock SL, Oupicky D. Enhanced gene and siRNA delivery by polycation-modified mesoporous silica nanoparticles loaded with chloroquine. *Pharm Res.* 2010; 27:2556–2568. [PubMed: 20730557]
70. Xia T, Kovochich M, Liong M, Meng H, Kabehie S, George S, Zink JJ, Nel AE. Polyethyleneimine Coating Enhances the Cellular Uptake of Mesoporous Silica Nanoparticles and Allows Safe Delivery of siRNA and DNA Constructs. *ACS Nano.* 2009
71. Ruiz-Hernández E, Baeza A, Vallet-Regí Ma. Smart Drug Delivery through DNA/Magnetic Nanoparticle Gates. *ACS Nano.* 2011; 5:1259–1266. [PubMed: 21250653]
72. Muhammad F, Guo M, Qi W, Sun F, Wang A, Guo Y, Zhu G. pH-Triggered controlled drug release from mesoporous silica nanoparticles via intracellular dissolution of ZnO nanolids. *J Am Chem Soc.* 2011; 133:8778–8781. [PubMed: 21574653]
73. Knezevic NZ, Trewyn BG, Lin VS. Functionalized mesoporous silica nanoparticle-based visible light responsive controlled release delivery system. *Chem Commun (Cambridge, U K).* 2011; 47:2817–2819.
74. Zhu CL, Lu CH, Song XY, Yang HH, Wang XR. Bioresponsive controlled release using mesoporous silica nanoparticles capped with aptamer-based molecular gate. *J Am Chem Soc.* 2011; 133:1278–1281. [PubMed: 21214180]
75. Schlossbauer A, Warncke S, Gramlich PME, Kecht J, Manetto A, Carell T, Bein T. A Programmable DNA-Based Molecular Valve for Colloidal Mesoporous Silica. *Angew Chem, Int Ed Engl.* 2010; 49:4734–4737. [PubMed: 20540129]
76. Venne A, Li S, Mandeville R, Kabanov A, Alakhov V. Hypersensitizing effect of pluronic L61 on cytotoxic activity, transport, and subcellular distribution of doxorubicin in multiple drug-resistant cells. *Cancer Res.* 1996; 56:3626–3629. [PubMed: 8705995]
77. Batrakova EV, Li S, Elmquist WF, Miller DW, Alakhov VY, Kabanov AV. Mechanism of sensitization of MDR cancer cells by Pluronic block copolymers: Selective energy depletion. *Br J Cancer.* 2001; 85:1987–1997. [PubMed: 11747344]
78. Alakhova DY, Rapoport NY, Batrakova EV, Timoshin AA, Li S, Nicholls D, Alakhov VY, Kabanov AV. Differential metabolic responses to pluronic in MDR and non-MDR cells: a novel pathway for chemosensitization of drug resistant cancers. *J Controlled Release.* 2010; 142:89–100.
79. Yang Z, Sahay G, Sriadibhatla S, Kabanov AV. Amphiphilic block copolymers enhance cellular uptake and nuclear entry of polyplex-delivered DNA. *Bioconjug Chem.* 2008; 19:1987–1994. [PubMed: 18729495]
80. Martin ME, Rice KG. A novel class of intrinsic proteasome inhibitory gene transfer peptides. *Bioconjug Chem.* 2008; 19:370–376. [PubMed: 18095640]

81. Li J, Zhu Y, Hazeldine ST, Li C, Oupicky D. Dual-function CXCR4 antagonist polyplexes to deliver gene therapy and inhibit cancer cell invasion. *Angew Chem Int Ed Engl.* 2012; 51:8740–8743. [PubMed: 22855422]
82. Luker KE, Luker GD. Functions of CXCL12 and CXCR4 in breast cancer. *Cancer Lett.* 2006; 238:30–41. [PubMed: 16046252]
83. Bergeron RJ, McManis JS, Liu CZ, Feng Y, Weimar WR, Luchetta GR, Wu Q, Ortiz-Ocasio J, Vinson JR, Kramer D, et al. Antiproliferative properties of polyamine analogues: a structure-activity study. *J Med Chem.* 1994; 37:3464–3476. [PubMed: 7932575]
84. Casero RA Jr, Woster PM. Terminally alkylated polyamine analogues as chemotherapeutic agents. *J Med Chem.* 2001; 44:1–26. [PubMed: 11141084]
85. Casero RA Jr, Marton LJ. Targeting polyamine metabolism and function in cancer and other hyperproliferative diseases. *Nat Rev Drug Discov.* 2007; 6:373–390. [PubMed: 17464296]
86. Kramer DL, Vujcic S, Diegelman P, Alderfer J, Miller JT, Black JD, Bergeron RJ, Porter CW. Polyamine analogue induction of the p53-p21WAF1/CIP1-Rb pathway and G1 arrest in human melanoma cells. *Cancer Res.* 1999; 59:1278–1286. [PubMed: 10096560]
87. Dong Y, Zhu Y, Li J, Zhou QH, Wu C, Oupicky D. Synthesis of bisethyl norspermine lipid prodrug as gene delivery vector targeting polyamine metabolism in breast cancer. *Mol Pharm.* 2012; 9:1654–1664. [PubMed: 22545813]

Figure 1.

Antitumor activity for mice bearing orthotopic U87 tumors using a combination of doxorubicin (DOX) with tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) gene therapy and a simultaneous dendrimer-based delivery system. Survival curves are shown for mice treated with the co-delivery system DGDPT/pORF-hTRAIL, which contains DOX-conjugated dendrimer (DGDPT) and complexed TRAIL plasmid (pORF-hTRAIL), (red line). Controls consisted of saline (black line), DOX only (blue line), DGDPT/pGL-3 co-delivery system containing a control Luc gene (pink line), DGDPT/pORF-hTRAIL co-delivery system without targeting ligand peptide (brown line), and TRAIL gene monotherapy DGPT/pORF-hTRAIL using DOX-free vector (green line) (*adapted from [57]*).

Figure 2.

A combination of systemic paclitaxel (PTX) therapy with local interleukin-12 (IL-12) gene therapy in immunogenic, PTX-sensitive EMT-6 murine breast cancer model. Tumor growth curves of mice treated with the combination of systemic PTX (PCT16mg) and local IL-12 plasmid (PCT16 mg+IL-12) compared with untreated control, local IL-12 monotherapy (wIL-12) and chemotherapy only (PCT16 mg) (*adapted from [25]*).

Figure 3. System for delivery of doxorubicin (DOX)/siRNA combination based on quantum dots (QDs) modified with β-cyclodextrin (β-CD) (*adapted from [33]*).

Figure 4. Mechanism of action of dual-function polycations as CXCR4 antagonists and gene-delivery vectors (*adapted from [81]*).

Table 1

Summary of drug-nucleic acid combinations in cancer treatment.

Nucleic acid	Drug	Target	Delivery method	Mode of delivery ^a	Ref.
ASCL1 siRNA	DOX	Neuroendocrine cancer (<i>in vitro</i>)	Gold nanorod		[8]
Bcl-2 siRNA	5-FU	Human colorectal cancer (<i>in vivo</i>)	Lipoplex	(Sequential)	[9]
Bcl-2 siRNA	DOX	Human hepatic cancer (<i>in vivo</i>)	Polyplex		[10]
Bcl-2 siRNA	DOX	Rat glioma (<i>in vivo</i>)	Polyplex		[11]
Bcl-2 siRNA	Cisplatin	Ehrlich ascites carcinoma (<i>in vivo</i>)	Polyplex	(Sequential)	[12]
Bcl-2 siRNA	DOX	Human ovarian cancer (<i>in vitro</i>)	Mesoporous silica nanoparticle		[13]
Bcl-2 siRNA	PTX	Human breast cancer (<i>in vitro</i>)	Polyplex		[14]
Bcl-2/MRP1 siRNA Bcl-2/MDR1 siRNA	DOX	Human multidrug resistant cancers including lung, breast and ovarian (<i>in vitro</i>)	Lipoplex		[15]

Nucleic acid	Drug	Target	Delivery method	Mode of delivery ^a	Ref.
Bcl-xL siRNA	Cisplatin	Human prostate cancer (<i>in vivo</i>)	Atelocollagen	(Sequential)	[16]
Choline kinase siRNA	5-FU	Human prostate cancer (<i>in vivo</i>)	Polyplex	(Sequential)	[17]
c-Myc siRNA	DOX	Human fibrosarcoma (<i>in vivo</i>)	Lipid-polymer nanoparticle		[18]
c-Myc/VEGF siRNA	DOX	Human fibrosarcoma (<i>in vivo</i>)	Lipid-polymer nanoparticle		[19]
Deoxycytidine kinase mutant plasmid	Bromovinyl-deoxyuridine	Human glioblastoma- astrocytoma (<i>in vivo</i>)	Virus	(Sequential)	[20]
EGFR siRNA	SAHA	Human glioblastoma (<i>in vitro</i>)	Polyplex		[21]
EGFR siRNA	DSGLA lipid	Human lung carcinoma (<i>in vivo</i>)	Lipid-polymer nanoparticle		[22]
Endostatin-yeast cytosine deaminase fusion gene	5-FU/cisplatin	Human ovarian cancer (<i>in vivo</i>)	Lipoplex	(Sequential)	[23]
FCU1 gene	5-FU	Head and neck squamous cell carcinoma (<i>in vivo</i>)	Virus	(Sequential)	[24]

Nucleic acid	Drug	Target	Delivery method	Mode of delivery ^a	Ref.
IL-12 plasmid	PTX	Mouse mammary adenocarcinoma (<i>in vivo</i>)	Lipid-polymer nanoparticle	(Sequential)	[25]
IL-24 gene	TMZ	Human glioma (<i>in vivo</i>)	Virus	(Sequential)	[26]
JIP1 siRNA	DOX	Human osteosarcoma (<i>in vitro</i>)	Lipoplex	(Sequential)	[27]
Mcl1 siRNA	Mitoxantrone	Human nasopharynx carcinoma (<i>in vivo</i>)	Lipoplex		[28]
Mcl1 siRNA	MEK inhibitor PD0325901	Human nasopharynx carcinoma (<i>in vivo</i>)	Lipoplex		[29]
Mcl1 siRNA	PTX	Human nasopharynx carcinoma (<i>in vivo</i>)	Lipoplex		[30]
Mcl1 siRNA	SAHA	Human nasopharynx carcinoma (<i>in vivo</i>)	Lipoplex		[31]
MDR-1(Pgp) siRNA	DOX	Human breast carcinoma (<i>in vivo</i>)	Polyplex		[32]
MDR-1(Pgp) siRNA	DOX	Human cervical carcinoma (<i>in vitro</i>)	Quantum dot		[33]

Nucleic acid	Drug	Target	Delivery method	Mode of delivery ^a	Ref.
MDR-1(Pgp) siRNA	PTX	Human ovarian adenocarcinoma (<i>in vitro</i>)	Polyplex/Polymeric nanoparticle	(Sequential)	[34]
MDR-1(Pgp) siRNA	PTX	Mouse mammary gland adenocarcinoma (<i>in vivo</i>)	Polyplex		[35]
MDR-1(Pgp) siRNA	DOX	Human breast cancer (<i>in vivo</i>)	Liposome	(Sequential)	[36]
MDR-1(Pgp) siRNA	DOX	Human breast cancer (<i>in vitro</i>)	Polyplex	(Sequential)	[37]
MDR-1(Pgp) siRNA	DOX	Human cervical carcinoma (<i>in vitro</i>)	Mesoporous silica nanoparticle		[38]
MDA-7/IL-24 plasmid	5-FU/DOX	Human colorectal cancer (<i>in vivo</i>)	Virus	(Simultaneous)	[39]
MVP siRNA	DOX	Human breast cancer (<i>in vivo</i>)	Polyplex		[40]
Notch3 siRNA	Platinum	Human ovarian cancer (<i>in vitro</i>)	Lipoplex	(Sequential)	[41]
Oncolytic adenovirus DNA	Dacarbazine	Human uveal melanoma (<i>in vitro</i>)	Virus	(Simultaneous)	[42]

Nucleic acid	Drug	Target	Delivery method	Mode of delivery ^a	Ref.
Oncolytic adenovirus DNA	TMZ	Human malignant melanoma (<i>in vitro</i>)	Virus	(Sequential)	[43]
p53 gene	DOX	NA (<i>in vitro</i>)	Microspheres		[44]
p53 gene	Cisplatin/DOX	Human osteosarcoma (<i>in vitro</i>)	Virus	(Simultaneous)	[45]
Plk1 siRNA	PTX	Human breast carcinoma (<i>in vivo</i>)	Polypex		[46]
Survivin siRNA	Cisplatin	Human head and neck squamous cell carcinoma (<i>in vitro</i>)	Virus	(Sequential)	[47]
Survivin siRNA	iNOS specific inhibitor + cisplatin/PTX	Human head and neck squamous cell carcinoma (<i>in vitro</i>)	Lipoplex	(Sequential)	[48]
Survivin shRNA	PTX	Human ovarian cancer (<i>in vivo</i>)	Polymeric micelle		[49]
Survivin shRNA	(-)-epigallocatechin -3-gallate (EGCG)	Human malignant neuroblastoma (<i>in vitro</i>)	Lipoplex	(Sequential)	[50]
Survivin/MDR-1 shRNA	DOX	Human breast cancer (<i>in vivo</i>)	Polypex	(Sequential)	[51]

Nucleic acid	Drug	Target	Delivery method	Mode of delivery ^a	Ref.
STAT3 siRNA	TMZ	Human glioma (<i>in vitro</i>)	Lipoplex	(Sequential)	[52]
TNF-	DOX	Murine neuroblastoma, human hepatoma, human colon carcinoma (<i>in vivo</i>)	Polyplex/Lipoplex	(Sequential)	[53]
TRAIL plasmid	Cisplatin	Human head and neck squamous cell carcinoma (<i>in vivo</i>)	Virus	(Simultaneous)	[54]
TRAIL plasmid	DOX	Human ovarian cancer (<i>in vivo</i>)	Polyplex		[55]
TRAIL plasmid	DOX	Human liver cancer (<i>in vivo</i>)	Polyplex		[56]
TRAIL plasmid	DOX	Human glioblastoma (<i>in vivo</i>)	Polyplex		[57]
VEGF siRNA	PTX	Human prostate adenocarcinoma (<i>in vitro</i>)	Polyplex		[58]
VEGF siRNA	DOX	Human hepatocarcinoma (<i>in vivo</i>)	Polymeric micelle		[59]
Anti-VEGF intraceptor Flt23k plasmid	Deslorelin-Docetaxel	Human lung cancer (<i>in vivo</i>)	Polyplex	(Simultaneous)	[60]

^aMode of delivery represented by the following three categories:

- 👉 delivery of nucleic acids in a nanocarrier combined with free drug given separately
- 👉 separated delivery of drug and nucleic acids in different nanocarriers
- 👉 co-delivery of drug and nucleic acids in the same nanocarrier

Table 2

Summary of benefits and limitations of different modes of delivery of drug-nucleic acid combinations.

Mode of Delivery ^b	Benefits	Limitations
<ul style="list-style-type: none"> • Easy optimization of doses of the two therapeutic agents • Ability to reach multiple therapeutic targets in different organs/populations of cells 	<ul style="list-style-type: none"> • Difficult to synchronize pharmacokinetics and biodistribution • Difficult to control drug-nucleic acid ratios at the target cells • Undesirable toxicity in healthy tissues caused by free drug
<ul style="list-style-type: none"> • Independent control of the rate of release and timing of drug action • Ability to reach multiple therapeutic targets in different organs/populations of cells • Decreased toxicity and controlled release of the therapeutics 	<ul style="list-style-type: none"> • Complex formulation • Difficult to synchronize pharmacokinetics and biodistribution
<ul style="list-style-type: none"> • Synchronized pharmacokinetics and biodistribution • Easy to control drug-nucleic acid ratios 	<ul style="list-style-type: none"> • Complex formulation • Difficult to independently control release of the two active agents

^bMode of delivery as described in Table 1.