Nano-sized polymers and liposomes designed to deliver combination therapy for cancer
Anat Eldar-Boock¹, Dina Polyak¹, Anna Scomparin and Ronit Satchi-Fainaro

The standard of care for cancer patients comprises more than one therapeutic agent. Treatment is complex since several drugs, administered by different routes, need to be coordinated, taking into consideration their side effects and mechanisms of resistance. Drug delivery systems (DDS), such as polymers and liposomes, are designed to improve the pharmacokinetics and efficacy of bioactive agents (drugs, proteins or oligonucleotides), while reducing systemic toxicity. Using DDS for co-delivery of several agents holds great potential since it targets simultaneously synergistic therapeutic agents increasing their selective accumulation at the tumor site and enhancing their activity allowing administration of lower doses of each agent, thus reducing their side effects. Taken together, implementation of smart DDS will hopefully result in increased patient’s compliance and better outcome. This review will focus on the latest developments of combination therapy for cancer using DDS.

Addresses
Department of Physiology and Pharmacology, Sackler School of Medicine, Tel Aviv University, Tel Aviv 69978, Israel

¹ These authors equally contributed to this manuscript.

Corresponding author: Satchi-Fainaro, Ronit (ronitsf@post.tau.ac.il)

Current Opinion in Biotechnology 2013, 24:682–689
This review comes from a themed issue on Nanobiotechnology
Edited by Michael C Jewett and Fernando Patolsky
For a complete overview see the Issue and the Editorial
Available online 29th May 2013
0958-1669/$ – see front matter, © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.copbio.2013.04.014

Introduction
Drug combinations for cancer therapy
The use of most anticancer drugs is limited by toxicity and development of drug-resistance. Administration of a combination of agents aiming at various targets and displaying different toxicity profiles can improve the therapeutic index by either enhanced efficacy or by comparable efficacy with reduced toxicity [1*]. The search after the optimal combination, drug synergism and dosing schedule between different agents will never cease. Currently, there are more than 9500 clinical trials for different combination therapies for cancer (http://clinicaltrials.gov/).

Concurrent with the concept of manipulating cancer via different routes, combinations include chemotherapies with diverse mechanisms of action, and drugs affecting the tumor stroma (e.g. anti-angiogenic drugs, immune system activating agents, growth inhibitors of cancer-associated fibroblasts (CAF), among others). Therapeutic agents may include a small molecule, a protein, small interfering RNA (siRNA) or microRNA (miR). Most chemotherapeutic agents are low-molecular weight hydrophobic compounds, which are administered systemically and exhibit non-specific biodistribution profile, short plasma circulation times and rapid systemic elimination. Consequently, relatively small amounts of the drug reach the target site, and therapy is associated with side effects and low efficacy [2,3]. As for proteins, siRNAs or miRs, their great promise for cancer therapy is still limited by their stability, immunogenicity and inability to cross cellular membranes [4]. The therapeutic index of these chemical and biological agents can be improved by using nano-sized delivery systems. Nano-sized drug delivery system (DDS), or nanocarrier, is a general name for a large group of multifunctional systems, usually 1–200 nm in size, used to deliver bioactive agents to their target sites [5]. When the DDS is combined with the active entity, targeting moiety and/or imaging modality it is termed as ‘nanomedicine’, including a large group of different nanotechnologies with various physico-chemical properties (polymer therapeutics (including dendrimers), liposomes, micelles, polymeric micelles, nanocrystals, carbon nanotubes, nano/micro-gels, nanocomplexes, organic and inorganic nanoparticles and more [6,7**]. (For a detailed summary of different nano-sized DDS see Box 1.)

The rational design of nanomedicines can promote a clear advantage for their use in cancer therapy. Exploitation of the leaky tumor blood vessels, together with the poor lymphatic drainage, allows for the selective accumulation of these macromolecules (defined as the enhanced permeability and retention (EPR) effect) [8,9]. Using nano-sized DDS for combination of therapeutic agents (e.g. drugs, proteins, nucleic acids) holds many advantages such as (i) increasing half-life by protecting the compound from degradation in the circulation, (ii) reducing
immunogenicity, (iii) increasing water-solubility of poorly-soluble drugs, (iv) enabling targeting to the site of action, (v) promoting cellular uptake and appropriate intracellular trafficking, (vi) the possibility to form an advanced complex drug delivery system, (vii) promoting synergism between the combined drugs while delivering and releasing them simultaneously at the target site, making use of different mechanisms of action, toxicity, resistance and side-effects. All these properties lead to increased efficacy and reduced toxicity, and may enable easier administration and increased patient compliance [10]. The exploitation of DDS for effective delivery of combinations of therapeutic agents in order to improve cancer therapy has been on the rise in the past few years. This review will present the latest pre-clinical and in-clinical-trials work that has been done in the last two years of research in the field of DDS for combination therapy.

**Drug–drug combination therapy**

Combination of two or more drugs, common approach in oncology, is a promising strategy to improve treatment efficacy, overcome undesirable toxicity, reduce the dose of each agent and reach multiple targets [11,12]. A step forward can be taken by loading different drugs onto a single carrier, allowing simultaneous delivery of both drugs. A proper, rational selection of the co-delivered drugs can increase the therapeutic index of the native drugs leading to an overall increased anticancer activity. Drugs with different mechanisms of action and different side effects can be co-delivered in order to act on the target cells via different fronts. Doxorubicin and paclitaxel are among the most common chemotherapeutics in clinical use. Doxorubicin is a hydrophilic compound which intercalates into the DNA strands and induces a series of biochemical events resulting in apoptosis. Paclitaxel is a highly hydrophobic drug, it stabilizes microtubules, hence disrupts cell mitosis and cell proliferation, and in turn causes cell apoptosis. The increased anticancer activity of the two drugs while administered concomitantly has been reported in several studies in mice and in humans [13,14], therefore it is possible to reduce the dose of every single therapeutic agent, decreasing the related side effects. These data gave a sound incentive for combining these two drugs on a single nanocarrier. Hopefully, the investigated combined systems will reach clinical trials soon.

Beyond synergism, other advantages of DDS is the ability to reduce the toxicity of the free drug (such as cardiovascular toxicity for doxorubicin), and to some point overcome multidrug resistance (MDR). The resistance of cancer cells to the cytotoxic effects of various unrelated chemotherapeutic agents represents a major obstacle in the clinical treatment of cancer. MDR correlates with efflux from tumor cells, mediated by P-glycoprotein (P-gp) transporters, multidrug resistance associated protein 1 (MRP1), topoisomerase II (Topo II) and glutathione transferase (GST-π) [15,16]. In order to improve treatment outcome and overcome MDR, co-delivery of chemotherapies together with another agent that will inhibit MDR holds many advantages.

Curcumin, a naturally occurring polyphenol extracted from the rhizome Curcuma longa, has a long history of use as an Asian spice and in traditional therapies. Curcumin has been described as a potent antioxidant, anti-inflammatory agent and chemo-sensitizer in cancer chemotherapy and was found to downregulate the intracellular levels of three major drug transporters, P-gp, MRP-1 and mitoxantrone resistance protein, all playing central roles in MDR mechanism [16]. The mechanism of synergistic activity and reduction of toxicity of curcumin-doxorubicin co-administration has been investigated in a mouse model following intraperitoneal injection of the two free agents [17]. Nevertheless, curcumin has a very poor bioavailability, therefore an adequate DDS can significantly improve the beneficial effect of the combination therapy. Polymeric nanoparticles were used for co-encapsulation of doxorubicin and curcumin. Curcumin-doxorubicin-NPs demonstrated increased efficacy on MDR cells *in vitro*, compared with the free drugs. The advantage of curcumin-doxorubicin-NPs in tissue biodistribution and overcoming MDR are currently under investigation in preclinical *in vivo* studies [16].

Paclitaxel resistance following activation of nuclear factor-κB (NF-κB) is one of the reasons of paclitaxel therapy.
failure [18]. Hence, co-administration of paclitaxel with a suppressor of NF-κB (such as parthenolide [19]) may be exploited to overcome paclitaxel-induced resistance. In colloidal drug delivery systems, such as lipid micelles, drugs may be incorporated into the DDS in a way which allows each drug to function against distinct intracellular targets at the same time [20]. This was demonstrated by Gill et al. that developed a formulation of paclitaxel and parthenolide in a modified vitamin E micellar system. Modified vitamin E can be used as a solubilizer and a vehicle for lipid-based DDS formulations. Moreover, it has the ability to overcome drug resistance by inhibiting P-gp. The delivery of paclitaxel and parthenolide in a single modified vitamin E micellar system was significantly superior to paclitaxel or parthenolide when given alone. Most importantly, paclitaxel-resistant cells were sensitized to chemotherapy when using these micelles as drug carriers in vitro [21].

Combination therapy can also target different cell types in the target tissue. For instance, cancer cells depend upon endothelial cells (ECs) for survival and growth, therefore damaging the proliferating tumor ECs amplifies the therapeutic effect [22]. Consequently, combination of anticancer drugs and anti-angiogenic agents seems a promising strategy. One such example is that of a low molecular weight heparin (LMWH) taurocholate conjugate (LHT7) and Histone deacetylase inhibitor. LHT7 is an effective angiogenesis inhibitor with low anti-coagulant activity [23]. Histone deacetylase inhibitors, such as suberoylanilide hydroxamic acid (SAHA), are one of the new classes of anticancer drugs affecting cell cycle, apoptosis, and protein expression [24]. Co-delivery of LHT7 with SAHA on a cationic nano-liposome provided synergistic anti-tumor effects, although their consequent co-treatment did not reveal any significant advantage [25].

Another combination suggested was of the potent anti-angiogenic agent TNP-470 and alendronate conjugated with an N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer backbone. TNP-470 is a synthetic analog of fumagillin, that selectively inhibits endothelial growth in vitro [26]. Alendronate, an aminobisphosphonate, has high affinity to the bone mineral hydroxyapatite. It inhibits osteoclast function and bone resorption and has direct anti-tumor and anti-angiogenic effects, therefore can be used for the treatment of bone metastases or osteosarcoma [27]. The polymer-drug conjugate of alendronate-TNP-470 showed increased anti-tumor efficacy and decreased toxicity compared to the combination of the free drugs, and specifically inhibited tumor-induced neovascularization [12*]. Alendronate was also combined with paclitaxel in two different DDSs: (1) a self-assembled micelle for the combination of paclitaxel and alendronate. (2) Polymer-paclitaxel-alendronate conjugate. Both micellar and polymeric conjugates with paclitaxel-alendronate exhibited an improved pharmacokinetic profile and efficacy compared with the free drugs, and both were better tolerated [28,29*].

In a different approach, the combination of the anti-angiogenic agent Combretastatin A-4 (CA-4) with doxorubicin has been proved to be efficient in increasing the anticancer activity of doxorubicin. CA-4 is a strong cell growth and tubulin inhibitor, causing selective and irreversible damage to the neovasculature of tumors. Ligand targeted-liposomal formulation of CA-4 and doxorubicin significantly prolonged the concentration of the two drugs at the tumor site. CA-4 was released first, causing vascular shutdown and cutting off the supply of nutrients and oxygen. Then, doxorubicin played its role in killing tumor cells [30]. The drug-to-drug and drugs-to-lipids ratio is crucial to the activity of the DDS, and optimization is required.

The choice of an optimal DDS plays a pivotal role in the efficacy of the combination therapy. In fact, the DDS must be selected according to the different physico-chemical properties of the active moieties, in order to exploit the distinct characteristics of each agent (e.g. charge, hydrophobicity, reactive functions for site-specific conjugation) [10**]. On the basis of the unique characteristics, different DDS are usually used for each combination, as elaborated in Table 1.

Drug–peptide combinations

Combination therapy is not limited to drug–drug combinations. In fact, several examples of drug–peptide combination are presented in the literature. A major role in this topic is played by integrins peptidic-inhibitors. Integrins are a class of receptors involved in the mechanism of cell adhesion. They play a key role in tumor angiogenesis and metastasis [42,43]. α3β3 integrin is overexpressed on proliferating endothelial cells such as those present in growing tumors, as well as on some tumor cells. α3β3 integrin specifically binds to an Arg-Gly-Asp (RGD) peptide sequence.

During the years, different RGD peptidomimetics (RGD-PM) were synthesized, and used as anti-angiogenic therapy for cancer (e.g. cilengitide, now in clinical trial Phase III), or as targeting moieties to endothelial and cancer cells. Because of its intrinsic anticancer activity, addition of RGD sequence to any DDS considerably improves treatment efficacy. Danhier et al. extensively reviewed RGD combinations with different agents [44]. Several new delivery systems for the co-delivery of chemotherapeutic drugs and RGD are presented.

Cyclic RGD (cRGD)-modified nanoparticles were loaded with 5-fluorouracil, a pyrimidine analog, that induces cell cycle arrest and apoptosis. The cRGD NPs significantly
### Table 1

<table>
<thead>
<tr>
<th>DDS/Peptide combinations</th>
<th>Nanocarrier</th>
<th>Agent 1</th>
<th>Agent 2</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Drug and drug combinations</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polymeric nanoparticles</td>
<td>Biodegradable polybutyl cyanoacrylate (PBCA)</td>
<td>Doxorubicin (DNA intercalating agent)</td>
<td>Curcumin (an antioxidant, anti-inflammatory agent, chemosensitizer, and down regulator of P-gp to overcome MDR)</td>
<td>[16]</td>
</tr>
<tr>
<td>Liposome</td>
<td>Polyethylene glycol (PEG)</td>
<td>Paclitaxel (inhibits microtubule disassembly)</td>
<td>Parthenolide (NF-κB inhibitor to overcome MDR)</td>
<td>[21]</td>
</tr>
<tr>
<td>Liposome</td>
<td>1,2-Distearoyl-sn-glycero-3-phosphoethanolamine N-[poly(ethylene-glycol)] (DSPE-PEG)</td>
<td>Doxorubicin (DNA intercalating agent)</td>
<td>CA-4 (anti-angiogenic agent)</td>
<td>[30]</td>
</tr>
<tr>
<td>Liposome-Nanolipoplex</td>
<td>Cationic N,N-dioleylglutamide, dioleyl-sn-glycero-3-phosphoethanolamine, cholesterol, and PEG–DSPE</td>
<td>Low molecular weight heparin (LMWH) taurocholate conjugate (LHT7) (an anti-angiogenic agent)</td>
<td>SAHA (Histone deacetylase inhibitors, affects cell cycle, apoptosis, and protein expressions)</td>
<td>[25]</td>
</tr>
<tr>
<td>Polymer</td>
<td>N-(2-hydroxypropyl)methacrylamide</td>
<td>TNP-470 (an anti-angiogenic agent)</td>
<td>Alendronate (inhibits osteoclast function and bone resorption, has anti-tumor and anti-angiogenic effects)</td>
<td>[12,26]</td>
</tr>
<tr>
<td>Dendrimer</td>
<td>H2N-PEG-dendrimer-(COOH)4</td>
<td>Paclitaxel (inhibits microtubule disassembly)</td>
<td>Alendronate (inhibits osteoclast function and bone resorption, has anti-tumor and anti-angiogenic effects)</td>
<td>[29]</td>
</tr>
<tr>
<td>Polymer</td>
<td>N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer</td>
<td>Paclitaxel (inhibits microtubule disassembly)</td>
<td>Alendronate (inhibits osteoclast function and bone resorption, has anti-tumor and anti-angiogenic effects)</td>
<td>[28]</td>
</tr>
<tr>
<td><strong>Drug and peptide combinations</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polymeric core–shell nanoparticles</td>
<td>Monomethoxy (polyethylene glycol) poly(ε-caprolactone)-poly(lysine) (mPEG–PLGA–PLL)</td>
<td>5FU (a pyrimidine analog, inducing cell cycle arrest and apoptosis)</td>
<td>c(RGD) (an anti-angiogenic agent)</td>
<td>[31]</td>
</tr>
<tr>
<td>Polymeric core–shell nanoparticles</td>
<td>poly(ε-caprolactone-co-glycolic acid)-block-polyethylene glycol (PLGA–PEG)</td>
<td>Pt(Iv), a cisplatin produg (binds nuclear DNA)</td>
<td>c(RGDfK) (an anti-angiogenic agent)</td>
<td>[32]</td>
</tr>
<tr>
<td>Polymer</td>
<td>Polyethylene glycol (PEG)</td>
<td>Doxorubicin (DNA intercalating agent)</td>
<td>E-[(c(RGDfK))3] (an anti-angiogenic agent)</td>
<td>[33]</td>
</tr>
<tr>
<td>Polymer</td>
<td>Polyglutamic acid (PGA)</td>
<td>Paclitaxel (inhibits microtubule disassembly)</td>
<td>E-[(c(RGDfK))3] (an anti-angiogenic agent)</td>
<td>[34]</td>
</tr>
<tr>
<td><strong>Drug and RNAi combinations</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cationic micelle</td>
<td>Poly-dimethylaminoethyl methacrylate (PDMAEMA–PCL–PDMAEMA)</td>
<td>Paclitaxel (inhibits microtubule disassembly)</td>
<td>Anti-VEGF siRNA (anti-angiogenic by silencing VEGF recruitment of neovascularization)</td>
<td>[35]</td>
</tr>
<tr>
<td>Cationic micelle</td>
<td>Polyethyleneimine (PEI)-stearic acid (SA)</td>
<td>Doxorubicin (DNA intercalating agent)</td>
<td>Anti-VEGF siRNA (anti-angiogenic by silencing VEGF recruitment of neovascularization)</td>
<td>[36]</td>
</tr>
<tr>
<td>Cationic nanoparticles</td>
<td>Palmitoleic acid</td>
<td>MTO</td>
<td>mcl-1 (Bcl-2 family gene possessing an anti-apoptotic property) siRNA</td>
<td>[37]</td>
</tr>
<tr>
<td>Cationic micelle</td>
<td>Polyethyleneimine (PEI)-poly(ethylene glycol) (PEG)</td>
<td>Doxorubicin (DNA intercalating agent)</td>
<td>Bcl-2 siRNA (Bcl-2 family gene possessing an anti-apoptotic property)</td>
<td>[38]</td>
</tr>
<tr>
<td>Cationic nanoparticles</td>
<td>PLGA–PEI</td>
<td>Paclitaxel (inhibits microtubule disassembly)</td>
<td>P-gp targeted siRNA (silencing MDR mechanism)</td>
<td>[38]</td>
</tr>
<tr>
<td>Lipid nanoparticles</td>
<td>Liposome-polycation-DNA (LPD) and anionic liposome-polycation-DNA (LPD-l)</td>
<td>doxorubicin (DNA intercalating agent)</td>
<td>c-Myc siRNA</td>
<td>[39]</td>
</tr>
<tr>
<td><strong>RNAi and RNAi combinations</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>polymer-SNALP</td>
<td>Poly (b-amino esters) (PAEs) stable nucleic acid lipid particles</td>
<td>Mdr-1-shRNA siRNA for vascular endothelial growth factor (VEGF)-A (in order to reduce angiogenesis)</td>
<td>Survivin-shRNA siRNA for kinesin spindle protein (KSP) (to disrupt cell division and induce apoptosis)</td>
<td>[40,41]</td>
</tr>
</tbody>
</table>
enhanced the cytotoxicity of 5-fluorouracil on cancer cells in culture [31].

Another particulated delivery system combined RGD-PM, c(RGDFK) together with cisplatin prodrug. Cisplatin (Pt(II)) is one of the most widely used anticancer drugs, yet only 5–10% of the active drug Pt(II) reaches its target site. A promising strategy for enhancing the delivery of Pt(II) is to employ Pt(IV) complexes as prodrug that can be intracellularly activated by reduction to generate Pt(II). The RGD-Pt(IV)-encapsulated NPs displayed moderately enhanced cytotoxicity and efficacy, yet were better tolerated as compared to cisplatin in its conventional form [32].

RGD-PM was also conjugated to a linear polymer chain (PEG) together with doxorubicin. The resulting doxorubicin-PEG-E-[c(RGDfK)2] conjugate actively and selectively targeted endothelial and tumor cells overexpressing α,β3 integrin, and demonstrated higher cytotoxicity than free doxorubicin to both tumor and endothelial cells [33].

Another example is a polyglutamic acid (PGA) conjugate of paclitaxel and RGD-PM. This conjugate significantly augmented the antitumor activity of both free and sole-conjugated paclitaxel. Inclusion of an RGD-PM leads to a selective anti-angiogenic mechanism of action and the conjugate internalization via receptor-mediated endocytosis presents an alternative route to overcome acquired MDR [34].

Despite the majority of the published work on RGD peptide–drug combination, there are some studies also on other peptides able to inhibit integrins or other receptors overexpressed on tumor endothelial cells, as the FNIII14, a fibronectin derived peptide that inhibits β1-integrin [45], or the somatostatin analog–camptothecin conjugate [46].

**Drug–RNAi combinations**

In the last decade we observed an exponential use of RNAi in cancer therapy. As a consequence, combinations of classic anticancer drugs and siRNA have recently emerged as a new anticancer therapy modality [36].

RNA interference (RNAi) is a process that induces sequence-specific and post-transcriptional gene silencing by using siRNA, miR or small-hairpin RNA (shRNA). siRNA or miR are incorporated into a RNA-induced silencing complex, which binds and degrades complementary mRNA, leading to target gene silencing.

The attractiveness of siRNA by contrast to other methods arises from its extremely specific inhibitory activity [47]. However, its in vivo stability is very low, mainly due to enzymatic degradation and aggregation with serum proteins [48]. Incorporation of siRNAs into synthetic vectors, such as polymeric carriers, can overcome those difficulties, and allow better delivery and enhanced biological efficacy of the complex. Furthermore, DDS-mediated cancer therapy studies have reported significant improvement with combination therapies employing potential anticancer siRNAs and drugs. Different research approaches for conjoint drug-siRNA delivery may be categorized by the rationale of the selected combination and the biological processes it aims to target.

**Enhancement of the chemotherapeutic drug effect by addition of siRNA**

Co-delivery of siRNA and chemotherapy, aiming at different cellular mechanisms in cancer is a promising approach. On the basis of this rationale, doxorubicin or paclitaxel were loaded on cationic micelles together with anti-vascular endothelial growth factor (VEGF) siRNA, aiming at enhanced apoptosis of cancer cells and angiogenesis prevention by inhibiting VEGF secretion [35,36]. Although the uptake efficiency of doxorubicin/VEGF-siRNA micelles was improved, no evidence of better transfection efficiency was seen. Both systems were able to decrease VEGF secretion by the tumor cells, while only doxorubicin/VEGF-siRNA micelles exhibited enhanced anti-tumor effect, compared with the free drug. Another interesting approach is to exploit intrinsic cationic properties of an anti-tumoral compound and complex it with siRNA by electrostatic interactions. Chang et al. conjugated the cationic chemotherapy mitoxantrone with hydrophobic palmitoleic acid for the delivery of anti-mcl-1 (Bcl-2 family gene possessing an anti-apoptotic property) siRNA [37]. Palmitoleyl-mitoxantrone nanoparticles complexed with the siRNA significantly inhibited tumor growth, compared to untreated controls. Similarly, Cao et al. developed a hierarchical assembly approach for targeted co-delivery of Bcl-2 siRNA, here combined with doxorubicin that also resulted in an enhanced chemotherapeutic activity [38]. A thorough study is needed to learn the synergistic concentrations and ratios of siRNA with conventional chemotherapy in vivo.

**Re-sensitization of cancer cells to overcome MDR by silencing genes of interest**

Overcoming MDR in cancer can be achieved by silencing known genes responsible for cell resistance mechanism [49]. Patil et al. used nanoparticles to encapsulate a combination of anti-P-Gp siRNA with paclitaxel. This study demonstrated that inhibition of P-Gp expression enhanced paclitaxel accumulation and cytotoxicity in drug-resistant cells. In addition, in vivo studies showed some growth inhibition, but the optimal dose of this formulation still needs to be determined [50]. A similar approach was taken by Chen et al., using lipid nanoparticles, to deliver doxorubicin combined with anti-c-Myc siRNA to doxorubicin-resistant cells and tumors [39].
A drug combined with siRNA on the same delivery system guarantees similar pharmacokinetics and concomitant passive accumulation for both treatments in the tumor tissue. Although progress has been made in combination therapy, further investigation into the synergistic co-delivery of siRNA and chemical drugs is required to achieve more effective combination standards.

**Combination of siRNAs**
siRNA as cancer therapy is a relatively new strategy, yet there are around 30 clinical trials with RNAIs for various targets in general focusing on macular degeneration, cancer and antiviral therapy [51]. Despite the understanding that delivery of siRNA requires a DDS, combination of two different siRNAs on the same system is still unusual. One pre-clinical study utilized a new biodegradable polymer for the delivery of Mdr-1-shRNA and Survivin-shRNA, two related genes participating in MDR. The polymer condenses nucleic acid molecules at physiological pH and releases them into the cytoplasm at acidic endosomal environment. In this novel approach, two shRNAs aimed at two different mechanisms of MDR were packaged into one vector. These two shRNAs administered simultaneously can overcome MDR in tumor cells, when concurrently delivered with chemotherapy, by synergistically interfering two different genes [40]. This work paves the way for more complex delivery systems that will enable co-delivery of chemotherapy with several RNAIs.

Another siRNA co-delivery system is currently under Phase 1 clinical trial. A lipid nanoparticle was formulated with two siRNAs: one, targeting the expression of VEGF-A, in order to reduce angiogenesis, and the other, silencing kinesin spindle protein (KSP), to disrupt cell division and induce apoptosis. Early results showed pharmacologically relevant accumulation of both siRNAs in tumor tissue [41**]. First-in-man trial of lipid nanoparticle (LNP)-formulated RNAi therapeutic demonstrated safety and biweekly administration tolerability. In addition, the trial demonstrates the formulation’s pharmacokinetics, RNAi mechanism of action, and clinical anti-cancer activity in humans.

**Summary**
Combination therapy is certainly more complex than monotherapy. However, appropriate drug combinations can yield significant benefits, including lower case-fatality ratios, slower development of drug resistance and, in the long run, even cost savings [1*,52].

Combination of several therapies facilitates the blockade of several survival mechanisms in cancer cells and their microenvironment. Proliferation, cell survival, angiogenesis and acquired drug resistance are simultaneously inhibited, achieving a synergistic therapeutic effect. Similarly to the rational evolution of the systematic design of nano-sized DDS and the resulting nanomedicines, enlightened choice of the drugs and the ratio at which they should be loaded on the nanocarrier must be done after a careful evaluation rather than random empirical selection.

The concept of nanomedicines based on synthetic polymers was elaborated by Helmut Ringsdorf in 1975 [53] on the basis of Jatzkewitz [54] and Ushakov [55] work on polymer drugs conjugates. Many nanomedicines had been developed since exploiting polymers, micelles, liposomes, dendrimers and other nanoparticles. Each nanotechnology has its advantages and disadvantages, and in order to find the perfect system for a specific combination of anti-cancer agents one needs to consider a few aspects: (1) The physico-chemical properties of each agent, drug, protein or RNAi, and (2) The potential loading of each agent. Besides determining the efficacy and synergistic effect for each agent and their combination, their potential loading capability into the nanomedicine might be a limiting factor in terms of their synergistic ratio, (3) The release mechanism of each drug or agent from the DDS should consider the intra-cellular target of each agent, i.e. lysosome, cytoplasm or nucleus. Generally, non-targeted nanomedicines enter cells via endocytosis, where siRNAs must escape into the cytoplasm to create a biological effect, and chemotherapeutics should be released from the carrier and reach their intracellular target sites. Paclitaxel, for example, is active within the cytoplasm, while doxorubicin needs to cross the nuclear membrane to interact with the DNA.

Despite the complexity involved, integration of combination therapies and nano-sized DDS holds great promise for future cancer treatment. Understanding the aspects of nanotechnology and the relevant demands of different anti-cancer agents will yield better combinations and will result in more efficient and patient-complied cancer treatment.

**Acknowledgements**
The Sarchi-Famaro research laboratory is partially supported by The Association for International Cancer Research (AICR), German-Israel Foundation (GIF), Rimonim Consortium and the MAGNET Program of the Office of the Chief Scientist of the Israel Ministry of Industry, Trade & Labor, THE ISRAEL SCIENCE FOUNDATION (Grant No. 1309/10), the United States-Israel Binational Science Foundation (Grant No. 2007347), Swiss Bridge Award, and by grants from the Israel National Nanotechnology Initiative, Focal Technology Area (FTA) programs: Nanomedicine for Personalized Theranostics, and by The Leona M. and Harry B. Helmsley Nanotechnology Research Fund.

**References and recommended reading**
Papers of particular interest, published within the period of review, have been highlighted as:  
- of special interest  
- of outstanding interest  


An excellent review on combination therapy based on polymer-drug conjugates.


17. Sadzuka Y et al.: Beneficial effects of curcumin on antitumor activity and adverse reactions of doxorubicin. Int J Pharm 2012, 432:42-49. This study investigates the potential of curcumin as a protective agent against the toxic effects of doxorubicin.


