Chapter 16
Exploiting Nanocarriers for Combination Cancer Therapy

Yi Wen Kong*, Erik C. Dreaden*, Paula T. Hammond, and Michael B. Yaffe

Abstract Combination chemotherapy has vastly improved patient outcomes following treatment for cancer. Combining multiple drugs with non-overlapping mechanisms of action has been shown to forestall the development of drug resistance, leading to increased efficacy. Emerging insights into cancer pathophysiology from tumor genomics, metabolomics, and proteomics now present us with unprecedented opportunities to combine targeted molecular therapies together, or to combine molecular therapies with cytotoxic chemotherapy in a rationally designed manner based on unique molecular signatures. However, the clinical implementation of these improved drug combinations is frequently limited by overlapping drug toxicities. By using new nanotechnology platforms to enhance tumor targeting, and provide precise spatial and temporal control of drug delivery for each agent within a multi-drug regimen, it should be possible to mitigate these toxicity limitations and treat tumors with increasing safety, efficacy and durability. This chapter discusses recent efforts in developing nanoparticles to deliver multiple types of drugs for temporally-sequenced concurrent or sequential combination chemotherapy.

Keywords Nanomedicine • Systems pharmacology • Nanotechnology • Chemotherapy • Rational drug combinations • Synergy • Synthetic lethality • Cancer treatment • Mechanism of action • Molecular targeting • Silica nanoparticles • Micelles • Toxicity profile

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Abbreviations

17-AAG	17-allylamino-17-demethoxygeldanamycin
5-FU	5-fluorouracil
6MP	6-mercaptopurine (6MP)
AlClPc	aluminum chloride phthalocyanine
dsRNA	double-stranded RNA
EGFR	epidermal growth factor receptor
EPR	enhanced permeability and retention
HMSNs	hollow mesoporous silica nanoparticles
LbL	Layer-by-Layer
miRNA	microRNA
mRNA	messenger RNA
MMs	macromonomers
MOMP	nitrogen mustard with vincristine, methotrexate, and prednisone
MOPP	nitrogen mustard with vincristine, procarbazine, and prednisone
MPS	mononuclear phagocyte system
MSNs	mesoporous silica nanoparticles
MTD	maximum-tolerated-dose
NSAIDSs	nonsteroidal anti-inflammatory drugs
PCL	poly caprolactone
PDT	photodynamic therapy
PEG	poly(ethylene glycol)
PGLA	poly(D,L-lactide-co-glycolide)
PLA	polylactic acid
RNAi	RNA interference

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siRNA	small interfering RNA
SSRIs	serotonin reuptake inhibitors
TNBC	triple-negative breast cancer
UCNs	upconversion nanoparticles
UV	ultraviolet
VAMP	vincristine, amethopterin, 6MP, and prednisone

16.1 Introduction

Cancer is a complex collection of diseases that through deregulation of myriad cellular pathways achieves unchecked growth, metabolism, and migration, thus making it the second leading cause of death in the United States. Solid tumors in particular have complex cyto-architecture and diverse microenvironments, making single-agent therapy largely ineffective due to sub-populations of cells that are resistant to a given agent (Burrell et al. 2013; Hainaut and Plymoth 2013; Hanahan and Weinberg 2000, 2011). Efforts have been taken to better understand the genetic alterations and complex molecular mechanisms in cancer to identify more effective therapies.

Due to the past failure of mono-therapies, most cancer patients now receive some form of combination therapy as the standard of care treatment for their tumor and this approach has improved patient outcomes. However, for the most part, current regimens were not "designed", rather, they were empirically determined to be combinations of drugs with non-overlapping toxicities, so that each drug could be administered at near-maximal dosage. Since these drugs generally have different mechanisms of action, there tends to be minimal cross-resistance, decreasing the emergence of drug resistant tumors (Mayer and Janoff 2007; Harasym et al. 2007; Ramsey 2005; Zoli et al. 2001). Importantly, however, this does not mean that current combinations are the best cocktail of drugs to achieve lasting remissions in patients. Rather they are the best cocktail of drugs identified to date that avoid unmanageable toxicity. Recent advances in nano-scale drug carriers that can target tumors preferentially and limit systemic toxicity are now revolutionizing the way we approach combination therapy.

16.2 Drug Combinations for Cancer Treatment

16.2.1 A Brief History of Combination Cancer Therapy

16.2.1.1 Combinations of Independently Active Drugs

Following the discovery of **cytotoxic chemotherapy** by Goodman et al. (1946; Gilman and Philips 1946) in 1943 and Farber et al. (1948) in 1947, researchers and clinicians began focusing on strategies to prolong cancer remissions in patients and

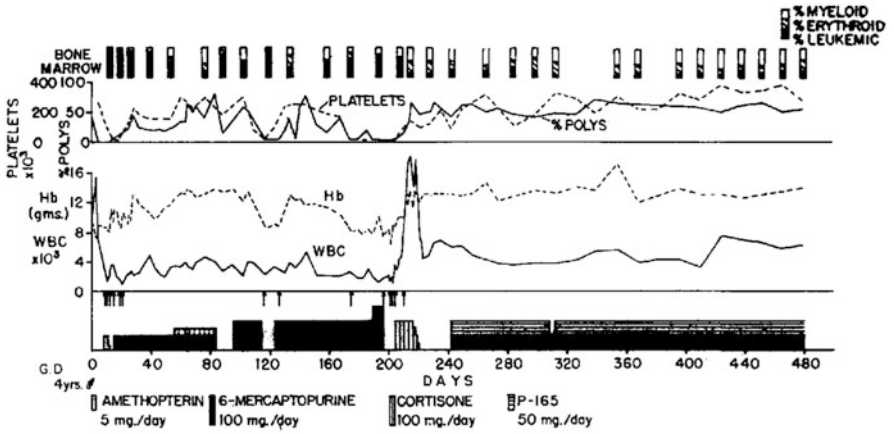

Fig. 16.1 An early example of sequential combination cancer therapy c.1954. Treatment regimen and corresponding blood counts for a 4 year old male with 6-mercaptopurine-resistent acute leukemia receiving amethopterin (antifolate), 6-mercaptopurine (purine antagonist), cortisone (steroid), and azaserine/P-165 (purine antagonist) at the Sloan-Kettering Institute (Reproduced with permission from Burchenal et al. (1954). Copyright 1954 John Wiley and Sons)

to delay drug resistance. Inspired by the observation of synergism between 6-mercaptopurine (6MP) and azaserine (O-diazoacetyl-L-serine), as well as 6MP and antifolates (Skipper et al. 1954) in preclinical mouse models of leukemia, Burchenal and colleagues conducted pilot studies (Burchenal et al. 1954) and later a randomized trial (Heyn et al. 1960) in children with acute leukemia receiving sequential combinations of 6MP, azaserine, and steroids (Fig. 16.1). While widespread improvements in overall survival were not observed, Frei et al. later demonstrated greatly enhanced remission rates in children with acute leukemia treated with 6MP and antifolates in 1961 – noting that "advantage derives from the two drugs acting independently on the patient" (Frei et al. 1961). With the discovery of antineoplastic activity from plant alkaloids from *Vinca rosea* at Eli Lilly, Freireich et al. (1964) quickly developed a "quadruple combination therapy" known as VAMP (vincristine, amethopterin, 6MP, and prednisone) which augmented remission rates in patients with acute leukemia treated in 1964. VAMP soon gave way to MOMP (Devita et al. 1965) (nitrogen mustard with vincristine, methotrexate, and prednisone) and then to MOPP (Devita et al. 1970) (nitrogen mustard with vincristine, procarbazine, and prednisone), the latter of which demonstrated startling activity in patients with Hodgkin's lymphoma – nearly 80 % displayed complete remission, and of these, 60 % demonstrated disease free survival. Throughout the advent of MOPP, the rationale for combination chemotherapy relied heavily on two fundamental underlying concepts: independent drug action and non-overlapping toxicity. As stated by Vincent DeVita: "*differing mechanisms of action and various dose-limiting toxicities could presumably overcome [low therapeutic index]*" (Devita et al. 1970).

16.2.1.2 Rational Drug Combinations Targeting a Shared Mechanism of Action

In 1982, *in vitro* studies demonstrated that pretreatment of cells with leucovorin (folinic acid), an innocuous nontoxic agent, to cancer cells could enhance the biochemical effects of the nucleoside analog, 5-fluorouracil (5-FU). Researchers found that leucovorin augmented inhibition of thymidylate synthase, the canonical target of 5-FU, depleting cellular nucleotide levels and inducing apoptosis (Pritchard et al. 2013; Longley et al. 2003). Preclinical studies in tumor xenograft-bearing mice, and later pilot studies in patients (Machover et al. 1982) showed clear benefits in response rates from the combination therapy versus that seen after treatment with 5-FU alone, although the improvements in overall survival were more moderate. The combination, still applied today as part of FOLFOX, FOLFIRI, and FOLFIRINOX treatment regimens, marked a significant departure from the strategy originally established by Frei, Freireich, and Zubrod. These drug combinations came about from "trial and error"/empirical testing in patients and are used not because they are the most effective treatment for tumor killing, but because they are capable of achieving the maximal tumor reduction within the maximal toxicity tolerated by patients. With the advancement of targeted drug delivery, "rational drug combinations" can now be used. Rather than combining drugs with independent activity or dose-limiting toxic effects, *rational drug combinations* could act cooperatively on overlapping molecular targets to selectively to kill cancer cells.

16.2.1.3 Molecularly Targeted Therapies

The discovery that multidrug transporter proteins could play a key role in the development of *adaptive resistance* to chemotherapy (Rothenberg and Ling 1989), led to the initiation of clinical trials in the late 1980s to concurrently inhibit promiscuous drug efflux pumps during the administration of cytotoxic anti-tumor agents. Here, competitive substrates of the multidrug transporters – already approved to treat other conditions (e.g. cyclosporine and verapamil) – were co-administered in patients with refractory disease. Although these trials, and those that followed over multiple generations of inhibitors, were largely disappointing (Fletcher et al. 2010; Kaye 1993), the general strategy of identifying and preemptively blocking the biological mechanisms responsible for adaptive resistance continued to guide a significant portion of subsequent work.

During this same period in the late 1980s, researchers were also beginning to understand structure-activity relationships for a novel small molecule contraceptive agent that showed unusually selective antitumor activity in breast cancer patients – tamoxifen (Jordan 2003). The drug was found to selectively inhibit the estrogen receptor, its 'target' protein, in breast tumors. These findings led to a shift in the focus of commercial anti-cancer drug discovery away from enhanced non-specific cell killing towards rational target inhibition. So-called 'molecularly **targeted therapies**' against the BCR-ABL fusion protein (i.e. imatinib/Gleevec) (Capdeville

et al. 2002), **monoclonal antibody** therapies (e.g. trastuzumab/Herceptin, and related molecules targeting EGFR family members) (Hudis 2007), and **recombinant proteins** (e.g. interleukin-2) (Dranoff 2004) followed soon afterward. Rational drug combinations incorporating these molecularly-targeted drugs added further complexity to prior combination therapeutic approaches.

16.2.1.4 Large-Scale Screens, Nucleic Acid Therapies, and Beyond

Later, with the discovery of RNA interference in mammalian cells in 2001 (Elbashir et al. 2001), large scale loss-of-function screens were used to identify a subset of new molecular targets for cancer therapy (Ngo et al. 2006; Luo et al. 2008), as well as new gene combinations whose pairwise loss blocked cancer cell survival, resulting in '*synthetic lethality*' (Kaelin 2005; Luo et al. 2009). A number of these early discoveries were complicated by poor reproducibility, despite improvements in **small interfering RNA** (siRNA) and **messenger RNA** (mRNA) delivery (Kormann et al. 2011). Improvements in **CRISPR-Cas9** technology have overcome many of these difficulties (Platt et al. 2014), further expanding this toolkit to include amplified or synthetic protein expression, in addition to genetic loss of function. Although it is currently unclear to what extent **epigenetic modifiers** (e.g. chromatin regulators) (Floyd et al. 2013; Keung et al. 2014), **immune checkpoint antagonists** (Mahoney et al. 2015), or **chimeric proteins/receptors** (Kalos et al. 2011; Morsut et al. 2016) will contribute to future multiplexed combination therapies, the number of possible pairwise combinations of the above drug classes alone provides ample opportunities for the creation of new and highly potent therapies with durable treatment responses.

16.2.2 Challenges in Delivering Drug Combinations to Tumors

16.2.2.1 Co-delivery

Implementation of drug combinations generally requires co-localization of each agent within the malignant cells for efficient tumor cell killing. This presents a variety of complex intrinsic challenges due to the unique physiochemical properties of each drug such as size, charge, hydrophobicity, and stability, among others. For example, current frontline two-agent therapy for ovarian cancer requires co-delivery of cisplatin and paclitaxel. Although both drugs are roughly neutral at physiological pH, paclitaxel is more than double the molecular weight of cisplatin and its relative hydrophobicity (octanol:water partition coefficient) is more than five logs higher than cisplatin. Cellular colocalization in the complex tumor microenvironment is thus severely constrained. Further challenges include colocalization of either drug with bevacizumab – a monoclonal antibody directed against the pro-angiogenic cytokine VEGF-A, which is 1000 times larger in molecular weight, as part of

platinum-sensitive disease therapy, or liposomal doxorubicin, a topoisomerase inhibitor whose nanoparticle is almost 10,000 times larger than the free small molecule paclitaxel – as part of platinum-insensitive disease therapy. Interestingly, Wittrup and coworkers (Schmidt and Wittrup 2009) have modeled tumor uptake data for biomolecules of varying size and affinity and found that intermediate-sized targeting agents (ca. 25 kDa, 5.3 nm dia) exhibit the lowest tumor uptake, whereas higher tumor uptake levels are observed for either smaller agents (e.g. peptides and small molecules) and larger agents (e.g. IgG, liposomes). Bawendi, Jain, and Fukumura (Stylianopoulos et al. 2012) have similarly examined size-dependent penetration of nanoscale particles into the interstitium of tumor xenografts and found that drug size is inversely correlated with tumor penetration (Fig. 16.2a). Because intratumoral distribution profiles of drugs are often heterogeneous, enhanced or diminished cell killing can thus occur in a spatially dependent manner. Using inorganic colloids as model drug carriers, Chan and coworkers also found that 20 nm particles efficiently penetrate and are retained in the tumor interstitium at significantly greater depths than comparable 40–100 nm particles (Perrault et al. 2009).

16.2.2.2 Stoichiometry/Ratiometric Dosing

Historically, combinations of free drugs are administered at their respective maximum-tolerated-doses (MTDs); however, it is now widely acknowledged that drug combinations can act synergistically at specific drug ratios, as well as additively or even antagonistically at other drug stoichiometries. For example, Dreaden et al. found that MEK and PI3K inhibitors, when co-administered, exhibit stoichiometry-dependent drug synergy *in vitro* (Fig. 16.2b). Likewise, optimally synergistic pairwise combinations of irinotecan/floxuridine (Batist et al. 2009), cytarabine/daunorubicin (Tardi et al. 2009a), irinotecan/cisplatin (Tardi et al. 2009b), paclitaxel/tanespimycin (17-AAG) (Katragadda et al. 2013), and quercetin/vincristine (Wong et al. 2010) have also been identified.

16.2.2.3 Drug Sequence and Timing

Cellular responses to perturbations occur in a time-dependent manner and drug combinations that exploit these response networks can often maximize therapeutic potential through sequence- and time-staggered delivery. Although conventional delivery methods such as intravenous or intraperitoneal infusion can be staged manually, poor drug colocalization and unfavorable drug stoichiometry within tumors can abrogate the therapeutic potential of even the most potent rational combination therapies. Lee et al. (2012), for example, recently employed a systems-based approach to understand how targeted cancer therapies rewire oncogenic cell signaling networks, and methods by which this ‘dynamic rewiring’ can be exploited to improve tumor cell killing (Fig. 16.2c). Interesting, they found that time-staggered

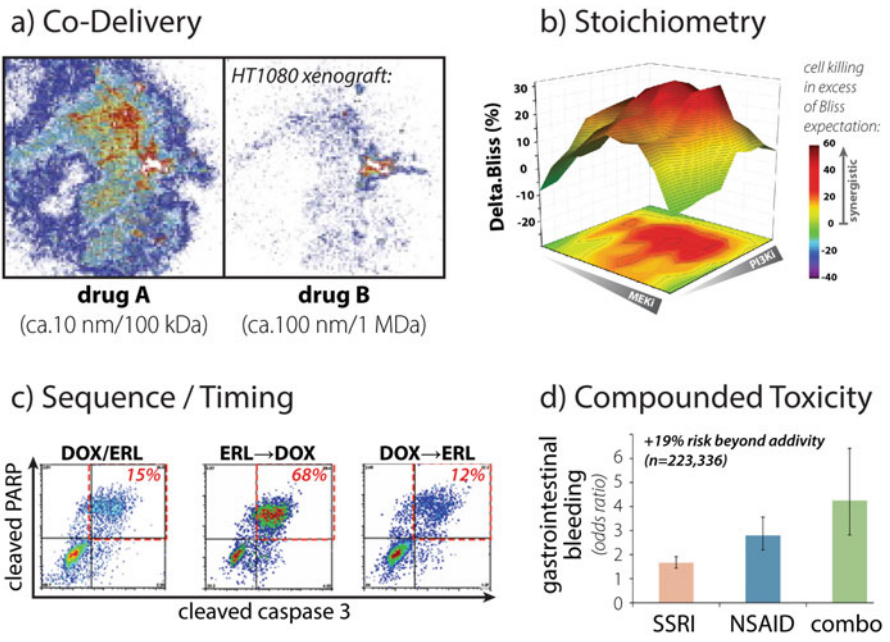

Fig. 16.2 Challenges in delivering drug combinations**.** (**a**) Drugs with differing targets often display disparate physiochemical properties that, here, affect intratumoral distribution, but also pharmacokinetics, pharmacodynamics, and tissue disposition. Model drug A is 10-fold smaller than B and penetrates more deeply into HT1080 tumor xenografts at 6 h as measured by fluorescence microscopy of hitological tissue sections. (**b**) In many canses, multiplexed combinations exhibit optimally synergistic drug stoichiometry that is difficult to recapitulate using free drug compounds. Here, small molecule inhibitors of Mek and PI3K are synergistic toxic only over a narrow range of drug ratios *in vitro* as measured by CellTiter Glo. (**c**) The sequence and timing with which drugs modulate (rewire) complex cell signaling networks also determines capacity for cell killing. Here, combinations of the cytotoxic chemotherapeutic, doxorubicin, and erlotinib, an inhibitor of EGFR, optimally induce apoptosis (double positive flow cytometry) in a sequence-dependent manner *in vitro*. (**d**) Dose-limiting toxic effects can also act synergistically, here increasing the relative risk of gastrointestinal bleeding 19 % beyond additivity when selective serotonin reuptake inhibitors (SSRIs) and nonsteroidal anti-inflammatory drugs (NSAIDSs) are combined in patients (n = 223,336) (Reproduced with permission from (**a**) Stylianopoulos et al. (2012), (**b**) Dreaden et al. (2015), (**c**) Lee et al. (2012), (d) Anglin et al. (2014). Copyright (**a**) 2012 Elsevier, (**b**) 2015 American Association of Cancer Research, (**c**) 2012 Cell Press, (**d**) 2014 Nature Publishing Group)

inhibition of epidermal growth factor receptor (EGFR), but not simultaneous co-administration, could sensitize a subset of triple negative breast cancer cells to DNA damaging chemotherapy. Not only was sequence a critical determinant for enhanced cell death, but a time delay of as little as 4 h could dramatically alter apoptotic response *in vitro*. In a related approach, Seino et al. (2016) recently found that JNK pathway may play a key role in adaptive resistance of ovarian cancer cells towards frontline platinum/taxane therapy. Although concurrent targeted inhibition of JNK induced contrasting effects on cisplatin (enhanced cytotoxicity) and paclitaxel (decreased cytotoxicity), time-staggered inhibition of JNK prior to chemotherapy

greatly augmented *in vitro* cell killing in a time-dependent manner. These findings also suggest that basal JNK activity may correlate with drug resistance in ovarian cancer. Sengupta and coworkers (Goldman et al. 2015) have also examined drug-induced tumor cell perturbations and found that taxane therapy induces a transient cell state characterized by Src family kinase (SFK)/Hck pathway activation and suppression of apoptosis. By studying the *in vivo* induction kinetics of this transient state, the authors could preemptively block anti-apoptotic responses *in vivo* through targeted inhibition of SFK/Hck staggered approximately 6 days after taxane therapy.

16.2.2.4 Compounding and Overlapping Toxicity

As the focus of combination cancer therapies shifts towards synergy and network rewiring, compounded dose-limiting toxic effects again present a significant challenge to multi-drug treatment regimens. Drug interactions are often complex to predict, and dose-limiting toxic effects – like therapeutic effects – can occur synergistically. For example, combined administration of common therapeutics such as nonsteroidal anti-inflammatory drugs (NSAIDSs) with selective serotonin reuptake inhibitors (SSRIs) increases the relative risk of gastrointestinal bleeding 19 % beyond the additive expectation (Anglin et al. 2014) (Fig. 16.2d). Similarly, doxocubicin and trastuzumab (anti-HER2/ErbB2; Herceptin) – commonly co-administered in breast cancer patients – are known to induce Type I and Type II cardiotoxicity, respectively, effects which compound the risk of dose-limiting cardiotoxic events in patients (Cardinale et al. 2010). More recently, combined blockade of MAPK and PI3K pathway signaling has been shown to synergistically kill a variety of solid tumors *and* pre-emptively block resistance-associated signaling in preclinical animal models (Engelman et al. 2008). A retrospective Phase I clinical study of patients with advanced solid tumors receiving small molecule inhibitors of MAPK or PI3K pathway signaling, alone or in combination, found that simultaneous blockade significantly decreased average tumor burden relative to monotherapy (Shimizu et al. 2012); however, these favorable outcomes were accompanied by a 2.0-fold increase in the prevalence of dose-limiting toxicity and a 3.0-fold increase in the prevalence of drug-related high grade (>III) adverse events, primarily hepatic-related.

16.3 Nanoparticle Formulations to Optimize Anti-cancer Combination Therapies

Nanomaterials can be used to co-deliver multimodal cancer therapeutic agents to achieve maximum therapeutic effects (Hu and Zhang 2012). Some of the nanomaterials used to study combination therapy include liposomes, polymer-drug conjugates, dendrimers, and polymeric micelles (Peer et al. 2007). We will discuss a few notable examples in detail below.

16.3.1 Mesoporous Silica Nanoparticles

Mesoporous silica nanoparticles (MSNs) exhibit a range of features that are amenable to drug delivery (Baek et al. 2015). They have high surface area, which allows for large amounts of drug loading, tunable porosity and size, structural diversity, easily modifiable chemistry and suitability for functionalization, and are biocompatible. MSNs have been used extensively as multifunctional nanocarrier systems by combination or hybridization with biomolecules, drugs, and other nanoparticles, and can be stimulated by signals such as pH, optical signal, redox reaction, òr electric and magnetic fields (Baek et al. 2015).

Lipid-coated MSN have recently been used to delivery synergistic gemcitabine and paclitaxel to both subcutaneous and orthotopic pancreatic tumors in mice. Mice with both subcutaneous and orthotopic tumors receiving systemic gemcitabine and paclitaxel loaded MSN achieved more effective tumor shrinkage than those receiving individual or free drug. The authors also observed elimination of metastatic foci without evidence of local or systemic toxicity (Meng et al. 2015).

Another way to use MSNs is for the combination of photodynamic and chemotherapies. One such example is the use of MSNs loaded with aluminum chloride phthalocyanine (AlClPc) and cisplatin for cancer treatment. Vivero-Escoto and colleagues showed that these MSNs are taken up by HeLa cells, and upon light exposure, the AlClPc-cisplatin-MSN combination was more cytotoxic then the AlClPc-MSN and cisplatin-MSN controls. These data suggest that there is great potential for the use of MSN platforms as nanocarriers for combination photodynamic and chemotherapies to treat cancer (Vivero-Escoto and Elnagheeb 2015). In another example, Zhang and colleagues synthesized a polymeric prodrug (doxorubicin)-coated hollow mesoporous silica nanoparticles (HMSNs) with an NIR absorbing dye IR825 loaded into the hollow cavity of the HMSN to form a multifunctional hybrid HMSNs-DOX/IR825 (Zhang et al. 2016). Cancer cells efficiently took up the hybrid nanoparticle, and the conjugated doxorubcin was successfully released in the cellular environment. *In vitro* cytotoxicity study showed that anticancer activity of HMSNs-DOX/IR825 was significantly improved by the NIR irradiation, suggesting that the hybrid nanoparticle could potentially be used for combined photothermal-chemotherapy of cancer (Zhang et al. 2016).

16.3.2 Self-Assembly Copolymer Carriers – Micelles

Many self-assembling molecules are amphiphilic, comprising of both hydrophobic and hydrophilic domains. Amphiphilic copolymers can self-assemble into micelles, vesicles, and molecular gels composed of tubules, fibrils, and fibers (Giddi et al. 2007; Nishiyama and Kataoka 2006; Rösler et al. 2001).

Micelles are amphiphilic molecules that self-assemble into a spherical structure with a hydrophobic core and hydrophilic exterior making it suitable for encapsulat-

ing hydrophobic cancer drugs (Jhaveri and Torchilin 2014). It is estimated that about 40 % of marketed drugs and up to 75 % compounds under development are poorly water soluble (Jhaveri and Torchilin 2014; Di et al. 2009; Williams et al. 2013). Polymeric micelles can be used for combination therapy by loading multiple anticancer agents in polymeric micelles in a one-step drug-loading process without chemical modification of drugs (Shin et al. 2011). Multi-drug release may occur by simple hydrolysis or be triggered by an acidic pH and/or lysosomal enzymes, and can be tuned by chemical linkage for concurrent or sequential delivery (Duncan 2006; Greco and Vicent 2009).

Shin and colleagues showed that they could encapsulate paclitaxel, rapamycin, and 17-allylamino-17-demethoxygeldanamycin (17-AAG) in PEG-*b*-PLA micelles without changing the pharmacokinetics of each drug at low doses. The pharmacokinetic profiles were however altered when drugs are delivered at higher doses (Shin et al. 2012). Rapamycin and 17-AAG acts concurrently to inhibit the PI3K/Akt/mTOR and Ras/Raf/MEK/ERK signaling pathways, enhancing cancer cell killing by paclitaxel. Bae and colleagues directly conjugated doxorubicin and 17-hydroxy ethylamino-17-demethoxygeldanamycin (GDM-OH) to a poly(ethylene glycol)-poly(aspartate hydrazide) block copolymers through acid-labile hydrazone bonds. The pH-sensitive micelles were combined and appeared to minimize a schedule-dependent change in combined drug efficacy when compared to the free drug combination (Bae et al. 2010). Karaca and colleagues, reported the use of methoxy poly(ethyleneglycol)-block-ploy(2-methyl-2-carboxyl-propylenecarbonate)-graft-dodecanol (mPEG-b-PCC-g-DC) copolymer to conjugate gemcitabine and encapsulate a Hedgehog inhibitor, Vismodegib (GDC-0449) into its hydrophobic core for the treatment of pancreatic ductal adenocarcinoma (PDAC). The *in vivo* stability of gemcitabine increased significantly after conjugation, and the drug combination, when administered to athymic nude mice bearing subcutaneous tumors generated using MIA PaCa-2 cells, efficiently inhibited tumor growth (Karaca et al. 2016).

16.3.3 Nanotechnology Approaches to Enhance Co-delivery

By physically confining drug combinations within a single carrier, pharmacokinetics for multiple drugs can be unified, ensuring all particle-treated cells receive a pairwise combination of drugs – maximizing therapeutic potential. One key challenge in this area involves combining three general categories of drugs, all of which display differing combinations of physiochemical properties: (i) large and hydrophilic proteins, (ii) small and hydrophobic small molecules, and (iii) moderately sized, hydrophilic, and highly anionic nucleic acids. Targeted, nanoscale delivery of therapeutic proteins remains an area currently underexplored in cancer therapy – particularly immunotherapy. Fahmy and coworkers recently addressed this challenge in the development of a combination cancer immunotherapy that reverts immunosuppressive tumor microenvironments (Fig. 16.3a) (Park et al. 2012). Using the cytokine, IL-2, which stimulates NK cell and cytotoxic T lymphocyte activity,

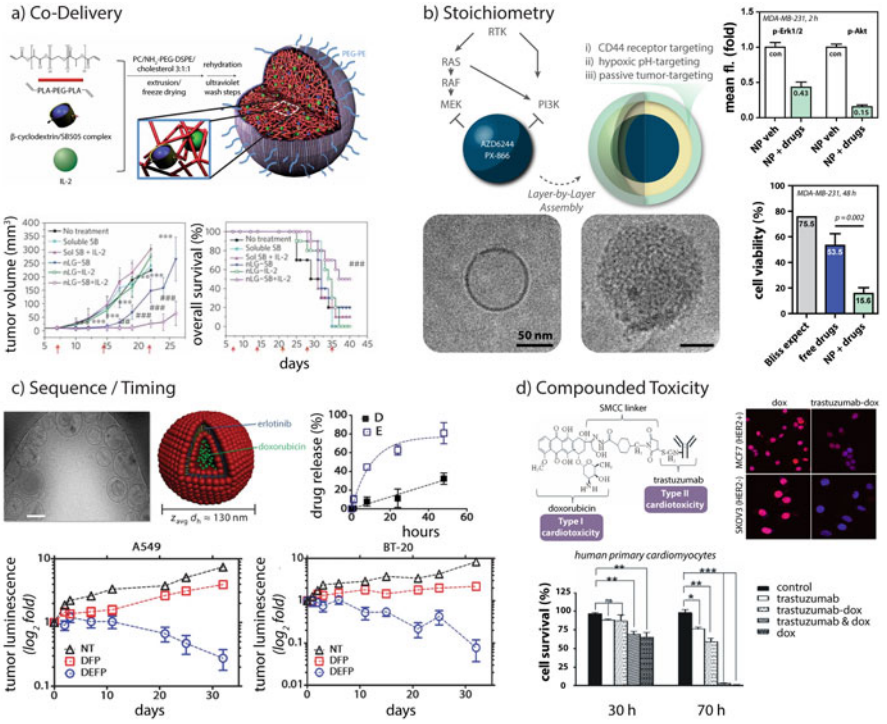

Fig. 16.3 Nanomedicines Overcome Challenges in Combination Drug Delivery. (**a**) Bulky and hydrophilic proteins are notably difficult to co-deliver with small, hydrophobic molecular inhibitors. Here, IL-2 is entrapped in a photo-polymerizable hydrogel matrix composed of poly(ethylene glycol), polylactide, and a small molecule/β-cyclodextrin inclusion complex. The hydrogel core is encapsulated by a liposomal shell and photocured under ultraviolet light. Systemically administered 'nanolipogels' decreased tumor burden and improved overall survival better than single drug-loaded particles or intratumoral injection of free drugs in immunocompetent, subcutaneous tumor xenograft mouse models (B16-F10). IL-2 promotes a hostile tumor microenvironment by stimulating NK cell and cytotoxic T lymphocyte activity, while a TGF-β signal blockade (SB505124) does the same while additionally depleting tumor-promoting regulatory T lymphocytes (Tregs). (**b**) The delivery of synergistic drug ratios can also be 'pre-programmed' into nanoscale drug carriers. Here, a hydrophobic inhibitor of MAPK pathway signaling is co-encapsulated with a hydrophilic inhibitor of the PI3K pathway at a pre-defined synergistic drug ratio. Layer-by-Layer (LbL) polymer assembly around the drug-containing liposomal core facilitated both affinity-based and microenvironment-responsive tumor targeting, while simultaneously blocking both pathways and enhancing drug synergy beyond that of the free drug combination *in vitro* and *in vivo*. (**c**) Time-staggered delivery can be achieved through structural partitioning of drugs within nanoscale drug carriers. Here, a hydrophobic inhibitor of EGFR (erlotinib) partitions into the lipid bilayer of a nanoscale lipid vesicle, while a hydrophilic genotoxic agent, doxorubicin, is loaded into the aqueous particle core. Initial release of erlotinib 'dynamically rewires' cell signaling in lung and breast tumors in a manner that enhances subsequent cell killing by doxorubicin in a time-dependent manner. Systemically administered, folate receptor-targeting constructs induce a partial response in lung (A549) and breast (BT-20) tumor xenograft-bearing mice, while mice receiving single drug-loaded particles (doxorubicin) exhibit progressive disease. (**d**) Nanomedicines can also mitigate dose-limiting side effects of drugs with overlapping toxicity profiles. Although, doxocubicin and

in combination with TGF-β signal blockade, which does the same while additionally depleting tumor-promoting regulatory T lymphocytes (Tregs), the authors were able to develop a systemically administerable drug carrier that decreased tumor burden and improved overall survival when compared to single drug-loaded particles or to intratumoral injection of free drugs in immunocompetent, subcutaneous tumor xenograft mouse models (B16-F10). To achieve this, IL-2 was entrapped in a photo-polymerizable hydrogel matrix composed of poly(ethylene glycol) (PEG), polylactide, and a small molecule/β-cyclodextrin inclusion complex. The hydrogel core is encapsulated by a liposomal shell and photocured under ultraviolet light to obtain PEG-stabilized 'nanolipogels'. A variety of other novel co-delivered drug combinations are listed in Table 16.1.

16.3.4 Nanotechnology Solutions: Stoichiometry/Ratiometric Dosing

Through ratiometric drug loading into nanoscale carriers, intracellular drug concentrations can be 'pre-programmed' for drug synergy, thus maximizing therapeutic potential and avoiding possible antagonistic interactions resulting from spatially and temporally heterogeneous delivery of free drug compounds. For example, Dreaden et al 2015. co-encapsulated a hydrophobic inhibitor of MAPK pathway signaling with a hydrophilic inhibitor of the PI3K pathway at a pre-defined synergistic drug ratio (Fig. 16.3b) (Dreaden et al. 2015). Layer-by-Layer (LbL) polymer assembly around the drug-containing liposomal core facilitated both affinity-based and microenvironment-responsive tumor targeting, while simultaneously blocking both pathways and enhancing drug synergy beyond that of the free drug combination *in vitro* and *in vivo*. A related therapeutic in clinical development, liposomal irinotecan/floxuridine (CPX-1, 1:1 mol; Celator), co-encapsulates drugs at a previously identified synergistic drug ratio and maintains this drug stoichiometry both in plasma and in the tumor bed (Batist et al. 2009). Interestingly, efficacy of the liposomal formulation in tumor-bearing mice was superior to both free drugs dosed at their respective MTDs and the additive expectation from both singly loaded

Fig. 16.3 (continued) trastuzumab (anti-HER2/ErbB2; Herceptin) – alone – induce Type I and Type II cardiotoxicity, respectively, the two are often co-administered in breast cancer patients. Engineering of an trastuzumab-doxorubicin antibody drug conjugate (ADC) rescues doxorubicin toxicity towards human primary cardiomyocytes by limiting cellular uptake to HER2-expressing cells. Here, a heterobifunctional linker (SMCC) containing an amine-reactive NHS ester and a thiol-reactive malimide crosslinks doxorubicin and IgG, respectively (Reprinted with permission from (a) Park et al. 2012, (b) Dreaden et al. 2015, (c) Morton et al. 2014, and (d) Zhang et al. 2013). Copyright (**a**) 2012 Nature Publishing Group, (**b**) 2015 American Association of Cancer Research, (**c**) 2014 American Association for the Advancement of Science, and (**d**) 2013 original authors under the Creative Commons Attribution License)

Table 16.1 Selected examples of nanotechnology-enabled combination cancer therapies

Material	Cytotoxic	Small molecule	Protein	Nucleic acid	Indication	References
Liposome	5:1 cytarabine and daunorubicin				Acute myeloid leukemia	Tardi et al. (2009a)
Liposome	1:1 irinotecan and floxuridine				Colorectal cancer	Batist et al. (2009)
Liposome	7:1 irinotecan and cisplatin				Small-cell lung cancer	Tardi et al. (2009b)
Liposome	6-mercaptopurine and daunorubicin				Acute lymphocytic leukemia	Agrawal et al. (2005)
Liposome	1:2 quercetin and vincristine				Breast cancer	Wong and Chiu (2010)
Polymer nanoparticle	Doxorubicin and docetaxel				Prostate cancer	Zhang et al. (2007)
Polymer conjugate	Platinum, doxorubicin, and camptothecin				Ovarian cancer	Liao et al. (2014)
Polymer conjugate	Gemcitabine and doxorubicin				Prostate cancer	Lammers et al. (2009)
Polymersome (LbL)	5-fluorouracil, irinotecan, oxaliplatin				Pancreatic cancer	Li et al. (2015)
Drug self-assembly	Irinotecan and chlorambucil				Breast cancer	Huang et al. (2014)
Liposome/silica hybrid	Doxorubicin, 5-fluorouracil, and cisplatin				Hepatocellular carcinoma	Ashley et al. (2011)
Silica	Paclitaxel and gemcitabine				Pancreatic cancer	Meng et al. (2015)
Polymer/liposome hybrid	Combretastatin and doxorubicin				Lung carcinoma, melanoma	Sengupta et al. (2005)

Emulsion	Paclitaxel	Curcumin			Ovarian cancer	Ganta and Amiji (2009)
Liposome	Doxorubicin	Verapamil			Leukemia	Wu et al. (2007)
Liposome (LbL)	Doxorubicin	Erlotinib			Breast, lung cancer	Morton et al. (2014)
Polymer nanoparticle	Paclitaxel	Ceramide			Ovarian cancer	van Vlerken et al. (2010)
Polymer nanoparticle	Paclitaxel	Tariquidar			Various cancers	Patil et al. (2009)
Polymer nanoparticle	Vincristine	Verapamil			Breast cancer	Song et al. (2009)
Polymer nanoparticle	Doxorubicin	Cyclosporin A			Various cancers	Emilienne Soma et al. (2000)
Polymer conjugate	Doxorubicin	Wortmannin			Breast cancer	Bae et al. (2007)
Dendrimer	Methotrexate	All-trans retinoic acid			Leukemia	Tekade et al. (2008)
Dendrimer	Methotrexate	All-trans retinoic acid			Leukemia	Tekade et al. (2009)
Dendrimer	Paclitaxel	Alendronate			Bone metastases	Clementi et al. (2011)
Liposome (LbL)		Selumetinib and PX-866			Breast, lung cancer	Dreaden et al. (2015)
Liposome		SB505124	IL-2		Melanoma	Park et al. (2012)
Liposome	Doxorubicin		TRAIL		Breast cancer	Jiang et al. (2014)
Polymer nanoparticle	Paclitaxel		TRAIL		Breast cancer	Lee et al. (2011)
Polymer nanoparticle	Paclitaxel		Anti-EGFR		Lung cancer	Karra et al. (2013)

(continued)

Table 16.1 (continued)

Material	Cytotoxic	Small molecule	Protein	Nucleic acid	Indication	References
Polymer conjugate	Camptothecin		Anti-HER2		Breast cancer	Han and Davis (2013)
Polymer nanoparticle	Doxorubicin		Anti-HER2		Breast cancer	Shi et al. (2009)
Polymer nanoparticle	Docetaxel and cisplatin		Anti-HER2		Breast cancer	Mi et al. (2013)
Nanocapsule	Paclitaxel		Caspase-3		Cervical cancer	Kim et al. (2015)
Graphene oxide	Doxorubicin		TRAIL		Lung, colorectal cancer	Jiang et al. (2015)
Polymer nanoparticle		Sorafenib		Survivin shRNA	Hepatocellular carcinoma	Shen et al. (2014)
Liposome (LbL)	Doxorubicin			MRP-1 and Bcl-2 siRNA	Lung cancer	Saad et al. (2008)
Polymer nanoparticle	Paclitaxel			Bcl-2 siRNA	Breast cancer	Wang et al. (2006)
Polymer nanoparticle	Paclitaxel			VEGF siRNA	Prostate cancer	Zhu et al. (2010)
Polymer nanoparticle	Doxorubicin			Bcl-xL shRNA	Prostate cancer	Kim et al. (2010)
Polymer nanoparticle	Doxorubicin			MDR-1 siRNA	Breast cancer	Misra et al. (2014)
Dendrimer	Doxorubicin			Luc siRNA	Glioblastoma	Kaneshiro and Lu (2009)
Dendrimer	Paclitaxel			Akt siRNA	Ovarian cancer	Kala et al. (2014)
Silica	Doxorubicin			P-gp siRNA	Breast cancer	Meng et al. (2013)
Silica	Doxorubicin			Bcl-2 siRNA	Ovarian cancer	Chen et al. (2009a)

liposomes. Strikingly, a liposomal formulation encapsulating a previously identified antagonistic drug ratio (10:1 mol) was less effective than its singly loaded counterpart, suggesting a putative role for heterogeneous combination drug delivery in promoting resistance to therapy. Similar approaches employing cytarabine/daunorubicin (CPX-351, 5:1 mol) (Tardi et al. 2009a), irinotecan/cisplatin (CPX-571, 7:1 mol) (Tardi et al. 2009b), and paclitaxel/tanespimycin (17-AAG) (Katragadda et al. 2013), quercetin/vincristine (1:2 mol), and doxorubicin/camptothecin/Pt (1:2.5:3.6 mol) (Liao et al. 2014) are currently under investigation.

16.3.5 Nanotechnology Approaches to Tailor Drug Combination Timing and Sequence

Another powerful property of nanoscale drug carriers is their capacity to not only spatially regulate drug release in the body, but also to temporally control the sequence and kinetics of therapeutics released. To exploit the observation by Lee et al. that time-staggered inhibition of EGFR could sensitize breast cancer cells to DNA damaging chemotherapy, Yaffe, Hammond, and coworkers engineered a nanoscale drug carrier which achieved staged drug delivery through structural partitioning of drugs within a liposomal vesicle (Fig. 16.3c) (Morton et al. 2014). Here, a hydrophobic inhibitor of EGFR, erlotinib, partitions into the lipid bilayer of the vesicle, while a hydrophilic genotoxic agent, doxorubicin, is loaded into the aqueous particle core. The initial release of erlotinib from these nanoparticles 'dynamically rewired' cell signaling in lung and breast tumors in a manner that recapitulates optimally staggered delivery kinetics seen with free drug administration, enhancing subsequent cell killing by doxorubicin. Systemically administered, folate receptor-targeted Erlotinib/doxorubicin nanoparticles were shown to induce a partial response in both lung (A549) and breast (BT-20) tumor xenografts in nude mice, while mice receiving only single drug-loaded nanoparticles (doxorubicin) exhibited progressive disease.

Gnanasammandhan and colleagues described a noninvasive method to deliver drugs that allow for a high degree of spatial and temporal control. Upconversion nanoparticles (UCNs) were used to convert deeply penetrating near-infrared (NIR) light to UV-visible wavelengths that match the absorption spectrum of photosensitive therapeutics. This allowed for the use of deep-penetrating and biologically friendly NIR light for photoactivation (Gnanasammandhan et al. 2016). The UCNs were used for photodynamic therapy (PDT) and photoactivated control of gene expression. For PDT, the UCNs are coated with polyethylene glycol (PEG) for stabilization and folic acid for tumor targeting and then loaded with photosensitizers that would be expected to kill cells by singlet oxygen production, whereas for the photoactivated control of gene expression, knockdown of essential tumor genes is achieved using UCNs loaded with caged nucleic acid (Gnanasammandhan et al. 2016).

To achieve controlled release of drugs, Liao and colleagues recently used two novel macromonomers (MMs) and a novel cross-linker as building blocks for the

construction of a multi-drug-loaded nanoparticle. **CPT-MM** and **DOX-MM** are branched MMs that release unmodified CPT and DOX in response to cell culture media and long-wavelength ultraviolet (UV) light, respectively (Liao et al. 2014).

16.3.6 Nanotechnology Approaches to Limit Compounding and Overlapping Toxicity

Combination drug carriers can overcome challenges from overlapping toxicity profiles by biasing tissue disposition away from off-target tissues or by decelerating bolus drug release in vital organs. To address cardiotoxicity from doxorubicin and trastuzumab (anti-HER2/ErbB2; Herceptin) combination therapy, Zhang et al. engineered an antibody-drug conjugate from the pair, thereby limiting cytotoxic doxorubicin delivery to cells expressing high levels of HER2, while blocking the compounded toxicity towards human primary cardiomyocytes (Zhang et al. 2013) (Fig. 16.3d). To address dose-limiting hepatotoxic effects from combined MAPK and PI3K pathway inhibition, Dreaden et al. engineered LbL nanoparticles that biased tissue disposition towards solid tumors and rescued both hepatic and renal tissue damage while improving antitumor efficacy *in vivo*. Similarly, Farokhzad and coworkers have found that aptamer-targeted PLGA nanoparticles can rescue the nephrotoxic effects of platinum chemotherapeutics while maintaining equivalent antitumor activity *in vivo* (Dhar et al. 2011; Kolishetti et al. 2010) and also accommodating the chemotherapeutic, docetaxel, in polylactide containing particles (Xu et al. 2013).

16.3.7 Combining Nucleic Acid Therapies with Other Drug Combinations

Since the discovery of RNA interference (RNAi) in 1997, there has been great interest in harnessing RNAi for the treatment of disease. RNAi is activated by double-stranded RNA (dsRNA), which includes short interfering RNA (siRNA) and microRNA (miRNA) and utilizes the endogenous RNAi pathway for the post-transcriptional silencing of gene expression. MicroRNAs form central nodal points in cancer development pathways and exert their effects by targeting various oncogenes and tumor suppressors (Kong et al. 2012; Zhang et al. 2007), while siRNAs can be used to efficiently silence the expression of any gene with high specificity. These include targets that are considered to be difficult to drug. Here we describe some platforms used to deliver RNAi-drug combinations.

One particular tumor type, for example, that could greatly benefit from RNAi therapy is triple-negative breast cancer (TNBC), which is characterized by the lack of progesterone, estrogen and HER2 receptors. It is non-responsive to conventional hormonal therapy (such as tamoxifen or aromatase inhibitors) or therapies that

target HER2 receptors, such as Herceptin (trastuzumab) (Foulkes et al. 2010). RNAi-based approaches can therefore be beneficial for the treatment of TNBC. One way to accomplish this is by modulating endogenous miRNA levels in TNBC. miR-221 and miR-205 have been shown to be up and down regulated in TNBC respectively (Nassirpour et al. 2013; Piovan et al. 2012). Conde and colleagues recently showed that they could deliver a miR-205 mimic and an antagomiRNA (miRNA inhibitor) using a self-assembled RNA-triple-helix structure which is conjugated to dendrimers to form stable triplex nanoparticles that can achieve nearly 90 % tumor shrinkage 2 weeks post-gel implantation in a triple-negative breast cancer mouse model (Conde et al. 2016).

A promising approach made available by nanotechnology is to combine siRNA and chemotherapeutics in a single platform. Deng and colleagues used a controlled layer-by-layer process to co-deliver siRNA against a drug-resistance pathway (multidrug resistance protein 1) and a chemotherapy drug (doxorubicin) to challenge a highly aggressive form of triple-negative breast cancer resulting in an 8-fold decrease in tumor volume compared to control treatments with no toxicity observed (Deng et al. 2013). Xu and colleagues combined siRNA against targets involved in error-prone translesion DNA synthesis pathway (REV1 and REV3L) with conventional DNA-damaging chemotherapy (cisplatin prodrug) through self-assembly of a biodegradable poly(lactide-coglycolide)-b-poly(ethylene glycol) diblock copolymer and a self-synthesized cationic lipid. This nanoparticle formulation had a synergistic effect on tumor inhibition in a xenograft mouse model of human lymph node carcinoma of the prostate that was noticeably more effective than platinum monotherapy (Xu et al. 2013).

Other carriers that have been used to deliver the siRNA based combinations include liposomes (Gabizon et al. 1994; Chen et al. 2009a, b, 2010a, b; Li et al. 2008), micelles (Zheng et al. 2013; Shim et al. 2011; Zhu et al. 2010), polymers poly (D,L-lactide-co-glycolide) (PLGA) (Li et al. 2001; Fonseca et al. 2002), poly lactic acid (PLA) (Tobío et al. 1998; Dong and Feng 2004), polycapro lactone (PCL) (Yang et al. 2006), dendrimers (Biswas et al. 2013; Kaneshiro and Lu 2009; Kulhari et al. 2011), natural chitosan polymeric nanoparticles (Wei et al. 2013; Nagpal et al. 2010), silica (Santra et al. 2001; Qhobosheane et al. 2001; Kneuer et al. 2000) and other inorganic nanoparticles e.g calcium, gold, quantum dots, etc. (Sokolova and Epple 2008).

16.4 Limitations to Developing Combination Chemotherapeutics, Tumor-Specific Targeting, and Enabling Approaches

Like Frei, Freireich, and Zubrod, drug discovery has historically focused on the development of compounds with independent antitumor activity – those intended for use as monotherapies. Modern approaches to combination development have, in contrast, been largely limited to off-patent cytotoxic drugs. To address the challenge of integrating patent-protected targeted therapies in combination approaches,

Merck and AstraZeneca initiated a seminal partnership in 2009 to share the costs of developing combination candidates, for example, AstraZeneca's MEK inhibitor (AZD6244) and Merck's protein kinase B inhibitor (MK-22060). Merck and Sanofi later followed with a similar agreement to investigate Merck's MEK inhibitor, MSC1936369B, in combination with Sanofi's PI3K/mTOR inhibitor, SAR245409, and class I PI3K inhibitor, SAR245408 . Bristol-Myers Squibb and Roche have likewise established agreements to combine Roche's vemurafenib (Zelboraf) with BMS's ipilimumab (Yervoy) for BRAF mutant metastatic melanoma. While highly promising, dose-limiting hepatotoxicity from the latter two combination approaches in Phase I clinical trials (Xu et al. 2013) highlights a key weakness of this approach: by neglecting combination effects during the discovery phase, more safe and/or effective combination candidates may be discarded simply as a result of exhibiting less potent independent antitumor activity. Although small molecule targeted therapies, in the past, have provided less incentive for early combination development (due to their single agent efficacy), the recent resurgence of cancer immunotherapy development will likely accelerate the integration of combination approaches earlier in the development pipeline, providing opportunities for the investigation of combinations with weak independent activity, but potent and safe combined therapeutic effects in the future.

A major but as-yet incompletely realized opportunity for nanoparticle therapeutics is the potential for tumor-specific targeting. Compounded organ-specific toxic effects such as those described above highlight a potential intrinsic advantage of – and challenge to – nanoscale drug delivery, whereby dose-limiting toxicities could be mitigated through nanoparticle-altered pharmacokinetics combined with favorable tumor tissue targeting profiles. The latter phenomenon can occur through size-dependent 'passive' tumor targeting or the 'active' targeting of tissues via stimuli-responsive behavior or affinity directed accumulation. While a number of recent publications seek to revisit the importance and prevalence of passive targeting in tumor delivery (Prabhakar et al. 2013; Park 2013) – largely attributed to the so-called enhanced permeability and retention (EPR) effect (Matsumura and Maeda 1986; Matsumoto et al. 2016) – nanoparticles are well known to preferentially accumulate in organs of the mononuclear phagocyte system (MPS), namely the liver and spleen, and to augment the accumulation of renally excreted drugs (i.e. <10 nm) in solid tumors. This property can be advantageous when designing treatments for hepatocellular carcinoma or immunotherapies, respectively; however, affinity directed targeting of tumor tissues remains an integral and underexplored challenge to the field. Chan and coworkers (Wilhelm et al. 2016), highlight this disparity in a recent retrospective literature analysis, noting only modest (0.5-fold) improvements in median tumor accumulation afforded by active targeting across multiple studies. In contrast, relatively smaller antibody-drug conjugates (ADCs) (Vaklavas and Forero-Torres 2012) and molecular polyconjugates (Rozema et al. 2007), while less prevalent, have demonstrated notably reproducible in vivo targeting capabilities.

Future research investigating specific targeting ligands including small molecules (e.g. folic acid, bisphosphonate, carbohydrates), peptides (e.g. GE11, RGD, knottin), and proteins (e.g. IgG, Fab fragments, Centyrins), is expected to improve both the effectiveness and reproducibility of affinity targeting strategies employed by the field, as well as subsequent treatment outcomes from rational drug combinations.

16.5 Outlook and Conclusions

The intersection between cancer biology and nanotechnology is an exciting and emerging area of current academic and industrial research. With ongoing efforts in both fields, combination anticancer treatments are continuing to evolve, raising hopes for unprecedented antitumor responses and reduced toxicity. The emergence of newly engineered combination drug delivery platforms should allow us to combine different classes of drugs into a single nanoparticle with tunable functionality over local or temporal control of drug delivery increasing safety, efficacy, and durability. The co-delivery of different cancer therapeutic agents provides promising options to overcome chemoresistance. Recent reports provide strong evidence that combining different drugs using nanoparticles improves tumor killing compared to single agent therapy. While these approaches hold great promise, there still remain key limitations in their proof of concept. Most nanocarrier studies are currently performed in preclinical models, and desperately need to be translated into human clinical trials, particularly since the biodistribution, localization, and release profiles of these drugs may differ in humans. It is also pertinent for the safety profiles of the various carriers used for the delivery of these therapeutic agents to be further studied, with special focus on their toxicity and immune response. Given the progress that has been made in the field during the past 5 years, the future of rationally designed and personalized combination therapy using customizable nanoparticle delivery platforms looks promising.

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References

Agrawal V, Paul MK, Mukhopadhyay AK (2005) 6-mercaptopurine and daunorubicin double drug liposomes—preparation, drug-drug interaction and characterization. J Liposome Res 15:141–155

Anglin R et al (2014) Risk of upper gastrointestinal bleeding with selective serotonin reuptake inhibitors with or without concurrent NonSteroidal anti-inflammatory use: a systematic review and meta-analysis. Am J Gastroenterol 109(6):811–819

Ashley CE et al (2011) The targeted delivery of multicomponent cargos to cancer cells by nanoporous particle-supported lipid bilayers. Nat Mater 10(5):389–397

Bae Y et al (2007) Mixed polymeric micelles for combination cancer chemotherapy through the concurrent delivery of multiple chemotherapeutic agents. J Control Release 122(3):324–330

Bae Y et al (2010) Mixed pH-sensitive polymeric micelles for combination drug delivery. Pharm Res 27(11):2421–2432

Baek S et al (2015) Smart multifunctional drug delivery towards anticancer therapy harmonized in mesoporous nanoparticles. Nanoscale 7(34):14191–14216

Batist G et al (2009) Safety, pharmacokinetics, and efficacy of CPX-1 liposome injection in patients with advanced solid tumors. Clin Cancer Res 15(2):692–700

Biswas S, Deshpande PP, Navarro G, Dodwadkar NS, Torchilin VP (2013) Lipid modified triblock PAMAM-based nanocarriers for siRNA drug co-delivery. Biomaterials 34(4):1289–1301, http://doi.org/10.1016/j.biomaterials.2012.10.024

Burchenal JH et al (1954) Clinical studies on 6-mercaptopurine. Ann N Y Acad Sci 60(2):359–368

Burrell RA et al (2013) The causes and consequences of genetic heterogeneity in cancer evolution. Nature 501(7467):338–345

Capdeville R et al (2002) Glivec (STI571, imatinib), a rationally developed, targeted anticancer drug. Nat Rev Drug Discov 1(7):493–502

Cardinale D et al (2010) Trastuzumab-induced cardiotoxicity: clinical and prognostic implications of troponin I evaluation. J Clin Oncol 28(25):3910–3916

Chen AM et al (2009a) Co-delivery of doxorubicin and Bcl-2 siRNA by mesoporous silica nanoparticles enhances the efficacy of chemotherapy in multidrug-resistant cancer cells. Small 5(23):2673–2677

Chen Y et al (2009b) Novel cationic lipid that delivers siRNA and enhances therapeutic effect in lung cancer cells. Mol Pharm 6(3):696–705

Chen Y, Bathula SR et al (2010a) Multifunctional nanoparticles delivering small interfering RNA and doxorubicin overcome drug resistance in cancer. J Biol Chem 285(29):22639–22650

Chen Y, Wu JJ, Huang L (2010b) Nanoparticles targeted with NGR motif deliver c-myc siRNA and doxorubicin for anticancer therapy. Mol Ther 18(4):828–834

Clementi C et al (2011) Dendritic poly(ethylene glycol) bearing paclitaxel and alendronate for targeting bone neoplasms. Mol Pharm 8(4):1063–1072

Conde J et al (2016) Self-assembled RNA-triple-helix hydrogel scaffold for microRNA modulation in the tumour microenvironment. Nat Mater 15(3):353–363

Deng ZJ, Morton SW, Ben-Akiva E, Dreaden EC, Shopsowitz KE, Hammond PT (2013) Layer-by-layer nanoparticles for systemic codelivery of an anticancer drug and siRNA for potential triple-negative breast cancer treatment. ACS Nano 7(11):9571–9584, http://doi.org/10.1021/nn4047925

Devita VT, Moxley JH, Brace K, Frei E III (1965) Intensive combination chemotherapy and X-irradiation in the treatment of Hodgkin's disease. Ant Intern Med 6:881–895

Devita VT, Serpick AA, Carbone PP (1970) Combination chemotherapy in the treatment of advanced Hodgkin's disease. Ann Intern Med 73(6):881–895, http://doi.org/10.7326/0003-4819-73-6-881

Di L, Kerns EH, Carter GT (2009) Drug-like property concepts in pharmaceutical design. Curr Pharm Des 15(19):2184–2194

Dhar S, Kolishetti N, Lippard SJ, Farokhzad OC (2011) Targeted delivery of a cisplatin prodrug for safer and more effective prostate cancer therapy in vivo. Proc Natl Acad Sci U S A 108(5):1850–1855, http://doi.org/10.1073/pnas.1011379108

Dong Y, Feng S-SS-S (2004) Methoxy poly(ethylene glycol)-poly(lactide) (MPEG-PLA) nanoparticles for controlled delivery of anticancer drugs. Biomaterials 25(14):2843–2849

Dranoff G (2004) Cytokines in cancer pathogenesis and cancer therapy. Nat Rev Cancer 4(1):11–22

Dreaden EC et al (2015) Tumor-targeted synergistic blockade of MAPK and PI3K from a layer-by-layer nanoparticle. Clin Cancer Res 21(19):4410–4419

Duncan R (2006) Polymer conjugates as anticancer nanomedicines. Nat Rev Cancer 6(9):688–701

Elbashir SM et al (2001) Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature 411(6836):494–498

Emilienne Soma C et al (2000) Reversion of multidrug resistance by co-encapsulation of doxorubicin and cyclosporin A in polyalkylcyanoacrylate nanoparticles. Biomaterials 21(1):1–7

Engelman JA et al (2008) Effective use of PI3K and MEK inhibitors to treat mutant Kras G12D and PIK3CA H1047R murine lung cancers. Nat Med 14(12):1351–1356

Farber S et al (1948) Temporary remissions in acute leukemia in children produced by folic acid antagonist, 4-aminopteroyl-glutamic acid (aminopterin). New England J Med 238(23):787–793

Fletcher JI et al (2010) ABC transporters in cancer: more than just drug efflux pumps. Nat Rev Cancer 10(2):147–156

Floyd SR et al (2013) The bromodomain protein Brd4 insulates chromatin from DNA damage signalling. Nature 498(7453):246–250

Fonseca C, Simões S, Gaspar R (2002) Paclitaxel-loaded PLGA nanoparticles: preparation, physicochemical characterization and in vitro anti-tumoral activity. J Control Release 83(2):273–286

Foulkes WD, Smith IE, Reis-Filho JS (2010) Triple-negative breast cancer. New England J Med 363(20):1938–1948

Frei E, Freireich EJ, Gehan E, Pinkel D, Holland JF, Selawry O, Haurani F, Spurr CL, Hayes DM, James GW, Rothberg H, Sodee DB, Rundles RW, Schroeder LR, Hoogstraten B, Wolman IJ, Traggis DG, Cooper T, Gendel BR, Ebaugh F, Taylor R (1961) Studies of sequential and combination antimetabolite therapy in acute leukemia: 6-mercaptopurine and methotrexate. Blood 18(4):431–454

Freireich EJ, Karon M, Frei E III (1964) Quadruple combination therapy (VAMP) for acute lymphocytic leukemia of childhood. Proc Am Ass Cancer Res 5:20

Gabizon A et al (1994) Prolonged circulation time and enhanced accumulation in malignant exudates of doxorubicin encapsulated in polyethylene-glycol coated liposomes. Cancer Res 54(4):987–992

Ganta S, Amiji M (2009) Coadministration of paclitaxel and curcumin in nanoemulsion formulations to overcome multidrug resistance in tumor cells. Mol Pharm 6(3):928–939

Giddi HS, Arunagirinathan MA, Bellare JR (2007) Self-assembled surfactant nano-structures important in drug delivery: a review. Indian J Exp Biol 45(2):133–159

Gilman A, Philips FS (1946) The biological actions and therapeutic applications of the B-chloroethyl amines and sulfides. Science (New York, NY) 103(2675):409–436

Gnanasammandhan MK et al (2016) Near-IR photoactivation using mesoporous silica-coated NaYF4:Yb, Er/Tm upconversion nanoparticles. Nat Protoc 11(4):688–713

Goldman A et al (2015) Temporally sequenced anticancer drugs overcome adaptive resistance by targeting a vulnerable chemotherapy-induced phenotypic transition. Nat Commun 6:6139

Goodman LS et al (1946) Nitrogen mustard therapy: use of methyl-bis(beta-chloroethyl)amine hydrochloride and tris(beta-chloroethyl)amine hydrochloride for Hodgkin's disease, lymphosarcoma, leukemia and certain allied and miscellaneous disorders. JAMA 132(3):126–132

Greco F, Vicent MJ (2009) Combination therapy: opportunities and challenges for polymer-drug conjugates as anticancer nanomedicines. Adv Drug Deliv Rev 61(13):1203–1213
Hainaut P, Plymoth A (2013) Targeting the hallmarks of cancer: towards a rational approach to next-generation cancer therapy. Curr Opin Oncol 25(1):50–51
Han H, Davis ME (2013) Single-antibody, targeted nanoparticle delivery of camptothecin. Mol Pharm 10(7):2558–2567
Hanahan D, Weinberg RA (2000) The hallmarks of cancer. Cell 100(1):57–70
Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144(5):646–674
Harasym TO et al (2007) Increased preclinical efficacy of irinotecan and floxuridine coencapsulated inside liposomes is associated with tumor delivery of synergistic drug ratios. Oncol Res 16(8):361–374
Heyn RM et al (1960) The comparison of 6-mercaptopurine with the combination of 6-mercaptopurine and azaserine in the treatment of acute leukemia in children: results of a cooperative study. Blood 15:350–359
Hu C-MJ, Zhang L (2012) Nanoparticle-based combination therapy toward overcoming drug resistance in cancer. Biochem Pharmacol 83(8):1104–1111
Huang P et al (2014) Combination of small molecule prodrug and nanodrug delivery: amphiphilic drug-drug conjugate for cancer therapy. J Am Chem Soc 136(33):11748–11756
Hudis CA (2007) Trastuzumab--mechanism of action and use in clinical practice. New England J Med 357(1):39–51
Jhaveri AM, Torchilin VP (2014) Multifunctional polymeric micelles for delivery of drugs and siRNA. Front Pharmacol 5:77
Jiang T et al (2014) Gel–liposome-mediated co-delivery of anticancer membrane-associated proteins and small-molecule drugs for enhanced therapeutic efficacy. Adv Funct Mater 24(16):2295–2304
Jiang T et al (2015) Furin-mediated sequential delivery of anticancer cytokine and small-molecule drug shuttled by graphene. Adv Mater 27(6):1021–1028
Jordan VC (2003) Tamoxifen: a most unlikely pioneering medicine. Nat Rev Drug Discov 2(3):205–213
Kaelin WG (2005) The concept of synthetic lethality in the context of anticancer therapy. Nat Rev Cancer 5(9):689–698
Kala S et al (2014) Combination of dendrimer-nanovector-mediated small interfering RNA delivery to target Akt with the clinical anticancer drug paclitaxel for effective and potent anticancer activity in treating ovarian cancer. J Med Chem 57(6):2634–2642
Kalos M et al (2011) T cells with chimeric antigen receptors have potent antitumor effects and Can establish memory in patients with advanced leukemia. Sci Transl Med 3(95):95ra73–95ra73
Kaneshiro TL, Lu Z-R (2009) Targeted intracellular codelivery of chemotherapeutics and nucleic acid with a well-defined dendrimer-based nanoglobular carrier. Biomaterials 30(29):5660–5666
Karaca, M. et al., 2016. Micelle Mixtures for Co-administration of Gemcitabine and GDC-0449 to treat Pancreatic Cancer. *Mol. Pharm.*, p.acs.molpharmaceut.5b00971.
Karra N et al (2013) Antibody conjugated PLGA nanoparticles for targeted delivery of paclitaxel palmitate: efficacy and biofate in a lung cancer mouse model. Small 9(24):4221–4236
Katragadda U et al (2013) Combined delivery of paclitaxel and tanespimycin via micellar nanocarriers: pharmacokinetics, efficacy and metabolomic analysis. PLoS One 8(3):e58619
Kaye SB (1993) P glycoprotein (P-gp) and drug resistance--time for reappraisal? Br J Cancer 67(4):641–643
Keung AJ et al (2014) Using targeted chromatin regulators to engineer combinatorial and spatial transcriptional regulation. Cell 158(1):110–120
Kim E et al (2010) Prostate cancer cell death produced by the co-delivery of Bcl-xL shRNA and doxorubicin using an aptamer-conjugated polyplex. Biomaterials 31(16):4592–4599
Kim CS et al (2015) Co-delivery of protein and small molecule therapeutics using nanoparticle-stabilized nanocapsules. Bioconjug Chem 26(5):950–954

Kneuer C et al (2000) Silica nanoparticles modified with aminosilanes as carriers for plasmid DNA. Int J Pharm 196(2):257–261
Kolishetti N et al (2010) Engineering of self-assembled nanoparticle platform for precisely controlled combination drug therapy. Proc Natl Acad Sci U S A 107(42):17939–17944
Kong YW et al (2012) microRNAs in cancer management. Lancet Oncol 13(6):e249–e258
Kormann MSD et al (2011) Expression of therapeutic proteins after delivery of chemically modified mRNA in mice. Nat Biotechnol 29(2):154–157
Kulhari H, Pooja D, Prajapati SK, Chauhan AS (2011) Performance evaluation of PAMAM dendrimer based simvastatin formulations. Int J Pharm 405(1–2):203–209, http://doi.org/10.1016/j.ijpharm.2010.12.002
Lammers T et al (2009) Simultaneous delivery of doxorubicin and gemcitabine to tumors in vivo using prototypic polymeric drug carriers. Biomaterials 30(20):3466–3475
Lee JH, Nan A (2012) Combination drug delivery approaches in metastatic breast cancer. J Drug Delivery 2012(8):1–17
Lee ALZ et al (2011) Synergistic anticancer effects achieved by co-delivery of TRAIL and paclitaxel using cationic polymeric micelles. Macromol Biosci 11(2):296–307
Lee MJ, Ye AS, Gardino AK, Heijink AM, Sorger PK, MacBeath G, Yaffe MB (2012) Sequential application of anticancer drugs enhances cell death by rewiring apoptotic signaling networks. Cell 149(4):780–794, http://doi.org/10.1016/j.cell.2012.03.031
Li Y et al (2001) PEGylated PLGA nanoparticles as protein carriers: synthesis, preparation and biodistribution in rats. J Control Release 71(2):203–211
Li S-D et al (2008) Tumor-targeted delivery of siRNA by self-assembled nanoparticles. Mol Ther 16(1):163–169
Li F et al (2015) Multiple layer-by-layer lipid-polymer hybrid nanoparticles for improved FOLFIRINOX chemotherapy in pancreatic tumor models. Adv Funct Mater 25(5):788–798
Liao L et al (2014) A convergent synthetic platform for single-nanoparticle combination cancer therapy: ratiometric loading and controlled release of cisplatin, doxorubicin, and camptothecin. J Am Chem Soc 136(16):5896–5899
Longley DB, Harkin DP, Johnston PG (2003) 5-Fluorouracil: mechanisms of action and clinical strategies. Nat Rev Cancer 3(5):330–338
Luo B et al (2008) Highly parallel identification of essential genes in cancer cells. Proc Natl Acad Sci U S A 105(51):20380–20385
Luo J et al (2009) A genome-wide RNAi screen identifies multiple synthetic lethal interactions with the Ras oncogene. Cell 137(5):835–848
Machover D et al (1982) Treatment of advanced colorectal and gastric adenocarcinomas with 5-FU combined with high-dose folinic acid: a pilot study. Cancer Treat Rep 66(10):1803–1807
Mahoney KM, Rennert PD, Freeman GJ (2015) Combination cancer immunotherapy and new immunomodulatory targets. Nat Rev Drug Discov 14(8):561–584
Matsumoto Y et al (2016) Vascular bursts enhance permeability of tumour blood vessels and improve nanoparticle delivery. Nat Nanotechnol 11(6):533–538
Matsumura Y, Maeda H (1986) A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs. Cancer Res 46(12):6387–6392
Mayer LD, Janoff AS (2007) Optimizing combination chemotherapy by controlling drug ratios. Mol Interv 7(4):216–223
Meng H, Mai WX, Zhang H, Xue M, Xia T, Lin S, Wang X, Zhao Y, Ji Z, Zink JI, Nel AE (2013) Codelivery of an optimal drug/siRNA combination using mesoporous silica nanoparticles to overcome drug resistance in breast cancer in vitro and in vivo. ACS Nano 7:994–1005
Meng H et al (2015) Use of a lipid-coated mesoporous silica nanoparticle platform for synergistic gemcitabine and paclitaxel delivery to human pancreatic cancer in mice. ACS Nano 9(4):3540–3557
Mi Y, Zhao J, Feng S-S (2013) Targeted co-delivery of docetaxel, cisplatin and herceptin by vitamin E TPGS-cisplatin prodrug nanoparticles for multimodality treatment of cancer. J Control Release 169(3):185–192

Misra R et al (2014) Reversal of multidrug resistance in vitro by co-delivery of MDR1 targeting siRNA and doxorubicin using a novel cationic poly(lactide-co-glycolide) nanoformulation. Int J Pharm 475(1–2):372–384
Morsut L et al (2016) Engineering customized cell sensing and response behaviors using synthetic notch receptors. Cell 164(4):780–791
Morton, S.W. et al., 2014. A nanoparticle-based combination chemotherapy delivery system for enhanced tumor killing by dynamic rewiring of signaling pathways. *Science signaling*, 7(325), pp.ra44–ra44. Available at: http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=24825919&retmode=ref&cmd=prlinks.
Moxley JH et al (1967) Intensive combination chemotherapy and X-irradiation in Hodgkin's disease. Cancer Res 27(7):1258–1263
Nagpal K, Singh SK, Mishra DN (2010) Chitosan nanoparticles: a promising system in novel drug delivery. Chem Pharm Bull 58(11):1423–1430
Nassirpour R et al (2013) miR-221 promotes tumorigenesis in human triple negative breast cancer cells. A. Ahmad, ed. PLoS One 8(4):e62170
Ngo VN et al (2006) A loss-of-function RNA interference screen for molecular targets in cancer. Nature 441(7089):106–110
Nishiyama N, Kataoka K (2006) Current state, achievements, and future prospects of polymeric micelles as nanocarriers for drug and gene delivery. Pharmacol Ther 112(3):630–648
Park K (2013) Facing the truth about nanotechnology in drug delivery. ACS Nano 7(9):7442–7447
Park J et al (2012) Combination delivery of TGF-β inhibitor and IL-2 by nanoscale liposomal polymeric gels enhances tumour immunotherapy. Nat Mater 11(10):895–905
Patil Y et al (2009) Nanoparticle-mediated simultaneous and targeted delivery of paclitaxel and tariquidar overcomes tumor drug resistance. J Control Release 136(1):21–29
Peer D et al (2007) Nanocarriers as an emerging platform for cancer therapy. Nat Nanotechnol 2(12):751–760
Perrault SD et al (2009) Mediating tumor targeting efficiency of nanoparticles through design. Nano Lett 9(5):1909–1915
Piovan C et al (2012) Oncosuppressive role of p53-induced miR-205 in triple negative breast cancer. Mol Oncol 6(4):458–472
Platt RJ et al (2014) CRISPR-Cas9 knockin mice for genome editing and cancer modeling. Cell 159(2):440–455
Prabhakar, U. et al., 2013. Challenges and key considerations of the enhanced permeability and retention effect for nanomedicine drug delivery in oncology. In Cancer research. American Association for Cancer Research, pp. 2412–2417.
Pritchard JR et al (2013) Defining principles of combination drug mechanisms of action. Proc Natl Acad Sci U S A 110(2):E170–E179
Qhobosheane M et al (2001) Biochemically functionalized silica nanoparticles. Analyst 126(8):1274–1278
Ramsey S (2005) What do we want from our investment in cancer research? Health Aff 24:W5R101–W5R104
Ribas A et al (2013) Hepatotoxicity with combination of vemurafenib and ipilimumab. New England J Med 368(14):1365–1366
Rösler A, Vandermeulen GW, Klok HA (2001) Advanced drug delivery devices via self-assembly of amphiphilic block copolymers. Adv Drug Deliv Rev 53(1):95–108
Rothenberg M, Ling V (1989) Multidrug resistance: molecular biology clinical relevance. J Natl Cancer Inst 81(12):907–910
Rozema DB et al (2007) Dynamic PolyConjugates for targeted in vivo delivery of siRNA to hepatocytes. Proc Natl Acad Sci U S A 104(32):12982–12987
Saad M, Garbuzenko OB, Minko T (2008) Co-delivery of siRNA and an anticancer drug for treatment of multidrug-resistant cancer. Nanomedicine 3(6):761–776
Santra S et al (2001) Conjugation of biomolecules with luminophore-doped silica nanoparticles for photostable biomarkers. Anal Chem 73(20):4988–4993

Schmidt MM, Wittrup KD (2009) A modeling analysis of the effects of molecular size and binding affinity on tumor targeting. Mol Cancer Ther 8(10):2861–2871
Seino M et al (2016) Time-staggered inhibition of JNK effectively sensitizes chemoresistant ovarian cancer cells to cisplatin and paclitaxel. Oncol Rep 35(1):593–601
Sengupta S et al (2005) Temporal targeting of tumour cells and neovasculature with a nanoscale delivery system. Nature 436(7050):568–572
Shen J et al (2014) Simultaneous inhibition of tumor growth and angiogenesis for resistant hepatocellular carcinoma by co-delivery of sorafenib and survivin small hairpin RNA. Mol Pharm 11(10):3342–3351
Shi M et al (2009) Doxorubicin-conjugated immuno-nanoparticles for intracellular anticancer drug delivery. Adv Funct Mater 19:1689–1696
Shim G et al (2011) Trilysinoyl oleylamide-based cationic liposomes for systemic co-delivery of siRNA and an anticancer drug. J Control Release 155(1):60–66
Shimizu T et al (2012) The clinical effect of the dual-targeting strategy involving PI3K/AKT/mTOR and RAS/MEK/ERK pathways in patients with advanced cancer. Clin Cancer Res 18(8):2316–2325
Shin H-C et al (2011) A 3-in-1 polymeric micelle nanocontainer for poorly water-soluble drugs. Mol Pharm 8(4):1257–1265
Shin H-C et al (2012) Pharmacokinetic study of 3-in-1 poly(ethylene glycol)-block-poly(D, L-lactic acid) micelles carrying paclitaxel, 17-allylamino-17-demethoxygeldanamycin, and rapamycin. J Control Release 163(1):93–99
Skipper HE et al (1954) Observations on the anticancer activity of 6-mercaptopurine. Cancer Res 14(4):294–298
Sokolova V, Epple M (2008) Inorganic nanoparticles as carriers of nucleic acids into cells. Angew Chem Int Ed 47(8):1382–1395, http://doi.org/10.1002/anie.200703039
Song XR et al (2009) Reversion of multidrug resistance by co-encapsulation of vincristine and verapamil in PLGA nanoparticles. Eur J Pharm Sci 37(3–4):300–305
Stylianopoulos T et al (2012) Multistage nanoparticles for improved delivery into tumor tissue. In: D Nejat (ed) Nanomedicine. Academic Press, pp 109–130
Tardi P, Johnstone S et al (2009a) In vivo maintenance of synergistic cytarabine:daunorubicin ratios greatly enhances therapeutic efficacy. Leuk Res 33(1):129–139
Tardi PG, Dos Santos N et al (2009b) Drug ratio-dependent antitumor activity of irinotecan and cisplatin combinations in vitro and in vivo. Mol Cancer Ther 8(8):2266–2275
Tekade RK et al (2008) Surface-engineered dendrimers for dual drug delivery: a receptor up-regulation and enhanced cancer targeting strategy. J Drug Target 16(10):758–772
Tekade RK et al (2009) Exploring dendrimer towards dual drug delivery: pH responsive simultaneous drug-release kinetics. J Microencapsul 26(4):287–296
Thiagarajan G, Greish K, Ghandehari H (2013) Charge affects the oral toxicity of poly(amidoamine) dendrimers. Eur J Pharm Biopharm 84(2):330–334
Tobío M et al (1998) Stealth PLA-PEG nanoparticles as protein carriers for nasal administration. Pharm Res 15(2):270–275
Vaklavas C, Forero-Torres A (2012) Safety and efficacy of brentuximab vedotin in patients with Hodgkin lymphoma or systemic anaplastic large cell lymphoma. Ther Adv Hematol 3(4):209–225
van Vlerken LE et al (2010) Augmentation of therapeutic efficacy in drug-resistant tumor models using ceramide coadministration in temporal-controlled polymer-blend nanoparticle delivery systems. AAPS J 12(2):171–180
Vivero-Escoto J, Elnagheeb M (2015) Mesoporous silica nanoparticles loaded with cisplatin and phthalocyanine for combination chemotherapy and photodynamic therapy in vitro. Nanomaterials 5(4):2302–2316
Wang Y et al (2006) Co-delivery of drugs and DNA from cationic core-shell nanoparticles self-assembled from a biodegradable copolymer. Nat Mater 5(10):791–796

Wei W, Lv P-P, Chen X-M, Yue Z-G, Fu Q, Liu S-Y et al (2013) Codelivery of mTERT siRNA and paclitaxel by chitosan-based nanoparticles promoted synergistic tumor suppression. Biomaterials 34(15):3912–3923, http://doi.org/10.1016/j.biomaterials.2013.02.030

Wilhelm S et al (2016) Analysis of nanoparticle delivery to tumours. Nat Rev Mater 1(5):16014

Williams HD et al (2013) Strategies to address low drug solubility in discovery and development. Pharmacol Rev 65(1):315–499

Wong M-Y, Chiu GNC (2010) Simultaneous liposomal delivery of quercetin and vincristine for enhanced estrogen-receptor-negative breast cancer treatment. Anticancer Drugs 21(4):401–410, http://doi.org/10.1097/CAD.0b013e328336e940

Wu J et al (2007) Reversal of multidrug resistance by transferrin-conjugated liposomes co-encapsulating doxorubicin and verapamil. J Pharm Pharm Sci 10(3):350–357

Xu X et al (2013) Enhancing tumor cell response to chemotherapy through nanoparticle-mediated codelivery of siRNA and cisplatin prodrug. Proc Natl Acad Sci U S A 110(46):18638–18643

Yang J et al (2006) Preparation of poly epsilon-caprolactone nanoparticles containing magnetite for magnetic drug carrier. Int J Pharm 324(2):185–190

Zhang L, Radovic-Moreno AF, Alexis F, Gu FX, Basto PA, Bagalkot V, Jon S, Langer RS, Farokhzad OC (2007) Co-delivery of hydrophobic and hydrophilic drugs from nanoparticle–aptamer bioconjugates. ChemMedChem 2:1268–1271

Zhang N et al (2013) Trastuzumab-doxorubicin conjugate provides enhanced anti-cancer potency and reduced cardiotoxicity. J Cancer Ther 04(01):308–322

Zhang Y et al (2016) Polymeric prodrug grafted hollow mesoporous silica nanoparticles encapsulating near-infrared absorbing dye for potent combined photothermal-chemotherapy. ACS Appl Mater Interfaces 8(11):6869–6879

Zheng C et al (2013) Polypeptide cationic micelles mediated co-delivery of docetaxel and siRNA for synergistic tumor therapy. Biomaterials 34(13):3431–3438

Zhu C et al (2010) Co-delivery of siRNA and paclitaxel into cancer cells by biodegradable cationic micelles based on PDMAEMA-PCL-PDMAEMA triblock copolymers. Biomaterials 31(8):2408–2416

Zoli W et al (2001) In vitro preclinical models for a rational design of chemotherapy combinations in human tumors. Crit Rev Oncol Hematol 37(1):69–82