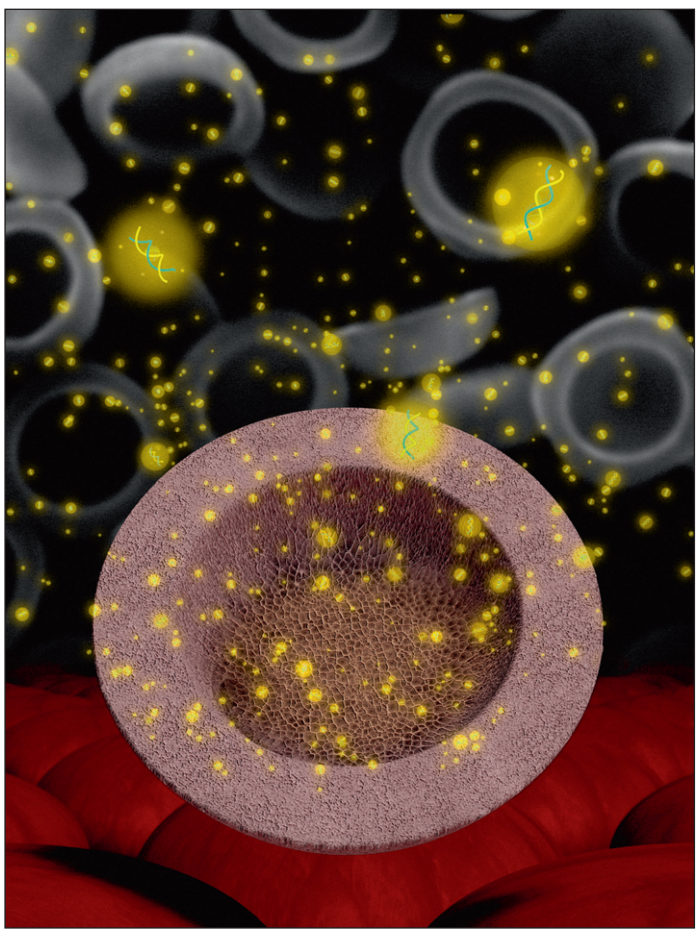
Molecular Oncology

A journal for discovery-driven translational cancer research



Targeted nanomedicine

Ischemia confounds microarray data

Early prediction of increased breast cancer risk

Targeting angiogenesis with FAK inhibitors

www.molecularoncology.org | Volume 5| Issue 6| December 2011

MOLECULAR ONCOLOGY 5 (2011) 492–503

available at www.sciencedirect.com

SciVerse ScienceDirect

www.elsevier.com/locate/molonc

Review

Molecular-targeted nanotherapies in cancer: Enabling treatment specificity

Elvin Blanco[a], *Angela Hsiao*[a], *Guillermo U. Ruiz-Esparza*[a], *Matthew G. Landry*[a], *Funda Meric-Bernstam*[b], *Mauro Ferrari*[a,*]

[a]*Department of Nanomedicine, The Methodist Hospital Research Institute, Houston, TX 77030, USA*
[b]*Department of Surgical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA*

ARTICLE INFO

Article history:
Received 12 October 2011
Accepted 13 October 2011
Available online 25 October 2011

Keywords:
Nanomedicine
Cancer
Molecular therapeutics
siRNA
Liposomes
Polymer micelles

ABSTRACT

Chemotherapy represents a mainstay and powerful adjuvant therapy in the treatment of cancer. The field has evolved from drugs possessing all-encompassing cell-killing effects to those with highly targeted, specific mechanisms of action; a direct byproduct of enhanced understanding of tumorigenic processes. However, advances regarding development of agents that target key molecules and dysregulated pathways have had only modest impacts on patient survival. Several biological barriers preclude adequate delivery of drugs to tumors, and remain a formidable challenge to overcome in chemotherapy. Currently, the field of nanomedicine is enabling the delivery of chemotherapeutics, including repositioned drugs and siRNAs, by giving rise to carriers that provide for protection from degradation, prolonged circulation times, and increased tumor accumulation, all the while resulting in reduced patient morbidity. This review aims to highlight several innovative, nanoparticle-based platforms with the potential of providing clinical translation of several novel chemotherapeutic agents. We will also summarize work regarding the development of a multistage drug delivery strategy, a robust carrier platform designed to overcome several biological barriers while *en route* to tumors.

1. Introduction

Cancer is a major cause of mortality worldwide (Jemal et al., 2011), with significant improvements in detection and therapy yielding modest impacts on patient survival. Chemotherapy represents a mainstay, powerful therapeutic cornerstone in the treatment and management of cancer, successfully supplementing strategies such as surgery and radiation therapy. To highlight the importance of chemotherapy, a recent review and meta-analysis of 17 randomized phase III clinical trials that included 30,672 breast cancer patients demonstrated that weekly administration of paclitaxel significantly diminished the risk of recurrence and mortality following surgical resection (Gines et al., 2011).

In the last century, innumerable compounds were isolated and screened for antitumoral purposes and included agents

* *Corresponding author*. Tel.: +1 713 441 8439; fax: +1 713 441 8235.
E-mail address: mferrari@tmhs.org (M. Ferrari).
1574-7891/$ – see front matter © 2011 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.molonc.2011.10.005

highly varied in origin with equally disparate mechanisms of action. As an example, consider the aforementioned paclitaxel and doxorubicin, one natural and the other from bacterial sources, which effectively kill tumor cells by microtubule hyperstabilization and DNA intercalation, respectively (Czeczuga-Semeniuk et al., 2004; Jin et al., 2010). In spite of these differences, the substantial arsenal of cytotoxic drugs initially employed in chemotherapy shared one common overarching thread, which was their inability to adequately discriminate between normal and tumor cells. Cell-killing was simply "selective" in the sense that these agents targeted rapidly replicating cells. Consequently, this lack of specificity gave rise to substantial morbidity in patients. One classic example is doxorubicin, now well-known to result in cardiotoxicity and acute myeloid leukemia in patients (Azim et al., 2011). As a result, clinicians were forced to accept the existence of an extremely narrow margin separating curative doses from those that resulted in severe toxic side effects.

The last few decades have witnessed a significant surge in the understanding of underlying mechanisms of tumor initiation and progression, shedding light and insights on distinct molecular traits shared among the different cancers. Several of these were elegantly summarized by Hanahan and Weinberg, and include such properties as invasion and metastasis activation, angiogenic potentiation, and sustained proliferative signaling (Hanahan and Weinberg, 2000). Most recently, the list detailing the complexities behind cancer has been updated to include emerging hallmarks such as immune destruction avoidance and energy metabolism reprogramming (Hanahan and Weinberg, 2011). With the elucidation and a more thorough comprehension of these properties, as well as the genomic instability proving causal to these, came the ability to rationally design novel agents that could exploit key molecular targets or dysregulated pathways necessary for tumor cell survival. Their emergence over the last 20 years produced a shift in the chemotherapeutic landscape towards strategies that comprise a highly specific, more targeted mechanism of action than those afforded by traditional drugs. Novel agents such as bevacizumab (Avastin®), have recently surfaced clinically as viable therapeutics that target specific molecules (Figure 1) (Alvarez et al., 2010). A prime example of one such target is the human epidermal growth factor receptor 2 (HER2), a tyrosine kinase receptor essential for signal transduction involved in cell growth and differentiation (Mendelsohn and Baselga, 2000). Overexpression or amplification of HER2 occurs in approximately one-fourth of breast cancer patients, and has been associated with poor prognosis and survival (Morrow et al., 2009). As a result, trastuzumab

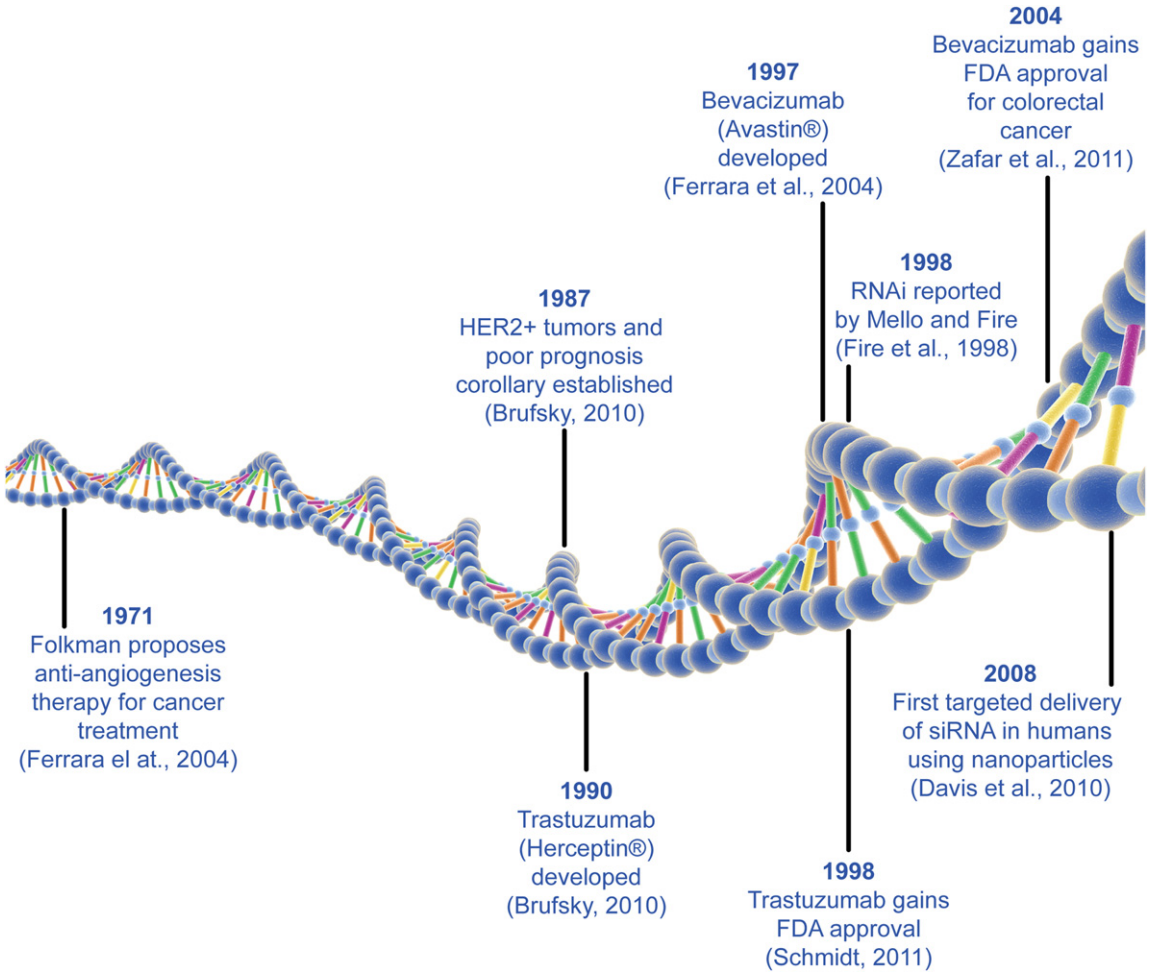


Figure 1 – Timeline of select milestones in molecular therapeutics for cancer treatment.

(Herceptin®), a recombinant monoclonal antibody that binds to HER2, was developed and is clinically used in the treatment of HER2 positive breast cancer patients, resulting in much improved outcomes (Pegram et al., 2000). Several drugs previously unassociated with cancer therapy are undergoing repositioning; explicitly, as more and more insights are discovered, so are the new indication or diseases in which they can be applied (Duenas-Gonzalez et al., 2008). And last but not least, a decade after the discovery of its potential in mammalian cells, RNA interference (RNAi) via small-interfering RNAs (siRNAs) has proven highly successful in its ability to knock down gene expression with immense specificity, ushering in a novel avenue for drug design and therapy (Petrocca and Lieberman, 2011).

With the emergence of novel agents such as trastuzumab and siRNAs, clinicians inch their way closer to Paul Erhlich's concept of a "magic bullet" for cancer therapy, or drugs highly specific for a particular tumor (Strebhardt and Ullrich, 2008). However, simply designing agents with heightened therapeutic affinity for targets is not enough to guarantee adequate treatment, since there is no assurance that this drug will reach its intended site upon injection. The major hindrance to clinical translation is the inability to overcome a number of obstacles present *en route* to the tumor. The most easily appreciable is the reticuloendothelial system (RES), a system comprised of monocytes and macrophages that clear foreign materials from circulation (Peer et al., 2007). Consequently, drug transport in blood is highly non-specific, accumulating in healthy organs, and resulting in lower tolerability and severe morbidity. Moreover, there are physical, biological barriers that exist in the body, obstacles oftentimes arising from tumorigenesis, that nonetheless prevent the drug from reaching its intended site (Michor et al., 2011). These include interstitial pressure gradients, abnormal blood flow in tumor microenvironments, and the existence of membranes to name a few (Ferrari, 2010a). The presence of these biobarriers indeed impedes drugs from reaching their intended destination at doses necessary to elicit an efficacious response.

Currently, the field of nanomedicine has given rise to several nanoscale (1–100 nm) platforms for biomedical applications that improve the delivery of both traditional and emerging therapeutics, essentially blazing a trail towards clinical translation of drugs deemed otherwise too toxic for systemic administration (Ferrari, 2005). Liposomes and polymer-drug conjugates were among the first nanoplatforms developed for these purposes, and are widely utilized in clinics (Duncan and Gaspar, 2011). Since their development, several different particle types have emerged with distinct properties, all the while maintaining the characteristics that make them ideal carrier systems. Nanoparticles either encapsulate or engraft the anticancer agent within a core, where the drug is protected from degradation and its solubility increased. The small size of nanoparticles aids in their evasion of the RES, and thus, results in longer circulation times. Chemically modifying the surface of nanoparticles with hydrating polymers such as poly(ethylene glycol) (PEG) also contributes to longer circulation times of the nanoparticle in the bloodstream. This sustained intravascular presence allows drugs to accumulate in tumors through the enhanced permeability and retention (EPR) effect, a transport phenomena arising from the presence of fenestrations in tumor blood vessels (Maeda, 2001). This heightened tumor accumulation, coupled with the inability to distribute evenly to all organs, results in less toxic side effects in patients and potentially heightened antitumor efficacies.

Over the last three decades, significant improvements in patient outcomes can be linked directly to the evolving therapeutic landscape. Herein, we aim to highlight significant strides towards the clinical translation of highly specific, molecular-based drugs for cancer therapy. Advances in nanomedicine stand to make immense contributions towards achieving this end in the safest and most efficacious manner, and as such, will constitute the main focus of this review. The innovative strategies and platforms presented herein will surely usher in a new era of chemotherapy, one that consists of tailor-made regimens best-suited for a specific tumor type, combined with enhanced delivery mechanisms that ensure maximal efficacy and reduced toxicity.

2. Traditional nanoparticle platforms and their applications in molecular therapy

2.1. *Liposomes*

Liposomes are spherical, bilayered membrane nanoconstructs with diameters typically approximating 100 nm (Figure 2A) (Torchilin, 2005). Composed of phospholipids with hydrophilic heads and hydrophobic long-chain tails, their morphology consists of an aqueous core surrounded by a hydrophobic membrane; the core capable of encapsulating a broad range of hydrophilic chemotherapeutic agents, including siRNA. Liposomes have the distinction of being the first nanoparticle platform to be approved by the FDA for clinical use, with a PEGylated liposomal formulation of doxorubicin (DOXIL®) gaining approval for treatment of Kaposi's sarcoma in 1995 (Gabizon et al., 2003; Gabizon, 2001). This formulation not only increased the circulation time of doxorubicin, but also diminished cardiotoxicity and patient morbidity (Hamilton et al., 2002). Following administration of liposomal doxorubicin once every three weeks in patients with Kaposi's sarcoma, 19 of 53 patients showed a partial response and one a complete response (Northfelt et al., 1997).

Since its arrival in the clinical arena, liposomal doxorubicin has been used as a vital component in various drug regimens, including those employing novel, emerging molecular therapies. Recently, liposomal doxorubicin was examined in a multicentre phase II study in combination with docetaxel and trastuzumab (Venturini et al., 2010). In the trial, thirty-one patients with metastatic HER2 positive breast cancer received liposomal doxorubicin, docetaxel, and trastuzumab for up to eight cycles, after which trastuzumab was administered alone for up to 52 weeks. The regimen was shown to have minimal cardiotoxicity and general side effects, and demonstrated promising overall response rates in patients with metastatic breast cancer.

Despite its main function as a therapeutic, trastuzumab and its analogs also serve as highly efficient targeting moieties for chemotherapeutic-containing nanoparticles (Colombo

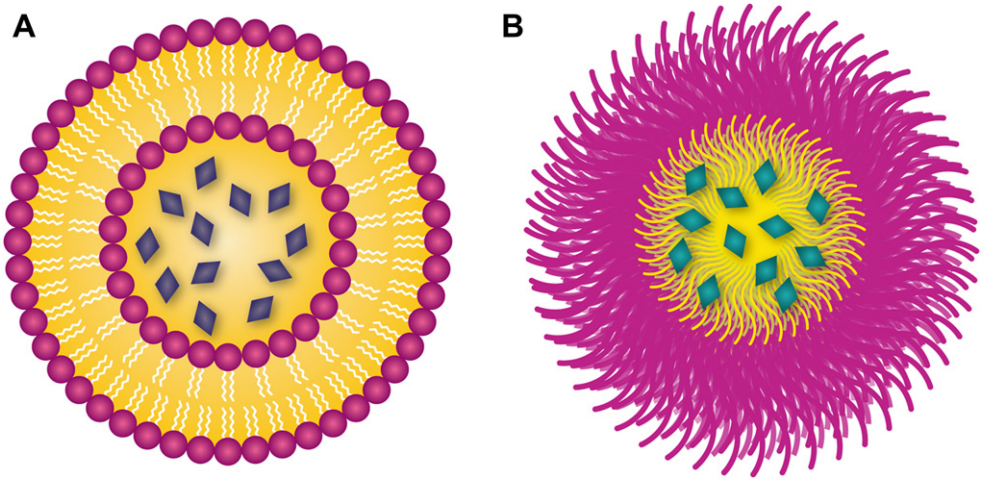


Figure 2 – Nanoparticle platforms for molecular therapy of cancer. A) Liposomes comprised of a lipid bilayer membrane encasing an aqueous core capable of encapsulating water-soluble agents. B) Polymer micelles consisting of a hydrating corona and hydrophobic core that can accommodate water insoluble drugs.

et al., 2010). A liposomal formulation encapsulating doxorubicin was developed to enhance targeting to HER2-overexpressing breast tumors by attaching several different MAb fragments, including a recombinant Fab' derived from trastuzumab (Park et al., 2002). Following experiments that demonstrated superior binding and internalization of anti-HER2 liposomes in HER2-overexpressing cells *in vitro*, Benz and coworkers examined the pharmacokinetics and antitumor activity of targeted liposomes in rat models. Results demonstrate that targeted liposomes had long circulation times, analogous to non-targeted liposomes, depicting a biphasic plasma profile for doxorubicin, with terminal half-lives approximating 12 h. When administered to rats intravenously at total doxorubicin doses of 15.0–22.5 mg/kg over three weeks, targeted doxorubicin liposomes yielded significant antitumor efficacy in four different HER2-overexpressing tumor models over controls that included free doxorubicin, liposomal doxorubicin, and trastuzumab alone. Cures were observed in 11 of 21 mice bearing BT474/SF tumors that received targeted liposomes, compared to no cures observed in control groups consisting of free doxorubicin or liposomal doxorubicin. In a more recent study, anti-HER2 liposomes were developed that contained topotecan, a topoisomerase I inhibitor (Drummond et al., 2010). Following functionalization with an anti-HER2 scFv F5 antibody, the liposomal formulation was intravenously administered to mice bearing BT474 breast tumors, in a treatment regimen consisting of 5 mg/kg of liposomes on days 14, 18, and 21 after tumor implantation. The targeted liposomal formulation proved highly efficacious, yielding 2-fold and 5-fold differences in antitumor activity compared to non-targeted liposomes and free topotecan, respectively, after the course of 53 days.

Bevacizumab is a recombinant humanized monoclonal antibody that inhibits VEGF, a growth factor ligand responsible for angiogenesis (Grothey and Ellis, 2008). Results from several phase III clinical trials comprising colorectal, non-small cell lung, and breast cancer demonstrate that bevacizumab results in superior patient response rates, and was FDA approved as a chemotherapeutic strategy in combination with several drugs such as paclitaxel (Gonzalez-Angulo et al., 2011). As in the case with trastuzumab, bevacizumab can be used as a targeting moiety to enhance the efficacy of nanoparticles. To this effect, Campbell and coworkers developed bevacizumab-labeled cationic liposomes to improve targeting to several pancreatic cancer cell lines including Capan–1, HPAF–II, and PANC–1 (Kuesters and Campbell, 2010). Bevacizumab-conjugated liposomes had modest impacts on cell viability *in vitro*, an effect shown to be cell-type specific, and demonstrated increased cellular uptake by PANC–1 cells grown in the presence of VEGF. Biodistribution studies in SCID mice showed that targeted liposomes had reduced uptake in the spleen and an approximate two-fold higher concentration in blood and Capan–1 tumors after 24 h, highlighting the potential of bevacizumab as a viable targeting ligand for nanoparticle-based chemotherapeutics in pancreatic cancer.

Small-interfering RNA fragments have been found to suppress gene expression with immense silencing efficiency and relatively low toxicity compared to traditional chemotherapeutics. siRNAs are 19–21 base-paired double-stranded RNA that post-transcriptionally silence genes, exerting effects at the translation level in the cell cytoplasm, its therapeutic target being mRNA (Figure 3) (Elbashir et al., 2001). Highly specific in their mechanism of action, they are capable of silencing regulators of oncogenesis at extremely low (picomolar to nanomolar) concentrations *in vivo*. While an exciting avenue for chemotherapy, siRNAs degrade extremely rapidly in physiological environments and are eliminated almost immediately from circulation upon injection (Zhang et al., 2006). Liposomes prove ideal carriers for biological agents such as siRNA because of their stable aqueous core. Moreover, it is possible to combine RNA-interfering strategies with traditional chemotherapeutics, as well as novel agents targeting unique pathways found dysregulated in cancers. One example is the Raf/MEK/extracellular signal-related kinase (ERK) pathway, which is essential for cellular proliferation, and found to be aberrant in

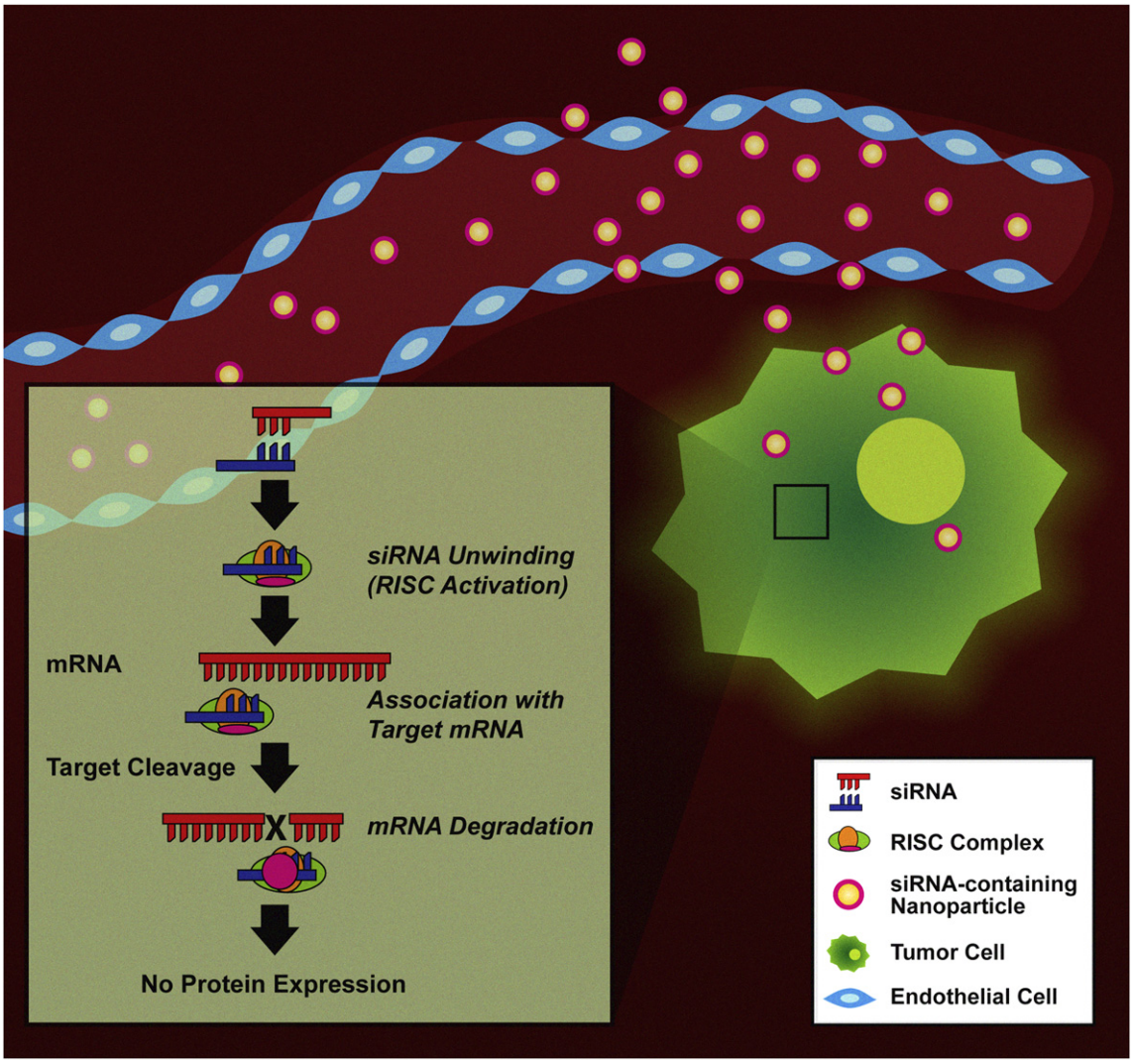


Figure 3 – Cellular-level schematic of nanoparticle-based delivery of siRNA. Nanoparticles provide biocompatible carrier platforms that protect siRNAs from degradation, increase circulation lifetime, and enhance accumulation in the tumor microenvironment. Once internalized in the cell, siRNA binds to the RISC complex and undergoes unwinding. The antisense RNA in complex with RISC binds to the corresponding mRNA, undergoing cleavage by the enzyme slicer, after which the mRNA is rendered inactive.

several cancers (Ciuffreda et al., 2009). As a result, several inhibitors of key proteins in the cascade have been developed as potential chemotherapeutics. Oh and coworkers developed liposomes encapsulating a Mcl1-specific siRNA (siMcl1) and a chemical MEK inhibitor (PD0325901), and examined the *in vitro* and *in vivo* antitumor efficacy of the platform (Kang et al., 2011). Following encapsulation and complexation of PD0325901 and siMcl1, respectively, the liposomal formulation was administered to KB cells. Western blot results showed that co-delivery of both agents significantly reduced expression of Mcl1 and pERK1/2 proteins, while *in vitro* growth inhibition assays showed a reduction in cell survival with the combination treatment. In mouse models of KB tumors, liposomes were administered intratumorally at a dose of 0.7 mg/kg siRNA and 0.72 mg/kg PD0325901 every other day for five total injections. Liposomes were found to significantly suppress tumor size (79% compared to controls), with western blots of extracted tumors showing *in vivo* gene-target silencing.

Antisense therapy represents a gene silencing strategy that stands to make a profound impact on cancer therapy, a strategy warranting nanoparticulate delivery for enhanced efficacy. In this approach, antisense oligonucleotides bind to complementary and specific mRNAs, rendering them inactive (Crooke, 2004). Kasid and coworkers examined, in a phase I clinical trial, a liposomal formulation, LErafAON, that encapsulates the *raf* antisense oligonucleotide, administered with the purpose of acting on c-*raf*, a protein that bestows cancer cells with resistance to radiation or chemotherapy (Dritschilo et al., 2006). The liposomal strategy is thus meant to serve as an adjuvant therapy that allows for sensitization of tumors to radiation therapy. In the trial, where the platform was administered to patients with advanced solid tumors undergoing radiation therapy, the c-*raf*-1 mRNA was inhibited in three of five evaluable patients, with a 2.0 mg/kg administered dose twice a week found to be well tolerated without producing severe radiation toxicity. Out of 12 evaluable patients, four

exhibited partial response, four had stable disease, and four showed progressive disease. From the partial response and stable disease patients, five were evaluable for c-*raf*-1 mRNA or Raf-1 protein expression. Three of the five patients exhibited c-*raf*-1 mRNA inhibition and four out of the five exhibited Raf-1 protein inhibition (Dritschilo et al., 2006; Moreira and Simoes, 2003).

The protein tyrosine kinase family also represents a novel avenue for targeted therapy, with enzymes pertaining to the src pathway comprising viable targets for inhibition. These kinases are essential in signaling pathways responsible for several cellular events including proliferation, secretion, and adhesion during tumorigenesis (Kim et al., 2009). Pyrazolopyrimidine derivatives have been shown to effectively inhibit c-src activity in several cancers including breast and thyroid. Given the immense potential of these agents, liposomal nanoparticles encapsulating Si 34, a novel compound with structural similarities to pyrazolopyrimidine derivatives, were developed with the hope of inhibiting epidermal growth factor (EGF)-stimulated src activation in the ARO thyroid cancer cell line (Celano et al., 2008). Both drugs were shown to greatly suppress growth of cells in a dose-dependent fashion, with a 72 h exposure showing a pronounced suppression of cyclin D1 expression. Upon encapsulation in liposomes, dose-dependent cell-killing was observed, albeit, at lower doses and shorter exposure times for liposomes than for free drugs. Further examination revealed apoptotic-cell death and significant reduction of EGF-induced migration after 48 h incubation. Moreover, an early onset of c-src and ERK phosphorylation inhibition was observed with Si 34 liposomes in ARO cells incubated with EGF. The antitumor effect of the liposomal formulation was examined *in vivo* in immunodeficient (NOD-SCID) mice bearing subcutaneous ARO cells. Following daily intravenous administration of 25 or 50 mg/kg of Si 34 liposomes during the course of 3 weeks, a 6-fold difference in tumor growth was observed between liposome-treated and control mice, highlighting the potential of pyrazolopyrimidine derivatives for chemotherapy.

Recently, there has been a major effort to target metastasis and tumor cell dissemination in sites such as bone marrow, the most common site of breast cancer metastasis. To this effect, the use of bisphosphonates, such as zoledronic acid, was explored as a treatment strategy, given its ability to inhibit the release of growth factors essential for cancer cell growth and differentiation in bone (Gnant, 2011). Emerging data from several clinical trials serves to highlight a potential anticancer effect of zoledronic acid, as well as chemotherapeutic synergy with established drugs (Ressler et al., 2011). While promising, zoledronic acid has an extremely rapid blood clearance and preferential accumulation in bone, necessitating encapsulation in nanoparticles. De Rosa and coworkers developed LipoZOL, a liposomal formulation of zoledronic acid, to increase circulation times, reduce accumulation in bone, and increase targeting to tumors (Marra et al., 2011). Resulting liposomes measured 200–240 nm in diameter, with a loading efficiency of zoledronic acid of ~75%. Confocal microscopy experiments demonstrated efficient uptake of liposomes in PC3 and LNCaP prostate cancer cells, and demonstrated potent growth inhibition in various cancer cell lines *in vitro*. *In vivo* studies were conducted in male mice injected with human PC3 cells, with liposomes administered intravenously 3 times per week for 3 weeks at zoledronic acid doses of 10 or 20 μg. Mice treated with 10 or 20 μg of zoledronic acid alone had tumor regressions of 16% and 22%, respectively, compared to a control group consisting of empty liposomes. In contrast, mice treated with 10 or 20 μg of LipoZOL showed tumor growth suppressions of 58% and 68%, respectively, in comparison to controls. Moreover, mice treated with LipoZOL had a tumor growth delay of 20–23 days compared to 1–2 days for all other groups, drastically increasing the overall survival of mice from 3 days to 31–47 days. Last but not least, LipoZOL was found to inhibit tumor-vessel formation, suggesting an anti-angiogenic effect in PC3 tumors.

2.2. *Polymer micelles*

Polymer micelles are supramolecular, spherical constructs formed from the self-assembly of amphiphilic-block copolymers in aqueous environments, ranging in size from 10 to 100 nm in diameter (Figure 2B) (Kataoka et al., 2001). The ensuing core-shell morphology of polymer micelles, consisting of a hydrophobic core and hydrophilic shell, makes them ideal carrier particles for lipophilic drugs (Blanco et al., 2009). Their chemical composition, which typically includes a hydrophilic block consisting of PEG, also opens several avenues for customization and functionalization (Murakami et al., 2011). As an example, targeting ligands can be incorporated on the corona-forming PEG, while specialty polymers can be incorporated for purposes of tailored drug release (Sutton et al., 2007). While lagging slightly behind liposomes in terms of clinical translation, polymer micelles of varying formulations and encapsulated drugs are currently being explored in several different clinical trials. Genexol-PM, a paclitaxel-containing micellar formulation composed of PEG-PLA, showed an overall response rate of 58.5%, with 19 partial responses and 5 complete responses in 41 patients, in a metastatic breast cancer phase II clinical trial where micelles were administered at a dose of 300 mg/m^2 every 3 weeks (Lee et al., 2008).

As in the case with liposomes, the antitumor effect of polymer micelles has been explored in combination with novel, molecular-targeted agents. Recently, Matsumura and coworkers explored the potential synergy and antitumor activity of NK012, a 7-ethyl-10-hydroxycamptothecin (SN-38) micellar formulation, and bevacizumab in human lung cancers (Kenmotsu et al., 2010). Nude mice bearing PC-14 or A549 lung adenocarcinoma xenografts, were administered at doses of 5 mg/kg and 30 mg/kg, respectively, with or without bevacizumab at a dose 5 mg/kg. At these combined doses of NK012 and bevacizumab, significant tumor growth inhibitions of 10-fold and 5-fold compared to saline controls were observed in PC-14 and A549 tumors, respectively, after 10 days. Moreover, the combination was shown to significantly suppress tumor growth compared to treatment groups receiving either agent alone.

Monoclonal antibodies like bevacizumab and trastuzumab can also be attached to polymer micelles for use as targeting ligands for enhanced treatment efficacy and imaging. Recently, quantum dots (QDs) have received considerable attention as powerful molecular imaging moieties given their fluorescence stability, high quantum yields, and tunable emission wavelengths (Smith et al., 2008). In an attempt to increase

the selective cellular uptake of near-IR QDs for imaging purposes, Lee and coworkers encapsulated near-infrared QDs within PEG-pentacosadiynoic acid (PCDA) polymer micelles functionalized with trastuzumab (Nurunnabi et al., 2010). The water-soluble, functionalized QD micelles were in the size range of 130–460 nm, were highly stable, and were shown to induce a dose-dependent effect on cell viability in KB and SK-BR3 cells, albeit with an enhanced effect in the latter cell line. Confocal experiments indeed demonstrated that increased uptake of trastuzumab-functionalized QD micelles occurred in SK-BR3 compared to KB cells. In light of these findings, the antitumor activity of this micellar platform was explored in female BALB/c-nu/nu mice bearing subcutaneous HER2-positive MDA-MB-231 tumors, in which treatment consisted of a single i.v. injection of a 10 mg/kg dose of QD micelles. Findings demonstrate that the functionalized micelles effectively suppressed tumor growth by approximately 6-fold compared to saline controls after 21 days, with the mechanism, while unclear, most likely relying on the presence of trastuzumab.

As mentioned previously, novel drugs from varying sources are emerging as powerful chemotherapeutic candidates. Interestingly, these repositioned drugs target specific enzymes or pathways, eliciting cell-killing effects that are far removed from the conventions of traditional chemotherapeutics. As an example, β-lapachone (β-lap) is a novel anticancer agent whose cell-killing effect is "bioactivated" by the enzyme NAD(P)H:quinone oxidoreductase 1 (NQO1), a flavoprotein found overexpressed in several cancers, including breast, prostate, and lung (Bey et al., 2007). In cancer cells where NQO1 is overexpressed, the agent undergoes futile cycling resulting in the generation of reactive oxygen species (ROS). ROS in turn lead to DNA single-strand breaks, hyperactivation of poly(ADP-ribose) polymerase-1 (PARP-1), and loss of NAD+ and ATP (Pink et al., 2000). *In vitro* experiments demonstrated that growth inhibition occurs in cells overexpressing NQO1 after a 2 h exposure, while cells in which NQO1 is absent are unaffected at equivalent concentrations. And while these results are promising, the insolubility of β-lap encumbers its clinical translation. Hence, Gao and coworkers developed polymer micelles encapsulating β-lap for treatment of non-small cell lung cancer (Blanco et al., 2010). Resulting micelles possessed diameters of ~30 nm, proved highly stable, and released drug in a biphasic manner over 96 h (Blanco et al., 2007). Micellar encapsulation increased the circulation time of β-lap, with an elimination phase half-life of 28 h, and yielded relatively high levels of tumor accumulation compared to other organs. Antitumor efficacy was examined in female nude mice bearing subcutaneous A549 lung tumors and orthotopic Lewis lung carcinoma. Following intravenous administration of a 50 mg/kg dose β-lap micelles every other day (total of 5 injections), A549 tumor growth suppression was apparent, with an approximate 2.5-fold difference observed between treatment and vehicle (empty micelle) controls. In the Lewis lung carcinoma model, an equivalent dose and dosing regimen resulted in a doubling of survival (16 days compared to 8 days in controls) in an otherwise very aggressive lung tumor model (Blanco et al., 2010).

Another viable target for molecular therapy in cancer is heat shock protein 90 (HSP90), a molecular chaperone which under normal conditions is responsible for prevention of protein aggregation (Khong and Spencer, 2011). HSP90 becomes overexpressed under conditions of stress, resulting in tumorigenesis and increased proliferation in a variety of cancers including lung, prostate, and breast. Tanespimycin, a derivative of the HSP90 inhibitor geldanamycin, has been explored clinically for chemotherapeutic purposes (Whitesell and Lindquist, 2005). The mechanism of action of tanespimycin involves the degradation of oncogenic signaling proteins, inducing cell death via apoptosis. In a phase I dose escalation study in twenty-nine patients with relapsed and refractory multiple myeloma, tanespimycin was administered on days 1, 4, 8, and 11 of a 3 week cycle, at doses progressively increased from 150 to 525 mg/m^2 (Richardson et al., 2010). Disease stabilization was observed in 11 of 16 patients who received the three lowest doses, and 5 of 13 patients who received the three highest doses. While effective, tanespimycin is not without dose limiting toxicity, and is still sparingly soluble in aqueous environments. Ghandehari and coworkers developed poly(styrene-co-maleic acid) (SMA) polymeric micelles encapsulating tanespimycin, and examined the *in vitro* and *in vivo* efficacy in prostate cancer models (Larson et al., 2011). Micelles measured approximately 75 nm in diameter, efficiently encapsulated tanespimycin (~93% loading efficiency), and released ~60% of the drug after 24 h. In nude mice bearing subcutaneous DU145 human prostate tumors, a single injection at a dose of 10 mg/kg yielded significant tumor growth suppression, with mean tumor volumes of treated mice remaining essentially unchanged from their initial volume of 100 mm^3. Contrastingly, tumors in mice treated with saline and free tanespimycin reached average volumes of 300 mm^3 by day 16.

As mentioned previously, for siRNA to be effective in cancer therapy, it must be stably encapsulated in nanoparticles that can reach tumors and undergo uptake by cancer cells, leading many to explore micellar technology as an avenue for siRNA delivery. Park and coworkers explored the use of a polyelectrolyte-based micellar VEGF siRNA delivery platform for treatment of prostate cancer (Kim et al., 2008). The micelles were approximately 99 nm in diameter, were shown to effectively inhibit VEGF expression in PC-3 cells *in vitro*, and were long circulating in the blood with adequate accumulation levels in tumors. The antitumor efficacy was examined *in vivo* in female nude mice bearing subcutaneous PC-3 tumors. The dosing regimen consisted of an injection of 1.5 nmol of siRNA on days 0, 4, 10, 18, and 28. Naked siRNA failed to have any effect on tumor growth, likely due to degradation effects. In contrast, VEGF siRNA-containing polymer micelles had an approximate 86% inhibitory effect compared to no treatment controls. Quantitative examination of microvessel density served to highlight the silencing effect that the micellar formulation had on microvessel formation, resulting in an approximate 78% reduction compared to no treatment controls.

3. CALAA-01 RNAi nanoparticles: clinical evidence of successful molecular therapy using targeted nanomedicine

In 2008, a team led by Mark Davis became the first to systemically deliver targeted nanoparticles containing siRNA in

a phase I clinical trial in human patients with solid cancers (Figure 1) (Davis et al., 2010). The nanoparticles consisted of a linear, cyclodextrin-based polymer (CDP), transferrin (Tf) protein targeting ligands, and anti-R2 siRNAs which interact with the cationic polymer, effectively protecting the siRNA, and producing nanoparticles less than 100 nm in diameter (Davis, 2009). In preclinical trials, the nanoparticle platform was administered to cynomolgus monkeys at doses of 3, 9, and 27 mg siRNA/kg over 17 days. Administered doses of 3 and 9 mg siRNA/kg were well tolerated but at 27 mg siRNA/kg, elevated levels of blood urea nitrogen and creatinine were present, suggesting possible kidney toxicity. Overall, there were no signs of toxicity that were overtly due to the treatment, with the preclinical study demonstrating that multiple, systemic doses of targeted nanoparticles containing siRNA could be safely administered to non-human primates (Heidel et al., 2007).

In the phase I clinical trial, patients with solid cancers were administered four 30 min intravenous injections of the siRNA platform on days 1, 3, 8, and 10 in a 21-day cycle (Davis et al., 2010). After 11 days of rest, a second 21-day cycle was administered, if deemed appropriate. To determine whether the targeted nanoparticle system could deliver the siRNA to the tumor site in humans, biopsies from 3 patients were analyzed after the final dose of cycle 1 and compared to their pretreatment archived tissue. Presence of nanoparticles in tumor cells was evident by staining for nanoparticles in tissue and confirmed via TEM analysis. Most importantly, the presence of an mRNA fragment was detected in a patient who underwent administration with the highest dose, demonstrating that siRNA-mediated cleavage occurred specifically where expected.

4. Multistage drug delivery: overcoming barriers to enable molecular therapy

We have developed a novel multistage platform for the delivery of chemotherapeutic agents, including emerging molecular therapeutics. The approach stems from the realization of mass transport differentials encountered in cancer, and the need to apply an understanding of "oncophysics" to overcome several of these biobarriers (Ferrari, 2010a). Therefore, our proposed multistage drug delivery strategy is designed in such a way to successfully circumnavigate several of the intricate biobarriers encountered by nanoparticles on their journey to tumor sites (Ferrari, 2008; Tasciotti et al., 2008). The platform aims to enhance site-specific delivery and release of therapeutics in tumors by encapsulating drug-containing nanoparticles within a carrier construct comprised of mesoporous silicon particles (MSPs) (Figure 4A). The multistage platform consists of the following components: 1) mesoporous silicon particles that house; 2) nanoparticles containing; 3) anticancer therapeutics. Porous silicon, on account of its biocompatibility, biodegradability, and FDA approval for clinical use, was chosen as the constituent material of the carrier particle. Photolithographic masks dictate the geometry and dimensions of the MSPs, while chemical composition and anodization conditions are used to tailor pore dimensions and porosity. It is now possible to obtain carrier particles ranging from 500 nm to 1.6 μm in size, in shapes that vary from hemispherical to discoidal, with mean pore sizes of 5–80 nm (Chiappini et al., 2010; Tasciotti et al., 2008). The large size of these particles makes them ideal for encapsulation of a large payload of nanoparticles, ranging from liposomes to carbon nanotubes. By combining *in silico* mathematical modeling with *in vitro* and *in vivo* experimentation, MSPs were rationally designed with ideal geometries and sizes for minimization of RES uptake and maximization of tumor uptake (Decuzzi et al., 2009). Moreover, the MSPs were designed to maximize margination, firm adhesion, and cellular internalization. Parallel-plate flow chamber experiments show that under controlled hydrodynamic conditions, non-spherical particles were not found in the center of blood flow, as in the case for spherical particles, but were found to drift laterally. This phenomenon in turn might increase potential interactions with vessel walls, including adherence and receptor-ligand interactions. Upon injection into mice bearing MDA-MB-231 breast tumors, hemispherical particles accumulated the highest in tumors compared to spherical, discoidal, and cylindrical particles (Decuzzi et al., 2010). In summary, findings regarding vessel margination and heightened accumulation in tumor tissue favor site-specific drug delivery of chemotherapeutics.

Upon arrival at the tumor site, MSPs undergo cellular internalization, with several MSP properties, such as chemical surface charge, vastly affecting cellular interactions. In an *in vitro* study where MSPs were administered to vascular endothelial cells, the cells were shown to rapidly internalize both negatively and positively charged particles, albeit, with positively-charged particles undergoing more internalization following serum opsonization (Serda et al., 2009). In regards to release of contents, nanoparticle release from MSPs was shown to be highly dependent on degradation kinetics of the MSP, which in turn is determined by degree of porosity. Results show that MSPs with a high degree of porosity degrade in a matter of hours, releasing their contents rapidly. In contrast, MSPs with low porosity take several days to degrade, resulting in sustained release of therapeutic agents over time. Moreover, degradation can also be modulated by functionalizing the surface of the MSP with materials such as PEG, which in turn affects release kinetics (Godin et al., 2010).

Given its ability to overcome biological barriers, successfully marginate to endothelial walls, effectively protect its cargo, and release its contents in a sustained fashion, the multistage delivery platform was hypothesized to be a promising vehicle for the *in vivo* translation of siRNA therapeutics (Ferrari, 2010b). The antitumor efficacy of the multistage drug delivery platform was examined *in vivo* in a mouse model of ovarian cancer, wherein liposomes containing an siRNA against EphA2, an oncogenic tyrosine kinase receptor, were encapsulated within MSPs (Tanaka et al., 2010). MSPs were successfully loaded with siRNA-containing liposomes, and the release of siRNA was found to be sustained over the course of weeks in physiological conditions. Mice were administered siRNA-containing liposomes twice a week for 3 weeks at a dose of 5 μg EphA2-siRNA, while a separate set of mice were given a single administration of multistage EphA2-siRNA-liposomes at the start of treatment at a dose of 15 μg EphA2-siRNA. Pronounced gene silencing of EphA2 protein in SKOV3ip1 tumors was observed after a single injection of

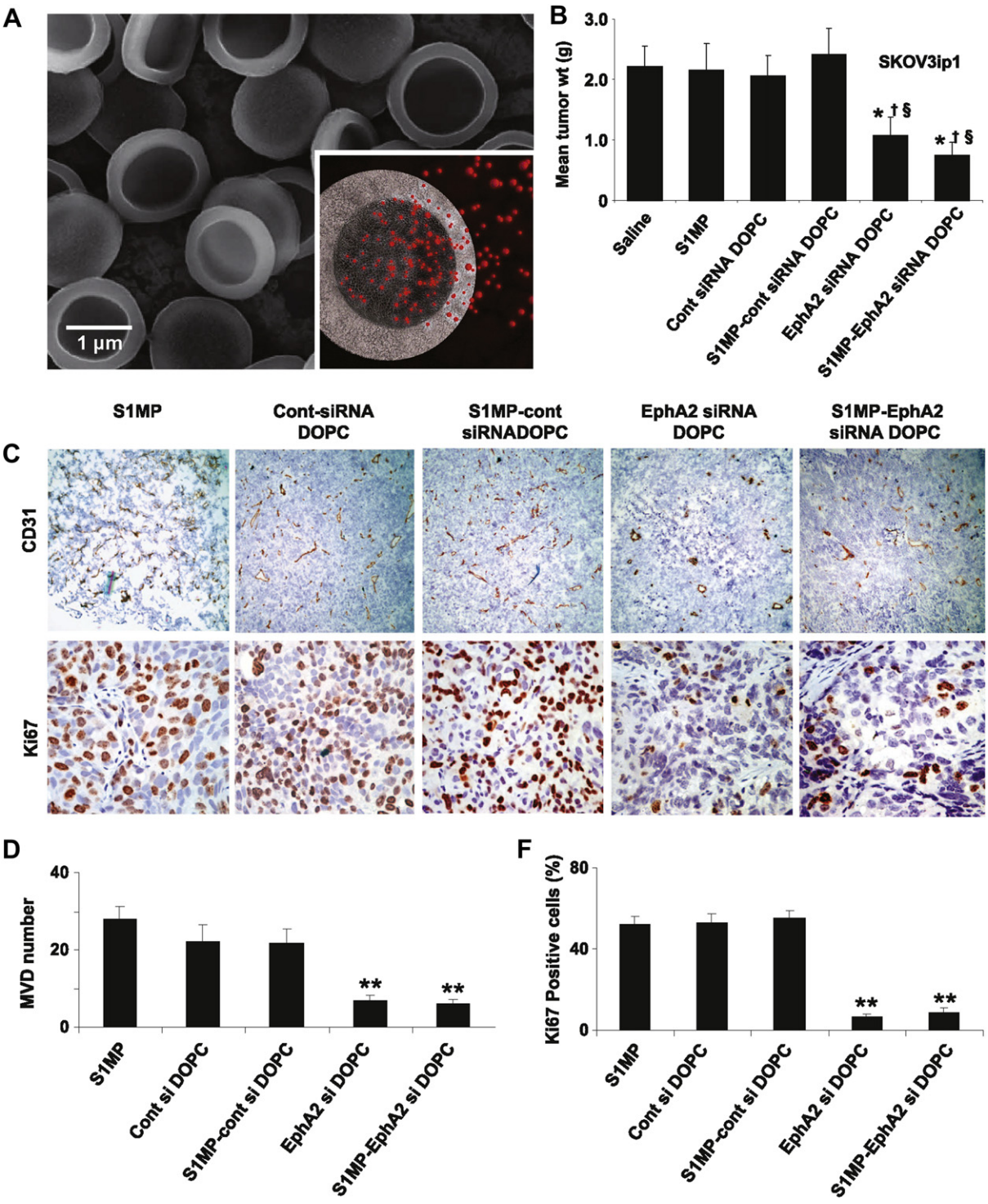


Figure 4 — siRNA-containing multistage particles for molecular-targeted cancer therapy. A) Scanning electron microscopy (SEM) image of mesoporous silicon particles, depicting the size and geometry of the platform. The inset represents a schematic of MSPs releasing drug-containing nanoparticles. B) *In vivo* antitumor efficacy of EphA2 siRNA-containing multistage particles in SKOV3ip1 cells. siRNA-liposomes (DOPC) were administered twice a week over the course of 3 weeks at a dose of 5 μg siRNA. Multistage particles (S1MP) were injected once at the start of the study at a dose of 15 μg siRNA. Statistical significance ($p \leq 0.05$) is represented by asterisks. C) and D) constitute representative immunohistochemistry images of CD31 and Ki67 stained tissues, respectively. DOPC and S1MP denote EphA2 siRNA-containing liposomes and MSPs, respectively. Dosing regimens were the same as previously mentioned. E) and F) represent quantitative estimates of mean number of vessels per field (CD31) and mean % of proliferative cells (Ki67), respectively, from representative sections of each treatment group. All figures are courtesy of AACR Publications.

the multistage siRNA formulation during the course of the three weeks, resulting in heightened tumor growth inhibition. As is evident in Figure 4B, an approximate 54% and 65% reduction in tumor weight occurred in mice treated with siRNA multistage particles compared to nonsilencing control siRNA-liposomes and multistage-non-silencing control-siRNA-liposomes, respectively. Interestingly, the antitumor efficacy of a single administration of multistage siRNA was comparably as effective, if not more, than repeated injections of EphA2-siRNA-liposomes. Downstream effects of sustained downregulation of EphA2, including microvessel density (CD31) and cell proliferation (Ki67), resulting from multistage siRNA treatment were examined in SKOV3ip1 ovarian tumors, revealing further insights into gene silencing and the antitumor effects observed. As is apparent in the representative immunohistochemistry sections comprising Figure 4C and D, there is a marked difference between CD31-positive vessels between multistage treatment and control groups, as well as between total numbers of Ki67-positive cells, respectively. Following quantification of microvessel density (CD31) and cell proliferation (Ki67), it is apparnet that mean microvessel density was significantly reduced by ~3-fold in tumors treated with multistage siRNA (Figure 4E). Figure 4F shows that the proliferation index was also significantly reduced by 20-fold when mice were treated with the multistage formulation.

5. Conclusions

Chemotherapy remains a vital cornerstone of cancer treatment, proving adjuvantly indispensable to patient survival. Initially, chemotherapeutics consisted of agents with immense cell-killing potential, but whose mechanism of action was incapable of distinguishing between malignant and healthy cells. Recently, chemotherapeutics have evolved into more specific agents as novel insights into key molecular targets emerged. Targeted biologics, including therapeutic antibodies and siRNAs, as well as repositioned drugs found to act on dysregulated pathways, are now being explored in clinics, either alone or in combination with preexisting chemotherapeutics. Despite significant improvements, the impact on patient survival remains modest and far from acceptable, with adequate, site-specific delivery even now lingering as a major hindrance to efficacious therapy. Nanomedicine is currently enabling the use of novel and traditional drugs otherwise deemed unsuitable for intravenous administration. Liposomes and polymer micelles have proven effective at solubilizing anticancer agents for injection into the bloodstream, leading to increments in drug half-life. Innovative strategies, such as the multistage drug delivery platform described herein, have the potential to overcome several of the biological barriers involved in drug delivery. By rationally designing the carrier with regards to size and geometry, the pharmacokinetics can be altered in such a way that enhances tumor accumulation. Moreover, the platform stably protects drugs, site-specifically releasing, in a sustained fashion, potentially a multitude of different agents encapsulated within the carrier particle. The latter property will prove important in the future, when insights into biomarkers and proteomics will allow clinicians to tailor drug regimens to specific tumor types, offering rationally-designed chemotherapy of a more personalized nature.

Acknowledgments

The authors gratefully acknowledge funding from DOD/BCRP (W81XWH-09-1-0212) awarded to MF. EB gratefully acknowledges support from postdoctoral fellowships from DOD/BCRP (W81XWH-11-1-0103) and the Susan G. Komen Breast Cancer Foundation (KG101394). MF serves on the Board of Directors of Arrowhead Research Corporation (NASDAQ:ARWR), Leonardo Biosystems, and NanoMedical Systems, and has a financial interest in these companies as a shareholder.

REFERENCES

Alvarez, R.H., Valero, V., Hortobagyi, G.N., 2010. Emerging targeted therapies for breast cancer. J. Clin. Oncol. 28, 3366–3379.

Azim Jr., H.A., de Azambuja, E., Colozza, M., Bines, J., Piccart, M.J., 2011. Long-term toxic effects of adjuvant chemotherapy in breast cancer. Ann. Oncol. 22, 1939–1947.

Bey, E.A., Bentle, M.S., Reinicke, K.E., Dong, Y., Yang, C.R., Girard, L., Minna, J.D., Bornmann, W.G., Gao, J., Boothman, D.A., 2007. An NQO1- and PARP-1-mediated cell death pathway induced in non-small-cell lung cancer cells by beta-lapachone. Proc. Natl. Acad. Sci. .U S A 104, 11832–11837.

Blanco, E., Bey, E.A., Dong, Y., Weinberg, B.D., Sutton, D.M., Boothman, D.A., Gao, J., 2007. Beta-lapachone-containing PEG-PLA polymer micelles as novel nanotherapeutics against NQO1-overexpressing tumor cells. J. Control Release 122, 365–374.

Blanco, E., Bey, E.A., Khemtong, C., Yang, S.G., Setti-Guthi, J., Chen, H., Kessinger, C.W., Carnevale, K.A., Bornmann, W.G., Boothman, D.A., Gao, J., 2010. Beta-lapachone micellar nanotherapeutics for non-small cell lung cancer therapy. Cancer Res. 70, 3896–3904.

Blanco, E., Kessinger, C.W., Sumer, B.D., Gao, J., 2009. Multifunctional micellar nanomedicine for cancer therapy. Exp. Biol. Med. (Maywood) 234, 123–131.

Brufsky, A., 2010. Trastuzumab-based therapy for patients with HER2-positive breast cancer: from early scientific development to foundation of care. Am. J. Clin. Oncol. 33, 186–195.

Celano, M., Schenone, S., Cosco, D., Navarra, M., Puxeddu, E., Racanicchi, L., Brullo, C., Varano, E., Alcaro, S., Ferretti, E., Botta, G., Filetti, S., Fresta, M., Botta, M., Russo, D., 2008. Cytotoxic effects of a novel pyrazolopyrimidine derivative entrapped in liposomes in anaplastic thyroid cancer cells in vitro and in xenograft tumors in vivo. Endocr. Relat. Cancer 15, 499–510.

Chiappini, C., Tasciotti, E., Fakhoury, J.R., Fine, D., Pullan, L., Wang, Y.C., Fu, L., Liu, X., Ferrari, M., 2010. Tailored porous silicon microparticles: fabrication and properties. Chemphyschem 11, 1029–1035.

Ciuffreda, L., Del Bufalo, D., Desideri, M., Di Sanza, C., Stoppacciaro, A., Ricciardi, M.R., Chiaretti, S., Tavolaro, S., Benassi, B., Bellacosa, A., Foa, R., Tafuri, A., Cognetti, F., Anichini, A., Zupi, G., Milella, M., 2009. Growth-inhibitory and antiangiogenic activity of the MEK inhibitor PD0325901 in malignant melanoma with or without BRAF mutations. Neoplasia 11, 720–731.

Colombo, M., Corsi, F., Foschi, D., Mazzantini, E., Mazzucchelli, S., Morasso, C., Occhipinti, E., Polito, L., Prosperi, D., Ronchi, S., Verderio, P., 2010. HER2 targeting as a two-sided strategy for breast cancer diagnosis and treatment: outlook and recent implications in nanomedical approaches. Pharm. Res. 62, 150–165.

Crooke, S.T., 2004. Progress in antisense technology. Annu. Rev. Med. 55, 61–95.

Czeczuga-Semeniuk, E., Wolczynski, S., Dabrowska, M., Dzieciol, J., Anchim, T., 2004. The effect of doxorubicin and retinoids on proliferation, necrosis and apoptosis in MCF-7 breast cancer cells. Folia. Histochem. Cytobiol. 42, 221–227.

Davis, M.E., 2009. The first targeted delivery of siRNA in humans via a self-assembling, cyclodextrin polymer-based nanoparticle: from concept to clinic. Mol. Pharm. 6, 659–668.

Davis, M.E., Zuckerman, J.E., Choi, C.H., Seligson, D., Tolcher, A., Alabi, C.A., Yen, Y., Heidel, J.D., Ribas, A., 2010. Evidence of RNAi in humans from systemically administered siRNA via targeted nanoparticles. Nature 464, 1067–1070.

Decuzzi, P., Godin, B., Tanaka, T., Lee, S.Y., Chiappini, C., Liu, X., Ferrari, M., 2010. Size and shape effects in the biodistribution of intravascularly injected particles. J. Control Release 141, 320–327.

Decuzzi, P., Pasqualini, R., Arap, W., Ferrari, M., 2009. Intravascular delivery of particulate systems: does geometry really matter? Pharm. Res. 26, 235–243.

Dritschilo, A., Huang, C.H., Rudin, C.M., Marshall, J., Collins, B., Dul, J.L., Zhang, C., Kumar, D., Gokhale, P.C., Ahmad, A., Ahmad, I., Sherman, J.W., Kasid, U.N., 2006. Phase I study of liposome-encapsulated c-raf antisense oligodeoxyribonucleotide infusion in combination with radiation therapy in patients with advanced malignancies. Clin. Cancer Res. 12, 1251–1259.

Drummond, D.C., Noble, C.O., Guo, Z., Hayes, M.E., Connolly-Ingram, C., Gabriel, B.S., Hann, B., Liu, B., Park, J.W., Hong, K., Benz, C.C., Marks, J.D., Kirpotin, D.B., 2010. Development of a highly stable and targetable nanoliposomal formulation of topotecan. J. Control Release 141, 13–21.

Duenas-Gonzalez, A., Garcia-Lopez, P., Herrera, L.A., Medina-Franco, J.L., Gonzalez-Fierro, A., Candelaria, M., 2008. The prince and the pauper. A tale of anticancer targeted agents. Mol. Cancer 7, 82.

Duncan, R., Gaspar, R., 2011. Nanomedicine(s) under the microscope. Mol. Pharm. In Press.

Elbashir, S.M., Harborth, J., Lendeckel, W., Yalcin, A., Weber, K., Tuschl, T., 2001. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature 411, 494–498.

Ferrara, N., Hillan, K.J., Gerber, H.P., Novotny, W., 2004. Discovery and development of bevacizumab, an anti-VEGF antibody for treating cancer. Nat. Rev. Drug Discov. 3, 391–400.

Ferrari, M., 2005. Cancer nanotechnology: opportunities and challenges. Nat. Rev. Cancer 5, 161–171.

Ferrari, M., 2008. Beyond drug delivery. Nat. Nanotechnology 3, 131–132.

Ferrari, M., 2010a. Frontiers in cancer nanomedicine: directing mass transport through biological barriers. Trends Biotechnol. 28, 181–188.

Ferrari, M., 2010b. Vectoring siRNA therapeutics into the clinic. Nat. Rev. Clin. Oncol. 7, 485–486.

Fire, A., Xu, S., Montgomery, M.K., Kostas, S.A., Driver, S.E., Mello, C.C., 1998. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature 391, 806–811.

Gabizon, A., Shmeeda, H., Barenholz, Y., 2003. Pharmacokinetics of pegylated liposomal Doxorubicin: review of animal and human studies. Clin. Pharm. 42, 419–436.

Gabizon, A.A., 2001. Pegylated liposomal doxorubicin: metamorphosis of an old drug into a new form of chemotherapy. Cancer Invest. 19, 424–436.

Gines, J., Sabater, E., Martorell, C., Grau, M., Monroy, M., Casado, M.A., 2011. Efficacy of taxanes as adjuvant treatment of breast cancer: a review and meta-analysis of randomised clinical trials. Clin. Transl. Oncol. 13, 485–498.

Gnant, M., 2011. Anticancer activity of bisphosphonates in breast cancer. Anticancer Agents Med. Chem..

Godin, B., Gu, J., Serda, R.E., Bhavane, R., Tasciotti, E., Chiappini, C., Liu, X., Tanaka, T., Decuzzi, P., Ferrari, M., 2010. Tailoring the degradation kinetics of mesoporous silicon structures through PEGylation. J. Biomed. Mater. Res. A 94, 1236–1243.

Gonzalez-Angulo, A.M., Hortobagyi, G.N., Ellis, L.M., 2011. Targeted therapies: peaking beneath the surface of recent bevacizumab trials. Nat. Rev. Clin. Oncol. 8, 319–320.

Grothey, A., Ellis, L.M., 2008. Targeting angiogenesis driven by vascular endothelial growth factors using antibody-based therapies. Cancer J. 14, 170–177.

Hamilton, A., Biganzoli, L., Coleman, R., Mauriac, L., Hennebert, P., Awada, A., Nooij, M., Beex, L., Piccart, M., Van Hoorebeeck, I., Bruning, P., de Valeriola, D., 2002. EORTC 10968: a phase I clinical and pharmacokinetic study of polyethylene glycol liposomal doxorubicin (Caelyx, Doxil) at a 6-week interval in patients with metastatic breast cancer. European Organization for Research and Treatment of Cancer. Ann. Oncol. 13, 910–918.

Hanahan, D., Weinberg, R.A., 2000. The hallmarks of cancer. Cell 100, 57–70.

Hanahan, D., Weinberg, R.A., 2011. Hallmarks of cancer: the next generation. Cell 144, 646–674.

Heidel, J.D., Yu, Z., Liu, J.Y., Rele, S.M., Liang, Y., Zeidan, R.K., Kornbrust, D.J., Davis, M.E., 2007. Administration in non-human primates of escalating intravenous doses of targeted nanoparticles containing ribonucleotide reductase subunit M2 siRNA. Proc. Natl. Acad. Sci. U S A 104, 5715–5721.

Jemal, A., Bray, F., Center, M.M., Ferlay, J., Ward, E., Forman, D., 2011. Global cancer statistics. CA Cancer J. Clin. 61, 69–90.

Jin, C., Li, H., He, Y., He, M., Bai, L., Cao, Y., Song, W., Dou, K., 2010. Combination chemotherapy of doxorubicin and paclitaxel for hepatocellular carcinoma in vitro and in vivo. J. Cancer Res. Clin. Oncol. 136, 267–274.

Kang, S.H., Cho, H.J., Shim, G., Lee, S., Kim, S.H., Choi, H.G., Kim, C.W., Oh, Y.K., 2011. Cationic liposomal co-delivery of small interfering RNA and a MEK inhibitor for enhanced anticancer efficacy. Pharm. Res. In Press.

Kataoka, K., Harada, A., Nagasaki, Y., 2001. Block copolymer micelles for drug delivery: design, characterization and biological significance. Adv. Drug Deliv. Rev. 47, 113–131.

Kenmotsu, H., Yasunaga, M., Goto, K., Nagano, T., Kuroda, J., Koga, Y., Takahashi, A., Nishiwaki, Y., Matsumura, Y., 2010. The antitumor activity of NK012, an SN-38-incorporating micelle, in combination with bevacizumab against lung cancer xenografts. Cancer 116, 4597–4604.

Khong, T., Spencer, A., 2011. Targeting heat shock protein 90 induces apoptosis and inhibits critical survival and proliferation pathways in multiple myeloma. Mol. Cancer Ther. 10, 1909–1917.

Kim, L.C., Song, L., Haura, E.B., 2009. Src kinases as therapeutic targets for cancer. Nat. Rev. Clin. Oncol. 6, 587–595.

Kim, S.H., Jeong, J.H., Lee, S.H., Kim, S.W., Park, T.G., 2008. Local and systemic delivery of VEGF siRNA using polyelectrolyte complex micelles for effective treatment of cancer. J. Control Release 129, 107–116.

Kuesters, G.M., Campbell, R.B., 2010. Conjugation of bevacizumab to cationic liposomes enhances their tumor-targeting potential. Nanomedicine (Lond) 5, 181–192.

Larson, N., Greish, K., Bauer, H., Maeda, H., Ghandehari, H., 2011. Synthesis and evaluation of poly(styrene-co-maleic acid) micellar nanocarriers for the delivery of tanespimycin. Int. J. Pharm. 420, 111–117.

Lee, K.S., Chung, H.C., Im, S.A., Park, Y.H., Kim, C.S., Kim, S.B., Rha, S.Y., Lee, M.Y., Ro, J., 2008. Multicenter phase II trial of Genexol-PM, a Cremophor-free, polymeric micelle formulation of paclitaxel, in patients with metastatic breast cancer. Breast Cancer Res. Treat. 108, 241–250.

Maeda, H., 2001. The enhanced permeability and retention (EPR) effect in tumor vasculature: the key role of tumor-selective macromolecular drug targeting. Adv. Enzyme. Regul. 41, 189–207.

Marra, M., Salzano, G., Leonetti, C., Tassone, P., Scarsella, M., Zappavigna, S., Calimeri, T., Franco, R., Liguori, G., Cigliana, G., Ascani, R., La Rotonda, M.I., Abbruzzese, A., Tagliaferri, P., Caraglia, M., De Rosa, G., 2011. Nanotechnologies to use bisphosphonates as potent anticancer agents: the effects of zoledronic acid encapsulated into liposomes. Nanomedicine In Press.

Mendelsohn, J., Baselga, J., 2000. The EGF receptor family as targets for cancer therapy. Oncogene 19, 6550–6565.

Michor, F., Liphardt, J., Ferrari, M., Widom, J., 2011. What does physics have to do with cancer? Nat. Rev. Cancer 11, 657–670.

Moreira, J.N., Simoes, S., 2003. Technology evaluation: LErafAON, NeoPharm. Curr. Opin. Mol. Ther. 5, 547–552.

Morrow, P.K., Zambrana, F., Esteva, F.J., 2009. Recent advances in systemic therapy: advances in systemic therapy for HER2-positive metastatic breast cancer. Breast Cancer Res. 11, 207.

Murakami, M., Cabral, H., Matsumoto, Y., Wu, S., Kano, M.R., Yamori, T., Nishiyama, N., Kataoka, K., 2011. Improving drug potency and efficacy by nanocarrier-mediated subcellular targeting. Sci. Transl. Med. 3 64ra62.

Northfelt, D.W., Dezube, B.J., Thommes, J.A., Levine, R., Von Roenn, J.H., Dosik, G.M., Rios, A., Krown, S.E., DuMond, C., Mamelok, R.D., 1997. Efficacy of pegylated-liposomal doxorubicin in the treatment of AIDS-related Kaposi's sarcoma after failure of standard chemotherapy. J. Clin. Oncol. 15, 653–659.

Nurunnabi, M., Cho, K.J., Choi, J.S., Huh, K.M., Lee, Y.K., 2010. Targeted near-IR QDs-loaded micelles for cancer therapy and imaging. Biomaterials 31, 5436–5444.

Park, J.W., Hong, K.L., Kirpotin, D.B., Colbern, G., Shalaby, R., Baselga, J., Shao, Y., Nielsen, U.B., Marks, J.D., Moore, D., Papahadjopoulos, D., Benz, C.C., 2002. Anti-HER2 immunoliposomes: enhanced efficacy attributable to targeted delivery. Clin. Cancer Res. 8, 1172–1181.

Peer, D., Karp, J.M., Hong, S., Farokhzad, O.C., Margalit, R., Langer, R., 2007. Nanocarriers as an emerging platform for cancer therapy. Nat. Nanotechnol. 2, 751–760.

Pegram, M.D., Konecny, G., Slamon, D.J., 2000. The molecular and cellular biology of HER2/neu gene amplification/overexpression and the clinical development of herceptin (trastuzumab) therapy for breast cancer. Cancer Treat. Res. 103, 57–75.

Petrocca, F., Lieberman, J., 2011. Promise and challenge of RNA interference-based therapy for cancer. J. Clin. Oncol. 29, 747–754.

Pink, J.J., Planchon, S.M., Tagliarino, C., Varnes, M.E., Siegel, D., Boothman, D.A., 2000. NAD(P)H: quinone oxidoreductase activity is the principal determinant of beta-lapachone cytotoxicity. J. Biol. Chem. 275, 5416–5424.

Ressler, S., Mlineritsch, B., Greil, R., 2011. Zoledronic acid for adjuvant use in patients with breast cancer. Expert Rev. Anticancer Ther. 11, 333–349.

Richardson, P.G., Chanan-Khan, A.A., Alsina, M., Albitar, M., Berman, D., Messina, M., Mitsiades, C.S., Anderson, K.C., 2010. Tanespimycin monotherapy in relapsed multiple myeloma: results of a phase 1 dose-escalation study. Br. J. Haematol. 150, 438–445.

Schmidt, C., 2011. How do you tell whether a breast cancer is HER2 positive? Ongoing studies keep debate in high gear. J. Natl. Cancer Inst. 103, 87–89.

Serda, R.E., Gu, J., Bhavane, R.C., Liu, X., Chiappini, C., Decuzzi, P., Ferrari, M., 2009. The association of silicon microparticles with endothelial cells in drug delivery to the vasculature. Biomaterials 30, 2440–2448.

Smith, A.M., Duan, H., Mohs, A.M., Nie, S., 2008. Bioconjugated quantum dots for in vivo molecular and cellular imaging. Adv. Drug Deliv. Rev. 60, 1226–1240.

Strebhardt, K., Ullrich, A., 2008. Paul Ehrlich's magic bullet concept: 100 years of progress. Nat. Rev. Cancer 8, 473–480.

Sutton, D., Nasongkla, N., Blanco, E., Gao, J., 2007. Functionalized micellar systems for cancer targeted drug delivery. Pharm. Res. 24, 1029–1046.

Tanaka, T., Mangala, L.S., Vivas-Mejia, P.E., Nieves-Alicea, R., Mann, A.P., Mora, E., Han, H.D., Shahzad, M.M., Liu, X., Bhavane, R., Gu, J., Fakhoury, J.R., Chiappini, C., Lu, C., Matsuo, K., Godin, B., Stone, R.L., Nick, A.M., Lopez-Berestein, G., Sood, A.K., Ferrari, M., 2010. Sustained small interfering RNA delivery by mesoporous silicon particles. Cancer Res. 70, 3687–3696.

Tasciotti, E., Liu, X., Bhavane, R., Plant, K., Leonard, A.D., Price, B.K., Cheng, M.M., Decuzzi, P., Tour, J.M., Robertson, F., Ferrari, M., 2008. Mesoporous silicon particles as a multistage delivery system for imaging and therapeutic applications. Nat. Nanotechnol. 3, 151–157.

Torchilin, V.P., 2005. Recent advances with liposomes as pharmaceutical carriers. Nat. Rev. Drug Discov. 4, 145–160.

Venturini, M., Bighin, C., Puglisi, F., Olmeo, N., Aitini, E., Colucci, G., Garrone, O., Paccagnella, A., Marini, G., Crino, L., Mansutti, M., Baconnet, B., Barbato, A., Del Mastro, L., 2010. A multicentre Phase II study of non-pegylated liposomal doxorubicin in combination with trastuzumab and docetaxel as first-line therapy in metastatic breast cancer. Breast 19, 333–338.

Whitesell, L., Lindquist, S.L., 2005. HSP90 and the chaperoning of cancer. Nat. Rev. Cancer 5, 761–772.

Zafar, S.Y., Malin, J.L., Grambow, S.C., Abbott, D.H., Schrag, D., Kolimaga, J.T., Zullig, L.L., Weeks, J.C., Fouad, M.N., Ayanian, J.Z., Wallace, R., Kahn, K.L., Ganz, P.A., Catalano, P., West, D.W., Provenzale, D., 2011. Early dissemination of bevacizumab for advanced colorectal cancer: a prospective cohort study. BMC Cancer 11, 354.

Zhang, C., Tang, N., Liu, X., Liang, W., Xu, W., Torchilin, V.P., 2006. siRNA-containing liposomes modified with polyarginine effectively silence the targeted gene. J. Control Release 112, 229–239.