ACS Biomaterials SCIENCE & ENGINEERING

Review

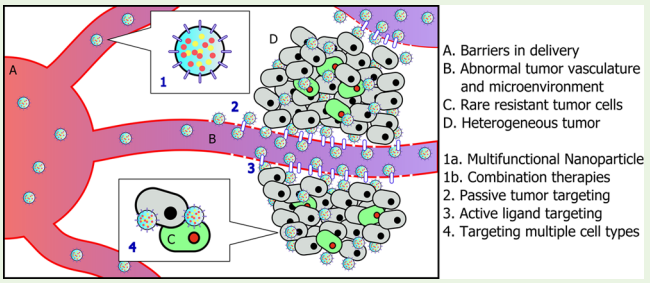
pubs.acs.org/journal/abseba

Nanotherapy for Cancer: Targeting and Multifunctionality in the Future of Cancer Therapies

Asiri Ediriwickrema and W. Mark Saltzman*

Department of Biomedical Engineering, Yale University, 55 Prospect Street, MEC 414, New Haven, Connecticut 06511, United States

ABSTRACT: Cancer continues to be a prevalent and lethal disease, despite advances in tumor biology research and chemotherapy development. Major obstacles in cancer treatment arise from tumor heterogeneity, drug resistance, and systemic toxicities. Nanoscale delivery systems, or nanotherapies, are increasing in importance as vehicles for antineoplastic agents because of their potential for targeting and multifunctionality. We discuss the current field of cancer therapy and potential strategies for addressing obstacles in cancer treatment with nanotherapies. Specifically, we review the strategies for rationally designing nanoparticles for targeted, multimodal delivery of therapeutic agents.



KEYWORDS: *nanoparticle, gene delivery, drug delivery, cancer therapy*

INTRODUCTION

History of the Field of Cancer Therapy. The first documented case of cancer was recorded in Egypt around 3000 BC, and surgical resections were historically the predominant mode of treatment.[1] Emile Grubbe began using X-rays to treat recurrent breast carcinoma in 1896, and surgery and radiotherapy continued to be the mainstay of treatment. It was not until 1948, when Sidney Farber evaluated the use of antifolate compounds for treating leukemia in children, that chemotherapy became a viable treatment option.[2,3] The ensuing decades have generated numerous advances in cancer biology and chemotherapy. The evaluation of the biochemical processes involved in drug resistance advanced in the 1960s, and resulted in the implementation of combination chemotherapies in 1965 and adjuvant chemotherapy in 1972.[3] Significant improvements in cancer mortality were first noticeable in the 1990s, and the field of targeted cancer therapies blossomed with new discoveries in cancer signaling pathways involved in tumor development, proliferation, and metastasis.

The completion of the Human Genome Project in 2003 created the potential for revolutionary advances in understanding many human diseases including cancer. Multiple international projects, like The Cancer Genome Atlas, were initiated to analyze the genetics of multiple cancer subtypes. Through extensive genome analysis of tissues from several patients, researchers are gaining a stronger understanding of the development, susceptibilities, and prognosis of individual cancers, and in turn learning how to design patient specific therapies. Consequently, the death rates for common cancers, such as prostate, breast, lung, and colorectal, are declining because of the development of new small molecules and immunotherapies, target specific screens, and new combination therapies.[4] In 2013, the five year survival for cancers from all sites has increased to 66.7%. Despite these promising advances, cancer is the second leading cause of death in the United States, with more than 1.6 million new cases annually, and approximately 580 000 deaths each year.[5] These numbers will likely increase with the expected aging of the population. Even as cancer survivors live longer, they are at a higher risk for developing new malignancies. In addition, the issue of drug resistance and tumor recurrence continue to hinder cancer therapies.

Cancer Biology and Rational Treatment Design. The birth of scientific oncology ensued when Rudolf Virchow examined blood samples from leukemia patients under the microscope in 1847. Currently, cancer pathogenesis is considered a complex multistep process where cells attain certain hallmark properties as a result of both genetic and epigenetic alterations.[6,7] Carcinogenesis is primarily a consequence of changes in the genetic code or gene expression. The affected genes can be categorized into three main groups: oncogenes, tumor suppressor genes, and mismatch-repair genes. Changes in gene expression may in turn allow cells to maintain proliferative signaling, evade growth suppressive signals, resist cell death, promote invasion and metastasis, confer replicative immortality, deregulate cellular energetics, promote genomic instability, and initiate angiogenesis.[8] Additionally, tumor interactions with adjacent stroma and the immune system can promote proliferation and metastasis through avoiding immune destruction and stimulating tumori-

Received: October 28, 2014
Accepted: January 13, 2015
Published: January 13, 2015

DOI: 10.1021/ab500084g
ACS Biomater. Sci. Eng. 2015, 1, 64−78

Table 1. Hallmarks of Cancer Pathogenesis and Therapeutic Implications[a]

hallmark of cancer pathogenesis[6]	cellular and molecular alterations[6,13,14]	potential targeted therapies
1. sustaining proliferative signaling	↑ MAP-kinase pathway, ↑ PI3K pathway, ↓ PTEN, mTOR kinase pathway	tyrosine kinase inhibitors,[15] proteasome inhibitors,[16] mTOR inhibitors,[17] PI3K inhibitors,[18,19] HDAC inhibitors[20]
2. evading growth suppressors	↓ TP53, ↓ RB, ↓ NF2, ↓ LKB1, TGF-β signaling	cyclin-dependent kinase inhibitors[21]
3. avoiding immune destruction	↓ CTLs, ↓ CD4+ Th1 cells, ↓ NK cells, ↓ PD-1 signaling, ↑ Tregs, ↑ MDSCs, TGF-β signaling	cancer vaccines,[22] ex vivo T cell modifications,[23] immune activating anti-CTLA4 mAb,[24] PD-1 agonists[25]
4. enabling replicative immortality	↑ telomerase, ↓ TP53	telomerase inhibitors[26]
5. tumor promoting inflammation	B lymphocytes, macrophages, mast cells, myeloids progenitors, necrosis, neutrophils, T lymphocytes, ↑ IL-1α, ↑ reactive oxygen species	anti-inflammatory drugs[27]
6. activating invasion and metastasis	↑ CCL5/RANTES, ↑ c-Met, ↑ CSF1, ↑ CCPs, ↑ heparanase, ↑ EMT, ↑ IL-4, ↑ matrix-degrading enzymes, ↑ N-cadherin, ↑ Wnt signaling, ↓ E-cadherin, snail, slug, TGF-β signaling, twist, Zeb1/2, macrophages, neoplastic stroma	inhibitors of HGF/c-Met[28]
7. inducing angiogenesis	↑ FGF family proteins, ↑ Ras, ↑ Myc, ↑ VEGFa, ↓ endostatin, ↓ plasmin, TGF-β signaling, ↓ TSP-1, endothelial cells	angiogenesis inhibitors[29]
8. genome instability and mutation	↓ BRCA, ↓ TP53	PARP inhibitors[30]
9. resisting cell death	↑ A1, ↑ Bcl-2, ↑ Bcl-xL, ↑ Bcl-w, ↑ Mcl-1, ↑ extrinsic growth factor signaling, ↓ Bax, ↓ Bak, ↓ BH3 proteins, ↓ TP53, ↓ extrinsic ligand-induced death pathways	proapoptotic BH3 mimetics,[31] Bcl-2 antagonists,[32] PARA therapy[33,34]
10. deregulating cellular energetics	↑ GLUT1, ↑ HIF, ↑ IDH1/2	aerobic glycolysis inhibitors[35]
11. deregulating autophagy	Beclin	autophagy inhibitors[13,36]
12. tumor microenvironment	cancer stem cells, endothelial cells (notch, neuropilin, Robo, and Eph-A/B singaling), fibroblasts, myofibroblasts, neoplastic stroma, pericytes, TGF-β signaling	antistem cell antibodies,[37] PDGF receptor inhibition[38]

[a]Bax, Bcl-2-associated X; Bcl, B-cell lymphoma; BRCA, breast cancer; CCL5/RANTES, Chemokine (C−C motif) ligand 5/regulated on activation, normal T cell expressed and secreted, CCPs, cysteine cathepsin proteases; CD4, cluster of differentiation 4; CSF, colony-stimulating factor; CTL, CD8+ cytotoxic T lymphocytes; EMT, epithelial-mesenchymal transition; EPH, ephrin type; FGF, fibroblast growth factor; GLUT, glucose transporter; HDAC, histone deacetylase; HGF, hepatocyte growth factor; HIF, hypoxia-inducible factor; IDH, isocitrate dehydrogenase; IL, interleukin; LKB1, liver kinase B1; MAP, mitogen-activated protein; MCL, myeloid cell leukemia; MDSCs, myeloid-derived suppressor cells; mTOR, mammalian target of rapamycin; NF2, neurofibromin 2 (merlin); NK, natural killer; PARA, proapoptotic receptor agonist; PD-1, programmed death-1; PDGF, platelet-derived growth factor; PI3K, phosphatidylinositide 3 kinase; PTEN, phosphatase and tensin homologue; RB, retinoblastoma; Robo, roundabout; TGF, transforming growth factor; T_h, T helper; Tregs, regulatory T cells; TSP, thrombospondin; VEGF, vascular endothelial growth factor; ZEB, zinc finger E-box-binding homeobox.

genic inflammation. Epigenetic factors can also promote carcinogenesis without directly conferring any genotypic variations. Deoxyribonucleic acid (DNA) methylation, histone modification, and gene silencing are involved in cancer pathogenesis.[9−12] The multiple, interconnected pathways complicates efforts for providing effective therapies. Therefore, a paradigm shift is underway as researchers are working to analyze individual tumors in order to design therapies for specific cancer phenotypes.

Understanding cancer pathogenesis has allowed for development of more effective therapies. For instance, cancer cells can receive increased proliferative signaling by up-regulating surface growth factor receptors such as EGFR. These discoveries have been translated into promising clinical therapies in the form of EGFR specific inhibitors. Similarly, by analyzing specific hallmarks necessary for cancer progression, new pipelines of therapeutics are being developed to treat the disease. Table 1 describes the multiple hallmarks of cancer pathogenesis, respective cellular and molecular alterations, and associated targeted therapies.[6,13,14] Molecular and genetic analysis allows physicians to detect, classify, monitor, and treat cancer more effectively. However, designing adequate therapies is difficult because of the intricacies of cancer biology and the vast heterogeneity of tumors.

Obstacles in Cancer Chemotherapy. Although our understanding of cancer pathogenesis is increasing, the disease process remains extremely complex and much is still unknown. Every cell lineage in the body can be affected. Inherent genomic instability and biological diversity in cancer cells can lead to treatment resistance. Only a small fraction of tumor cells is highly sensitive to therapy, and even those cells can develop resistance and progress into a more aggressive disease. Drug resistance can be either intrinsic or acquired. Intrinsic resistance occurs when tumor cells have decreased or no sensitivity to a therapeutic agent. Cells can develop resistance through a variety of mechanisms including decreased drug uptake, up-regulation of drug efflux transporters, aberrant cell cycle checkpoints, increased DNA repair, increased drug metabolism, induction of stress response genes, and inhibition of apoptosis.[39] Additionally, an individual cell can become resistant through its own unique variation, which further complicates cancer therapies. Acquired resistance occurs when neoplasms that were initially responsive to certain therapies becoming unresponsive. Similar to the development of antibiotic resistance in bacteria, use of chemotherapeutic agents can lead to selection for inherently resistant tumor cells. The characteristic genomic instability of many cancer subtypes and the mutagenic properties of chemotherapeutic interventions lead to new mutations that translate into drug resistance. Of note, hierarchies in signaling cascades involved in tumor development can diminish efficacy of targeted therapies if the new mutation overrides the targeted factor in the signaling cascade. For instance, mutations in the tumor suppressor gene, PTEN, can decrease efficacy of anti-HER2 immunotherapy, or

DOI: 10.1021/ab500084g
ACS Biomater. Sci. Eng. 2015, 1, 64−78

Table 2. Examples of Nanoparticle Therapeutics[a,63,64]

nanocarrier	name	formulation	indication	status
inorganic nanoparticle	Ferumoxide[65]	iron oxide MRI contrast agent	liver imaging	approved 1997
	CYT-6091[66]	TNFα-PEG-gold	solid tumors	phase I
liposome	Doxil[67]	liposomal doxorubicin	ovarian, breast cancer	approved 1995
micelle	NKTR-102[68]	PEG-micelle Irinotecan	colorectal and breast cancer	phase III
protein nanoparticle	Abraxane[69]	paclitaxel-albumin	metastatic breast cancer	approved 2005
polymeric micelle	Genexol-PM[70]	miceller paclitaxel	breast, lung, pancreatic cancer	phase II–IV
polymer-drug conjugate	Xyotax[71]	paclitaxel-poly-L-glutamic acid	breast, ovarian cancer	phase III
	Oncaspar[72]	PEG-L-asparaginase	acute lymphoblastic leukemia	approved 2006
polymer nanoparticle	BIND-014[73]	docetaxel-PLGA/PLA–PEG with targeting ligand	nonsmall cell lung cancer, prostate cancer	phase II
radio-immunoconjugate	Zevalin[74]	anti-CD20 conjugated to yttrium-90 or indium-111	non-Hodgkin's lymphoma	approved 2002

[a]PEG – polyethylene glycol; PLA, polylactic acid; PLGA, poly(lactic-*co*-glycolic) acid; TNF, tumor necrosis factor.

inactivation of TP53 can minimize the cytotoxicity of several cancer therapies.[40] Mutations and natural selection are fundamental in the development of resistance, as they are the primary drivers of cancer pathogenesis.

The two overarching models of carcinogenesis are the stochastic clonal evolution model and the hierarchal cancer stem cell model.[41] The original stochastic model describes tumor pathogenesis as the progression of somatic mutations that lead to isolation of a dominant cancerous clone that, through selective influences, eventually progresses into metastatic tumors. However, during the past two decades, studies have shown that certain cancer types arise from a more hierarchal organization of cells. These tumors are comprised of multiple cell subpopulations having a spectrum of proliferative and regenerative capabilities. This phenomenon was first described in human acute myeloid leukemia cells,[42] by isolation of rare leukemia initiating cells that have differentiation and proliferative capabilities similar to leukemic stem cells, and demonstration that these cells are responsible for the regenerative, self-perpetuating, and diverse nature of the tumor. The primitive tumor initiating cells are inherently quiescent and less susceptible to traditional agents that target rapidly proliferating tumor cells.[41,43] Subsequent studies have identified tumor initiating cells in breast cancer,[44,45] colon cancer,[46−48] melanoma,[49] and brain tumors.[50−52] Therefore, effective therapies will require targeting of these rare initiating cells since traditional chemotherapies target only the proliferative subset of the cancer.

In addition to the challenges presented by tumor cell biology, the tumor microenvironment and host factors can influence efficacy of current chemotherapy regimens. The tumor microenvironment, including both the abnormal vasculature and adjacent stromal cells, can impede drug delivery or increase drug clearance. Delivery to the tumor can be impaired in large, necrotic malignancies. Host factors, including decreased absorption, rapid metabolism, and increased clearance of agents, can lower serum drug concentrations. Additionally, drug solubility and size can hinder tumor delivery and penetration. Patients also have variable capacities to tolerate chemotherapy agents, and the development of side effects can significantly obstruct dosing and treatment duration.[39]

Because of tumor heterogeneity and the development of drug resistance, future therapeutic regimens will likely incorporate combination multimodal therapies. Mathematical models can be used to evaluate tumor response to targeted monotherapy and combination therapies.[53] These models suggest that dual therapy is often adequate for long-term disease control, but that patients with a larger initial disease burden may require triple therapy. Further, the models suggest that simultaneous therapy is more effective than sequential therapy.

Cancer Genetics, Tumor Profiling, and Personalized Medicine. With the discovery of numerous clinically relevant cancer genes, gene editing is becoming an increasingly relevant aspect of cancer therapy. Gene editing via RNA interference (RNAi), through small interfering RNAs (siRNA) or microRNAs (miRNA) delivery, peptide nucleic acids, and CRISPR-Cas technology can potentially silence any gene of interest.[54−56] New efforts seek to streamline evaluation of cancer genomes to allow for personalized therapies.[57] Improvements in sequencing technologies have evolved into new paradigms for analyzing tumor specimens; massively parallel screens can examine patient samples for potentially actionable targets.[57−59] Further, a pilot program, the Master Protocol, was recently approved by the Food and Drug Administration (FDA), which will connect patients to relevant drug therapy trials based on biomarkers.[60] Determining specific gene or biomarker expression profiles can align specific disease with the ideal, patient centered treatment regimen.

As the spectrum of genomic targets or abnormal signaling cascades widens, it may be advantageous to incorporate gene therapy along with targeted small molecules or immunotherapies. Gene delivery, however, can be either inefficient or dangerous. Similarly, most chemotherapeutics are highly toxic, especially after systemic delivery. Commonly used cytotoxic agents can act indiscriminately against both cancerous and healthy cells resulting in nausea and hair loss, neutropenia, peripheral neuropathies, kidney failure, encephalopathy, and heart disease.[61] The associated side effects of common chemotherapy drugs, as well as host factors and systemic delivery barriers, can severely limit dosing and, ultimately, treatment efficacy.

The complexities of cancer pathogenesis—coupled with the problems of drug resistance, side effects of therapy, and inadequate delivery to tumors—call for new solutions, especially new therapies that can overcome traditional barriers to effective treatment, while allowing for multifunctionality. Nanotechnology offers just such a solution, particularly nanoparticle drug delivery. In the next section, we discuss recent advances in the field, and promising strategies to address some of the many obstacles in cancer drug delivery.

IMPROVING CANCER THERAPIES WITH NANOTHERAPIES

Introduction to Nanotherapies. Nanotechnology offers the potential to improve drug solubility and stability, prolong drug half-lives in plasma, minimize off target effects, and

DOI: 10.1021/ab500084g
ACS Biomater. Sci. Eng. 2015, 1, 64−78

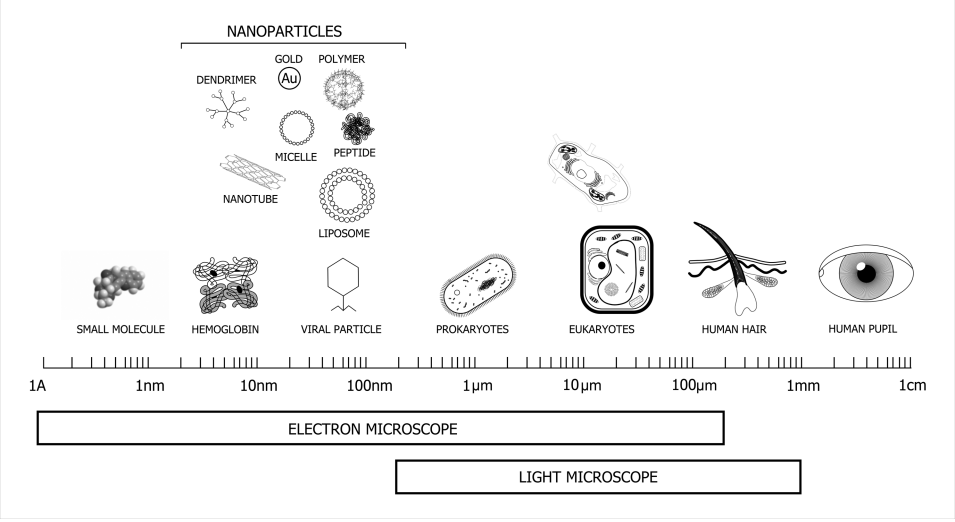


Figure 1. Relative sizes of nanoparticles compared to common biological structures. Illustration of nanoparticle size as compared to common biological structures and their associated length scale. An electron microscope is needed to visualize structures that are submicrometer in size.

concentrate drugs at a target site.[62] Nanotechnology is traditionally defined as submicron sized molecular devices or nanoparticles predominantly ranging from 5 to 500 nm in at least one dimension. Substantial past research effort has resulted in methods to incorporate therapeutic agents into biocompatible nanodevices including polymer nanoparticles, liposomes, micellar systems, inorganic nanoparticles, nanotubes, and dendrimers. Table 2 lists a few examples of nanoparticle therapeutics that are currently approved by the FDA or in clinical trials. For reference, Figure 1 compares various nanoparticles to common biological structures including hemoglobin, which is approximately 5 nm is size, to the human pupil, which is 1 000 000 times larger (4−9 mm). Nanovectors for drug delivery typically contain a core material or matrix, a therapeutic payload, and surface modifications. Of particular interest in this review are polymer nanoparticles, which have been studied extensively during the previous few decades.

Small Molecule Delivery. Folkman first described a method for incorporating proteins and macromolecules into polymers in 1964.[75] The field of polymer drug delivery has continued to evolve and generate an array of novel applications.[76,77] Many polymers are safe to use clinically, and the most extensively studied are poly(lactic acid) (PLA) and poly(lactic-*co*-glycolic acid) (PLGA), which was first approved by the FDA as a suture material in 1969 and, more recently, has been approved for delivery of peptides and proteins. There are multiple PLA and PLGA delivery systems on the market including Zoladex and Nutropin Depot, and several more in the pipeline.[78] PLGA nanoparticles are being formulated to target specific tumors and deliver a host of agents including chemotherapy drugs or RNAi.[79−81] Upon exposure to physiologic solutions, PLGA undergoes hydrolysis into biocompatible metabolites, glycolic acid (GA) and lactic acid (LA), which are eventually metabolized through the citric acid cycle. Biodegradable, polymer nanoparticles provide several distinct advantages as a drug delivery vectors including tunable payload release characteristics and superior pharmacokinetics.

PLGA particles are particularly useful for agents that have low solubility in water, and therefore are difficult to formulate as drugs. The majority of clinically available chemotherapeutic agents are lipophilic, and have low solubility in water. A common measurement of lipophilicity is the distribution coefficient, $\log(D)$, where D is the ratio of solute concentration in octanol to the solute concentration in aqueous buffer in both ionized and nonionized forms. $\text{Log}(D)$ values larger than zero indicate greater solute partitioning into the hydrophobic solvent relative to water.[82] Figure 2 illustrates the spectrum of distribution coefficients at physiologic conditions for currently approved antineoplastic agents.

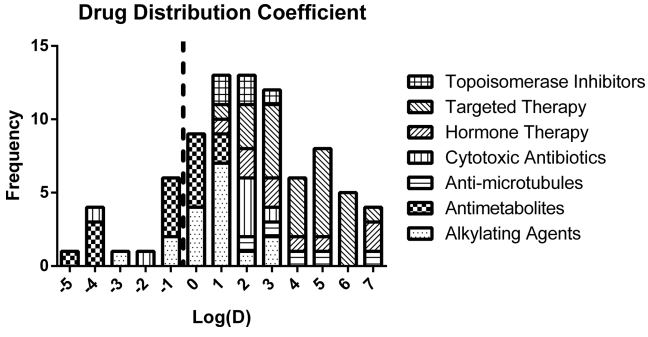


Figure 2. Distribution coefficient of common antineoplastic agents. The frequency distribution of antineoplastic agents by lipophilicity. The distribution coefficient (D) is a measure of lipophilicity, and $\log(D)$ values greater than zero indicate greater solubility in oil rather than water. The majority of clinically available antineoplastic agents are lipophilic.

The PLGA matrix releases encapsulated drugs at a sustained rate, allowing for both solubilization of drugs within the intravascular space and release over a long period. When compared to repeat free drug boluses, sustained release is more appropriate for maintaining drug concentrations within the therapeutic window. Free drug boluses result in pulsatile plasma concentrations. Levels above the minimal tolerated concentration may result in serious toxicity, and levels below the minimum effective concentration will be subtherapeutic (Figure 3). The ratio of LA to GA subunits can be adjusted to tune the rate of drug release, allowing for release profiles ranging from days to months.[83] Production of PLGA nanoparticles can be scaled to industrial levels, and the resulting particles can be

DOI: 10.1021/ab500084g
ACS Biomater. Sci. Eng. 2015, 1, 64−78

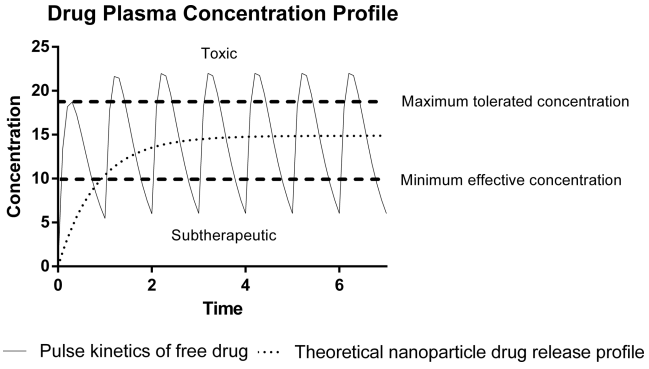


Figure 3. Nanoparticle pharmacokinetics. Drug plasma concentrations associated after repeated free drug boluses compared to a single nanoparticle dose. Because of rapid bioavailability and clearance of free drugs relative to drug encapsulated polymer nanoparticles, plasma concentrations will oscillate above and below the maximum tolerated concentration (MTC) and minimum effective concentration (MEC). Plasma drug levels above the MTC will result in systemic toxicity whereas drug levels below the MEC will be ineffective. Drug-loaded polymer nanoparticles theoretically release drugs via first-order rate kinetics resulting in a more stable plasma drug level.

stored for extended periods.[84] Encapsulating unstable small molecules or readily degradable proteins and oligonucleotides in a core polymeric matrix protects them from physiologic factors that would normally facilitate their clearance. Certain compounds are readily inactivated via hepatic metabolism or circulating proteases and endonucleases. Additionally, glomerular filtration in the kidneys rapidly clears compounds smaller than 10 nm. Although nanoparticles avoid renal clearance, they tend to accumulate in the mononuclear phagocyte system (MPS). But surface conjugation with polyethylene glycol (PEG) and other polymers improves particle circulation by reducing uptake into the MPS.[85,86] In turn, delivery via nanoparticles extends drug half-life, allowing for better control of circulating drug concentrations.

Introduction to Gene Delivery. Gene therapy is the cellular delivery of nucleic acids in order to modulate gene expression toward treating disease. Phenotypic modulation is achieved either through gene addition, gene correction, or gene knockdown.[87] Gene addition is generally the most common approach, and alters cell behavior by introducing genetic material and consequent proteins that are inherently missing in the host. Gene correction is less common, but growing in popularity, and utilizes technology—such as zinc finger nucleases, triplex forming oligonucleotides, or CRISPR-Cas—to alter or correct genomic sequences.[56,88−90] Finally, gene knockdown through RNAi has received significant enthusiasm. Because of the complex nature of cancer pathogenesis and multitude of signaling pathways involved in disease progression, isolating unique and singular molecular targets can become increasingly difficult. Often, tumor cells have altered transcription factor activity, influencing multiple pathways, which is difficult to target through small molecule drugs. Therefore, gene therapy can provide an alternative strategy for designing effective and specific therapies against cancer.

The U.S. FDA approved its first clinical trial in gene therapy in 1990. Michael Blease conducted an ex vivo gene therapy trial on two children with adenosine deaminase deficiency, a form of severe combined immunodeficiency (SCID).[91] Subsequent trials in treating SCID through ex vivo gene delivery, however, have demonstrated better long-term results.[92,93] In 1998, a team in Scandinavia demonstrated the first successful gene transfer from in vivo gene delivery into the brain.[94] Currently, there are more than 1,800 approved clinical trials using gene therapy worldwide.[95] Greater than 60% of current trials are designed to treat cancer, and viral vectors continue to be the most popular approach.[96] China was the first country to approve a commercial gene therapy, which is currently being used to treat head and neck cancer,[97] and there are multiple therapies nearing the final stages of clinical testing worldwide.[98] Of interest, the CTL019 trial, at the University of Pennsylvania, has shown promising results using chimeric antigen receptor therapy for treating B-cell neoplasms.[23,99,100] The patient's T-cells are modified ex vivo using a lentiviral vector to express chimeric surface antibodies against CD19, which is expressed on B-cells. Twelve of 14 pediatric patients with acute lymphoblastic leukemia have responded to therapy, and eight experienced complete remission. Twelve of 24 adult patients with chronic lymphocytic leukemia have responded to therapy, and five of those responders have attained complete remission.[101]

Methods of Gene Delivery. There has been significant progress in the field, despite earlier setbacks, including the death of 18 year-old Jesse Gelsinger in 1999,[102] and the development of T cell leukemia in multiple patients receiving gene therapies for SCID.[103,104] The dangers of viral gene therapy are due to the associated acute immune response, immunogenicity, and oncogenesis after integration of viral components into chromosomal DNA. Even the recently successful CTL019 therapy has significant toxicities including the development of cytokine release syndrome, which can progress into macrophage activation syndrome.[99] Safety concerns about viral vectors, as well as their limited payload capacity and the difficulty of large-scale production, have driven interest in synthetic vectors for gene delivery. Nonviral vectors are advantageous because of their safety profile, low cost, large-scale manufacturing potential, stability, and capacity for a larger nucleic acid payload.[105,106] The main limitation of nonviral vectors is their low transfection efficiency. Table 3 lists the array of different vectors for gene therapy, and their associated transfection efficiency and toxicity. Although most synthetic polymers were initially considered inert, certain polymers can influence multiple cellular processes, especially when combined with biologically active agents, primarily through interactions with biological membranes and modulation of gene expression profiles.[107] PLGA nanoparticles can deliver nucleic acids with minimal cytotoxicity, but they have relatively low transfection efficiency. Incorporation of counterions, like spermidine, and surface functionalization with cell targeting or cell penetrating peptides have improved DNA loading and particle transfection.[108,109] However, their transfection efficiency remains far lower than polycationic nanoparticle formulations and viruses.

Barriers in Gene Delivery. Among nonviral systems, cationic liposomes are currently the gold standard. The first gene therapy trial using cationic liposomes occurred in 1992, and approximately 13% of all gene therapy trials worldwide currently use liposomal nanoparticles. Toxicity, however, is a major concern for liposomes. Additionally, liposomes are heterogeneous and relatively unstable, causing significant obstacles for large-scale pharmaceutical production.[113] Liposomes are readily inactivated in the serum, which can lower the high transfection levels commonly seen in vitro.[114] Serum instability, clearance, and cytotoxicity are common obstacles facing many nonviral gene delivery vectors. Polycationic

DOI: 10.1021/ab500084g
ACS Biomater. Sci. Eng. 2015, 1, 64−78

Table 3. Methods of Gene Delivery[a,87,105,106,110−112]

category	gene delivery system	transfection efficacy	toxicity
inorganic	calcium phosphate	II	I/II
	gold		
	magnetic		
	silica		
	quantum dots		
cationic lipids	emulsions	II/III	II/III
	liposomes		
	lipid nanoparticles		
cationic polymers	PAMAM	II/III	II/III
	PbAE		
	PEI		
	terpolymers		
cationic peptide	GALA,KALA	II/III	II/III
	poly-L-lysine		
	protamine		
	self-assembling peptides		
polymer	chitosan	II	I/II
	copolymer micelles		
	PLGA, PLA		
	polymethacrylates		
hybrid	lipid-polycationic polymer	I/II	I/II
	PLGA-polycationic polymer		
	PLGA-lipid		
physical	needle	II/III	II/III
	ballistic DNA injection		
	electroporation		
	sonoporation		
	photoporation		
	magnetofection		
	hydroporation		
viral	retroviral	III	III
	adenoviral		
	adeno-associated		

[a]I, low; II, medium; III, high; GALA, glutamic acid-alanine-leucine-alanine; KALA, lysine-alanine-leucine-alanine; PAMAM, polyamido-amine; PbAE, poly(beta-amino ester); PEI, polyethylenimine; PLA, polylactic acid; PLGA, poly(lactic-*co*-glycolic acid).

polymers, like polyethylenimine (PEI), have high in vitro transfection potential but are cytotoxic. PEI induces channel formation on the mitochondrial membrane and subsequent caspase activation and apoptosis.[115] Other polycations, such as poly-L-lysine (PLL) and chitosan, are less toxic but provide lower transfection. Further, PLL can stimulate an immune response due to the introduction of foreign amino acid sequences, and chitosan, at high doses can result in hypolipidemia in vivo.[113]

In addition to cytotoxicity, multiple barriers hinder effective transfection by nonviral systems (Figure 4).[87,110,111] Gene loaded particles need to protect their payload from nucleolytic enzymes while in circulation, and ultimately penetrate into the target tissue at adequate concentrations. Polymers such as PLGA encapsulate nucleic acids, protecting them from endonucleases. Similarly, condensing the negative phosphate bonds of nucleic acid chains with cationic polymers into polyplexes also protects the oligonucleotides from degradation during circulation.

After escape from the vascular space into tumors, the particle must traverse the interstitial space toward the target cell.

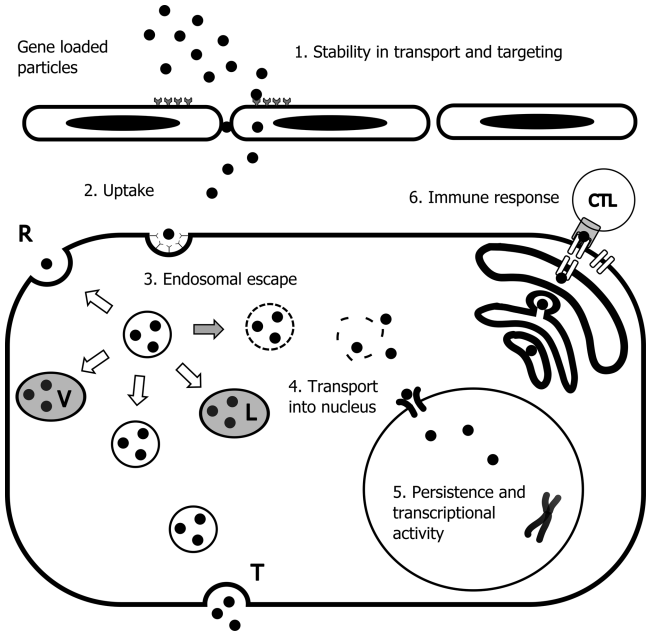


Figure 4. Barriers to gene therapy. The six major barriers for gene delivery. Gene-loaded particles need to be stable and need to protect their genetic cargo during transport in the circulatory system, while ultimately being able to localize at the target tissue.[1] After the tissue vasculature is penetrated, there needs to be efficient uptake of the particle into the cell.[2] After endocytosis, the particle needs to effectively escape the endosome[3] and transfer into the nucleus.[4] Once inside the nucleus, the transgene needs to persist and maintain adequate transcriptional activity.[5] During the entire process, these particles will need to evade the host immune response.[6] CTL, cytotoxic T lymphocyte; L, lysosome; V, vesicle; R, endosomal recycling; T, transcytosis.

Therefore, nanoparticle effectiveness depends on a variety of biophysiochemical characteristics of both the particle and cell surface. The cell surface is highly heterogeneous, both spatially and temporally, because of a variety of membrane structures, molecular interactions, and transport processes. Therefore, uptake of the vector into cells depends on both chance interaction and specific particle cell surface dynamics.[116] Surface charge and particle size are intimately associated with uptake efficiency. Cell surface proteins provide an overall negative charge on the plasma membrane, which readily interacts with the positive charge on certain nanocarriers. Electrostatic cell surface interactions primarily occur through positively charged vector interactions with cell surface proteoglycans. In fact, reduction in proteoglycan expression or function results in decreased transfection efficiencies.[117,118] Optimal particle diameters range from 50 to 120 nm, with smaller particles experiencing faster uptake.[119] Energy-dependent endocytosis is a primary route for cellular entry and can occur through multiple mechanisms. Recent studies report that clathrin- or caveolin-mediated endocytosis is capable of internalizing particles with diameters upward to 500 nm, yet internalization efficiency decreases with increased size.[120] Table 4 illustrates the five major endocytic pathways. Each route has its associated molecular players, compartment size, and intracellular fate.[121−123] There are multiple destinations for the early endosome, and the majority could render the genetic material ineffective. Figure 4 depicts different destinations for the endosomal cargo, and early escape appears to be necessary for subsequent gene activity. Endosomal acidification and

DOI: 10.1021/ab500084g
ACS Biomater. Sci. Eng. 2015, 1, 64−78

Table 4. Pathways for Endocytosis[a,119−123]

endocytic pathway	compartment size	relevant molecular players	intracellular fate	targeting modalities
phagocytosis	0.1−10 μm	actin, CDC42, PI(3), RHOA	phagosome, endosome, phagolysosme, lysosome	chitosan, mannose
macropinocytosis	50−1000 nm	ARF6, CDC42, nexins, Rab5, RAC1, rafts, ruffles	macropinosome, lysosome, TGN	AP, poly arginine peptides, TAT
clathrin coated vesicles	100−200 nm	actin, clathrins, dynamins, RHOA, SRC, TK	endosome, lysosome	antibody, RGD peptide, TAT, transferrin
noncoated vesicle (CLIC-D, CLIC-DI)	∼100−500 nm	ARF6, CDC42, flotillin, RHOA	endosome, lysosome	folate, transferrin
caveolae	40−100 nm	actin, caveolin, dynamin, intersectin, lipid rafts, PKC, SRC	endosome, lysosome, golgi, ER	anticaveaolae antibodies, AP, folate, TAT

[a]AP, antennapedia; CDC42, cell division cycle 42; CLIC-D, dynamin-dependent clathrin-independent carriers; CLIC-DI, dynamin- and clathrin-independent carriers; ER, endoplasmic reticulum; PI(3), Phosphoinositide 3; RGD, arginine-glycine-aspartic acid; TAT, transactivator of transcription; TGN, trans-Golgi network; TK, tyrosine kinases.

payload degradation through the lysosomal pathway is a common barrier for transfection. Sequestration within vesicles can also occur, as can externalization through trancytosis or endosomal recycling, all of which will reduce the effectiveness of the genetic payload. Additionally, there is significant variability in these pathways between different cell lines.[124]

Surface modification with ligands can facilitate particle targeting to tumors, as described above, and they can also facilitate uptake. Cell-penetrating peptides (CPPs) are short 30−35 amino acid peptides, often rich in arginine and lysine residues that promote cargo uptake via multiple mechanisms. Prototypic CPPs are the HIV transactivator of transcription (TAT) peptide and a peptide derived from antennapedia isolated from Drosophila antennae (AP).[125] Many studies have evaluated these peptides and their influence on cargo endocytosis. The specific mechanism for endocytosis varies, and several studies have suggested multiple routes of entry for individual CPPs.[126−128] Of interest, Zhou et al. evaluated histidine modifications of several CPPs, and found that flanking AP with five histidine residues (mAP) significantly increased nanoparticle transfection efficiencies.[108] Additionally, particle surface modifications with antireceptor antibodies, or surface conjugation with folate and transferrin can increase cargo uptake.[129−132] Table 4 summarizes targeting strategies for specific endocytic pathways.

Strategies to improve endosomal escape include utilizing fusogenic or pore-forming peptides or the proton sponge effect.[132] Certain viral peptides are known to promote endosomal disruption in a pH-dependent manner, and similar peptides have been synthesized to improve gene transfection when incorporated with DNA polyplexes.[133] These polymers are converted from hydrophilic to hydrophobic structures upon protonation in the endosome, which consequently allows for vesicle membrane lysis.[134] Cationic polyplexes, such as PEI or polyamidoamine (PAMAM) dendrimers, can also promote endosomal escape through the proton sponge mechanism.[135] During endosomal acidification, the protons pumped into the lumen via the vacuolar-ATPase are buffered by amines on the polymers. This buffering leads to an increase proton influx, which passively recruits chloride ions in order to maintain charge balance. The resulting accumulation of osmotic agents results in endosomal swelling and consequent lysis. Additionally, codelivery of chloroquine analogues has been shown to improve gene transfection through unclear mechanisms. There is some evidence that chloroquine can either act as a pH buffer, displace cationic complexes from nucleic acids, or alter biophysical properties of the released genetic material.[136]

If the nanocarrier does escape the endosome, depending on the genetic payload, the next challenge is nuclear targeting. Plasmid delivery requires translocation into the nucleus to attain transcription, whereas siRNA or miRNA activity resides in the cytoplasm. Additionally, cytosolic nucleases will eventually degrade cytosolic DNA or RNA, so effective translocation must occur prior to degradation. A variety of approaches appear to stabilize nucleic acids during transport. Polyplexes can potentially protect against cytosolic nucleases and travel along microtubules toward the nucleus via nonspecific charge interactions or even motor-protein driven transport.[137] Modulation of microtubules via histone deacetylase inhibitors has improved transfection by 10-fold.[138] Additionally, random redistribution during mitosis can result in gene uptake within the nucleus.[139] Nuclear localization signals (NLS), which are naturally occurring cationic peptides, are used to deliver proteins to the nucleus. Polyplexes may act similarly to NLS because of their inherent positive charge. NLS can also be conjugated to plasmids to improve nuclear targeting and transfection.[140] Co-delivery of *trans*-cyclohexane-1,2-diol (TCHD) has been shown to improve gene transfection through nonselective gating of the nuclear pore.[141] Within the nucleus, vector genome persistence may be an issue if the exogenous material does not integrate with the hose genome. The episomal DNA can persist in quiescent tissue; however, gene expression will become increasingly transient in rapidly dividing cells. Repeat dosing may then be required to sustain therapeutic transfection levels. Vector integration into the host genome can lead to greater persistence at the risk of gene disruption via insertional mutagenesis. Additionally, epigenetic alterations may disrupt gene expression regardless of genome integration. Persistence and sustained gene expression are vital for diseases requiring permanent gene expression. For acquired diseases like cancer, transient transfection may be adequate to achieve a therapeutic effect. Alternatively, minicircle DNA vectors lacking bacterial DNA have been expressed at high levels in vivo for extended periods, yet obstacles in their mass production have limited their use.[142] There has been significant strides to improve minicircle production using carefully engineered plasmids and culturing techniques.[87]

The host immune response is a significant barrier to efficient gene therapy. Particle components, extranuclear nucleic acids, and transgene products can activate an immune response. It is difficult to predict human immune responses because most animal models fail to replicate the human immune systems accurately. Generally, the immune response elicited by viral vectors is far more severe than that of nanoparticles, as most

DOI: 10.1021/ab500084g
ACS Biomater. Sci. Eng. 2015, 1, 64−78

particles are only as immunotoxic as their cargo. However, foreign DNA payloads can also illicit an interferon response that can potentially lead to immunotoxicity or decreased transfection efficiencies.[143] The inflammatory response may be due to the presence of unmethylated CpG dinucleotides present on plasmid DNA. Mutating the immunostimulatory CpG motifs or codelivering immunosuppressants can decrease the inflammatory response and elevate transgene expression.[144,145] Additionally, higher surface charges present on liposomes have induced secretion of cytokines including tumor necrosis factor (TNF), interleukin 12, nuclear factor $\kappa\beta$ (NF$\kappa\beta$), and interferon γ. However, these immunostimulatory effects may be beneficial in cancer therapies.[146−148]

The MPS plays a major role in nanoparticle clearance. Nanoparticles readily adsorb plasma proteins upon introduction to systemic circulation, and are consequently opsonized and phagocytized by the MPS. Due to hepatic and splenic filtration, particles tend to accumulate in those organs.[149] Smaller particles and a neutral surface charge result in lower levels of opsonization and phagocytosis. Surface modifications can reduce protein adsorption and entrapment within the MPS. For instance, PEG not only decreases protein adsorption and phagocytis into the MPS as discussed earlier, but it can also decrease platelet and erythrocyte interactions.[150] Unfortunately, the shielding effects of particle PEGylation are transient and can hinder target particle cell interactions as well. Poloxamer and poloxamine have been evaluated as potential alternatives to PEG, and have been shown to have similar benefits and drawbacks.[151,152] Additionally, surface functionalization with self-peptides, like CD47, can delay macrophage-mediated clearance.[153] Nanoparticle design is critically important to address the multiple barriers involved in gene transfection; Table 5 summarizes the common strategies utilized in particle engineering.

Targeting Tumors with Nanoparticles. Nanoparticle systems also have unique properties that allow for both passive and active targeting of tumors. Because of up regulation of proangiogenic signaling, most solid tumors are hypervascular. However, the new vessels have abnormal architecture and are highly permeable. The tumor mass also has poor lymphatic drainage, allowing for accumulation of macromolecules greater than approximately 40 kDa within its microenvironment. Nanoparticles exploit this feature, which is called the enhanced permeability and retention (EPR) effect, to target solid tumors. The ideal size range to benefit from the EPR effect is between 10 to 200 nm. Particles that are too small will be renally cleared, preventing accumulation into the tumor site, and particles that are too large will not adequately penetrate the tumor vasculature and interstitial space.[154,155]

Particle surface modifications can be incorporated to improve cell targeting and internalization while bypassing certain forms of multidrug resistance.[156] Nanoparticles coupled with surface ligands or antibodies can localize to tissue expressing the associated receptors or antigens and improve delivery efficacy.[157] Certain ligand receptor interactions will facilitate receptor-mediated endocytosis, which can further enhance payload delivery. Surface ligand or antibody coupling can achieve densities high enough to interact efficiently with target sites, and these techniques lend themselves well to cancer therapies.[158] New antibody-coated nanoparticles have even allowed for effective oral delivery.[159] Many tumors up-regulate growth factor receptors, such as ErbB2 in certain breast cancers, which can be targeted with anti-ErbB2 surface antibodies.[157]

Table 5. Strategies for Addressing Gene Delivery Barriers via Nanoparticles[a]

barrier	strategy
1. stability in transport and targeting	local delivery[156]
	encapsulation in lipid and polymer delivery systems
	tumor homing peptides[161]
	fabricate at optimal particle size for the EPR effect[155,164]
2. uptake	fabricate at optimal particle size for cellular uptake[119]
	ligand or CPP surface modifications[63,126−128,131,156,165]
3. endosomal escape	chloroquine analogues[136]
	pH sensitive, fusogenic, or synthetic peptides[132,133]
	histidine-rich peptides[132]
	"proton sponge" polymers[135]
4. transport into nucleus	nuclear pore gating with TCHD[141]
	nuclear targeting via NLS[124,132,140]
	transport along microtubules[137]
	HDAC inhibitors[138]
5. persistence and transcriptional activity	insertional vectors[87]
	minicircle DNA vectors[87,142]
	repeat dosing[87]
6. immune response	co-deliver immunosuppressants[145]
	fabricate at optimal particle size and surface charge[149]
	mutating immunostimulatory CpG motifs on plasmid DNA[144]
	surface modifications with PEG, poloxamer, or poloxamine[150,151]
	surface functionalization with self-peptides[153]

[a]EPR, enhanced permeation and retention; HDAC, histone deacetylase; PEG, polyethylene glycol; NLS, nuclear localization signal; TCHD, trans-cyclohexane-1,2-diol.

Various cancer lines up regulate surface antigens, including fetoprotein, human carcinoembryonic antigen, and human chorionic gonadotropin antigen, which provide targets for antibody mediated targeting.[63] Additionally, CPPs or targeting peptides can facilitate interactions with tumor cells or tumor endothelium. Established conjugation chemistries provide facile mechanisms for surface modifying polymer nanoparticles with targeting peptides. Zhou et al.[108] optimized multiple CPPs for improved particle endocytosis and Teesalu et al.[112,160] used the novel internalizing RGD (iRGD) peptide to target nanoparticles to the tumor endothelium. The RGD peptide sequence recognizes the $\alpha_v\beta_3/\alpha_v\beta_5$ integrins that are up-regulated on tumor endothelial cells. The cyclic iRGD peptide contains both the RGD sequence and a CendK/R element. The iRGD structure is readily cleaved by proteases, allowing for exposure of the CendK/R element and subsequent tissue penetration through binding of neuropilin-1.[161]

Active nanoparticle targeting as well as particle clearance by the liver, lungs, and MPS can be beneficial for treating advanced cancers and metastatic disease, which has proven to be resistant to conventional methods.[162] In addition to ligand-based targeting, nanoparticles can be delivered locally via intravenous catheters, inhalation, transdermal patches, or intravitreal administration.[156,163] Local delivery of chemotherapeutic agents can minimize several of the harmful side effects associated with common cancer therapies. New polymer delivery vehicles allow for targeted and combination cancer therapies, which can

DOI: 10.1021/ab500084g
ACS Biomater. Sci. Eng. 2015, 1, 64−78

ultimately decrease the development of drug resistance while simultaneously minimizing the side effect profile.

Combination Therapies. Delivering multiple agents in vivo is complicated because of their independent pharmacokinetics, biodistribution, and clearance.[166] Nanoparticle delivery systems can consolidate these properties into one vehicle and increase the likelihood that targeted tumor cells receive both agents at a ratiometric dose.[167] Therefore, once an optimal drug ratio is tuned in vitro, it can be translated to the clinic effectively. Combining multiple agents into one carrier can also streamline manufacturing and infusion processes, overcome batch-to-batch variability, and lower costs. The patient will also receive smaller doses of the nanocarriers, which can potentially lower toxicity. There have been several reports of codelivering multiple anticancer agents using nanocarriers, and some are reaching clinical trials.[168−172] Only a few of these approaches are capable of efficiently codelivering both small molecule drugs and genetic material in vivo.[170,173−175] As these carriers show considerable promise, improvements are still necessary to improve transfection potential, while maintaining ideal particle size, surface charge, loading, targeting, and biocompatibility. Additionally, evaluation of synergistic interactions is rare, especially when using nanoparticle vectors for codelivery, and several of the reported nanocarriers were only able to show improved anticancer effects at high doses. Table 6 highlights interesting nanoparticle formulations that effectively analyzed the delivery system for synergy, and were translated effectively to in vivo antitumor therapies.

Table 6. Combination Nanoparticle Formulations Translated to Effective in Vivo Antitumor Therapies[a]

formulation	therapeutics	synergy analysis
CPX-351 liposome (phase II clinical trials)[176]	cytarabine and daunorubicin	yes
CPX-1 liposome (phase II clinical trials)[177]	irinotecan and floxuridine	yes
CPX-571 liposome[178]	irinotecan and cisplatin	yes
pegylated liposome[179]	quercetin and vincristine	yes
triblock polymer micelle[180]	paclitaxel and Plk-1 siRNA	yes
PEGylated dendrimers[173,174]	doxorubicin and TRAIL encoded plasmid	yes
PLGA core with surface PEI and PEG[181]	camptothecin and TRAIL encoded plasmid	yes
PLGA core with block copolymer envelope[168]	doxorubicin and combretastatin	no
cationic amphiphilic copolymer[170]	paclitaxel and IL-12 encoded plasmid	no
pegylated liposome[175]	doxorubicin and c-Myc siRNA	no
aptamer-dendrimer conjugates[182]	doxorubicin and immune stimulating unmethylated CpG oligonucleotides.	no
dendritic PEG[183]	paclitaxel and alendronate	no

[a]IL-12, interleukin-12; PEG, polyethylene glycol; PEI, polyethylenimine; PLGA, poly(lactic-*co*-glycolic acid); Plk-1, polio-like kinase 1; siRNA, short interfering RNA.

Designing therapies with synergistic agents allows for reduced drug dosing and toxicity. Two agents act synergistically when their combined effect is greater than the sum of their individual effects. Analysis of synergism is complex and there are numerous methods for determining true synergism. Several of these methods contradict each other. Although commonly presented, the arithmetic sum of individual effects does not necessarily provide a cutoff for synergism, since you cannot have effect levels greater than one. Potency as well as efficacy is important in determining synergy, and therefore a dose response curve is necessary for accurate analysis. Chou and Talalay derived the combination index (CI) and median effect equation (MEE) in 1984, and have since established precedents for analyzing synergism.[184,185] The MEE is derived from the mass action law, and describes the behavior of many biological systems. In fact, the Michaelis−Menten, Hill, Henderson−Hasselbalch, and Scathcard equations can be derived from the MEE. Therefore, the mechanisms of action and conventional kinetic constants for the individual agents are not necessary to evaluate synergism via this method.

The linearized MEE uses dose response data to determine D_m, the median effective dose, and m, a Hill-type coefficient (eq 1). The coefficient for the linear regression is an indicator for the applicability of the Chou−Talalay analysis. At each effect level, the corresponding doses, $D_{alone,1}$, $D_{alone,2}$, $D_{comb,1}$, and $D_{comb,2}$, can be tabulated to determine a CI value using eq 2. CI values less than one indicate synergistic effects, values greater than one indicate antagonistic effects, and values equal to one indicate additive effects.[184,185] The doses for individual agents during the combined dose response analysis are determined using the known ratio between the two components. Finally, a calculated dose-reduction index (DRI) can provide the fold change in drug dosing required to achieve a similar effect (eq 3). A computer program, CompuSyn, is available to assist with the Chou−Talalay analysis.[186]

$$\log\left(\frac{f_a}{f_u}\right) = m\log(D) - m\log(D_m)$$

$$f_a + f_u = 1$$

$$\log(f_u^{-1} - 1) = m\log(D) - m\log(D_m) \quad (1)$$

$$\mathrm{CI} = \frac{D_{comb,1}}{D_{alone,1}} + \frac{D_{comb,2}}{D_{alone,2}} + \propto \frac{D_{comb,1}D_{comb,2}}{D_{alone,1}D_{alone,2}} \quad (2)$$

Where CI is combination index; $D_{alone,1}$, dose of drug 1; $D_{alone,2}$, dose of drug 2; $D_{comb,1}$, combination dose of drug 1; $D_{comb,2}$, combination dose of drug 2. For mutually exclusive drugs, $\propto = 0$, and for mutually nonexclusive drugs, $\propto = 1$.

$$\mathrm{DRI}_1 = D_{alone,1}/D_{comb,1} \quad (3)$$

Where DRI is dose-reduction index; $D_{alone,1}$, dose of drug 1; and $D_{comb,1}$, combination dose of drug 1.

CHALLENGES AND FUTURE DIRECTIONS

The EPR effect is often cited to support nanoparticle delivery into tumors. Yet, there is considerable variability of the EPR effect between not only the vasculature within tumors but also between tumor types and tumor models.[187] Therefore, it is difficult to predict clinical efficacy based on preliminary in vivo data. In fact, only 8% of successful animal studies are translated into clinical trials.[188] In clinical practice, it may be necessary to evaluate EPR activity in each specific patient with diagnostic nanoparticles. It is also possible to enhance the EPR effect by utilizing active tumor targeting, or coadministering agents to augment tumor vasculature and blood pressures.[189] Multiple studies evaluate nanoparticle efficacy using subcutaneous tumor

DOI: 10.1021/ab500084g
ACS Biomater. Sci. Eng. 2015, 1, 64−78

models, which may falsely overestimate high EPR vasculature. Although, there may be some limitations with utilizing the EPR effect, the technology to actively target to tumors can potentially overcome those shortcomings.[187] Metastatic, orthotopic, and genetically engineered animal models should provide more accurate cancer models, but need additional characterization. Advanced tumors will cause greater animal morbidity requiring earlier termination. It may be beneficial to utilize a hierarchal approach, where researchers screen with small subcutaneous xenografts and continue to evaluate therapies against larger tumors, orthotopic grafts, and ultimately with genetically engineered cancer models.[190]

Additional limitations for nanotherapy in cancer continue to be particle clearance and, in the case of gene delivery, inefficient transfection. This is complicated by the observation that particle properties that promote higher cellular uptake or gene transfection usually also produce greater cytotoxicity or clearance in vivo. In the case of combination therapies, careful synergy analysis is needed to provide the optimal ratio of therapeutics and potentially a lower loading requirement for the delivery vector. The field of cancer nanotherapy continues to make significant advancements through both increasing vector functionality and tumor targeting. There are many barriers to drug and gene delivery, yet the multifunctionality of nanoparticle systems allows for rational, stepwise optimizations for addressing these barriers. The evolving sophistication of nanotherapy systems allows for addressing heterogeneity and biological diversity that are now known to exist within the most aggressive cancers. For instance, new nanoparticle techniques are being used to target cancer stem cells which may be linked to treatment resistance.[191,192] And given their unique interactions with the immune system, nanoparticles are being evaluated as therapeutic vaccines, targeted monoclonal antibody treatments, and activators of cell based immune therapies.[193] The ability to deliver multimodal therapeutic agents can potentially provide synergistic therapies, leading to decreased dosing and associated toxicities. Improvements in materials design are providing new functionalities for therapeutic encapsulation and surface ligand conjugations for tumor targeting. Nanoparticle design has become increasingly sophisticated over the past few decades, and a rational approach to their design can potentially address many of the obstacles in current cancer therapies.

AUTHOR INFORMATION

Corresponding Author

*E-mail: mark.saltzman@yale.edu.

Author Contributions

Both authors contributed equally. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the Howard Hughes Medical Research Fellowship, the Yale School of Medicine Office of Student Research, and a grant from the National Institutes of Health (R01 CA149128).

REFERENCES

(1) The History of Cancer. http://www.cancer.org/acs/groups/cid/documents/webcontent/002048-pdf.pdf (accessed November 14, 2013).

(2) Mukherjee, S. *The Emperor of All Maladies: A Biography of Cancer*; Scribner: New York, 2010.

(3) Cravo, A.; Mrsny, R. A Time Travel Journey Through Cancer Therapies. In *Cancer Targeted Drug Delivery*, Bae, Y. H.; Mrsny, R. J.; Park, K., Eds. Springer: New York, 2013; pp 3−36.

(4) Edwards, B. K.; Noone, A.-M.; Mariotto, A. B.; Simard, E. P.; Boscoe, F. P.; Henley, S. J.; Jemal, A.; Cho, H.; Anderson, R. N.; Kohler, B. A.; Eheman, C. R.; Ward, E. M. Annual Report to the Nation on the status of cancer, 1975−2010, featuring prevalence of comorbidity and impact on survival among persons with lung, colorectal, breast, or prostate cancer. *Cancer* **2014**, *120*, 1290−1314.

(5) Cancer Facts & Figures 2013. http://www.cancer.org/acs/groups/content/@epidemiologysurveilance/documents/document/acspc-036845.pdf (accessed Novemmber 14, 2013).

(6) Hanahan, D.; Weinberg, R. A. Hallmarks of cancer: the next generation. *Cell* **2011**, *144*, 646−74.

(7) Luo, J.; Solimini, N. L.; Elledge, S. J. Principles of cancer therapy: oncogene and non-oncogene addiction. *Cell* **2009**, *136*, 823−37.

(8) Hanahan, D.; Weinberg, R. A. The hallmarks of cancer. *Cell* **2000**, *100*, 57−70.

(9) Esteller, M. Cancer epigenomics: DNA methylomes and histone-modification maps. *Nat. Rev. Genet* **2007**, *8*, 286−98.

(10) Biancotto, C.; Frige, G.; Minucci, S. Histone modification therapy of cancer. *Adv. Genet* **2010**, *70*, 341−86.

(11) Ellis, L.; Atadja, P. W.; Johnstone, R. W. Epigenetics in cancer: targeting chromatin modifications. *Mol. Cancer Ther* **2009**, *8*, 1409−20.

(12) Ganesan, A.; Nolan, L.; Crabb, S. J.; Packham, G. Epigenetic therapy: histone acetylation, DNA methylation and anti-cancer drug discovery. *Curr. Cancer Drug Targets* **2009**, *9*, 963−81.

(13) Yang, Z. J.; Chee, C. E.; Huang, S.; Sinicrope, F. A. The role of autophagy in cancer: therapeutic implications. *Mol. Cancer Ther* **2011**, *10*, 1533−41.

(14) Padua, D.; Massague, J. Roles of TGF beta in metastasis. *Cell Res.* **2009**, *19*, 89−102.

(15) Vokes, E. E.; Chu, E. Anti-EGFR therapies: clinical experience in colorectal, lung, and head and neck cancers. *Oncology (Williston Park)* **2006**, *20*, 15−25.

(16) Crawford, L. J.; Walker, B.; Irvine, A. E. Proteasome inhibitors in cancer therapy. *J. Cell Commun. Signal* **2011**, *5*, 101−10.

(17) Zhou, H.; Luo, Y.; Huang, S. Updates of mTOR inhibitors. *Anticancer Agents Med. Chem.* **2010**, *10*, 571−81.

(18) Holmes, D. PI3K pathway inhibitors approach junction. *Nat. Rev. Drug Discovery* **2011**, *10*, 563−4.

(19) Rodon, J.; Dienstmann, R.; Serra, V.; Tabernero, J. Development of PI3K inhibitors: lessons learned from early clinical trials. *Nat. Rev. Clin Oncol* **2013**, *10*, 143−53.

(20) West, A. C.; Johnstone, R. W. New and emerging HDAC inhibitors for cancer treatment. *J. Clin. Invest.* **2014**, *124*, 30−9.

(21) Dai, Y.; Grant, S. Cyclin-dependent kinase inhibitors. *Curr. Opin. Pharmacol.* **2003**, *3*, 362−370.

(22) Foon, K. A.; Safa, M. M. Activating the Immune System to Fight Cancer. *Oncology Issues* **2008**, 19.

(23) Porter, D. L.; Levine, B. L.; Kalos, M.; Bagg, A.; June, C. H. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. *N Engl. J. Med.* **2011**, *365*, 725−33.

(24) Fong, L.; Small, E. J. Anti-cytotoxic T-lymphocyte antigen-4 antibody: the first in an emerging class of immunomodulatory antibodies for cancer treatment. *J. Clin. Oncol.* **2008**, *26*, 5275−83.

(25) Topalian, S. L.; Drake, C. G.; Pardoll, D. M. Targeting the PD-1/B7-H1(PD-L1) pathway to activate anti-tumor immunity. *Curr. Opin. Immunol.* **2012**, *24*, 207−12.

(26) Shay, J. W.; Wright, W. E. Telomerase therapeutics for cancer: challenges and new directions. *Nat. Rev. Drug Discovery* **2006**, *5*, 577−84.

DOI: 10.1021/ab500084g
ACS Biomater. Sci. Eng. 2015, 1, 64−78

(27) Rayburn, E. R.; Ezell, S. J.; Zhang, R. Anti-Inflammatory Agents for Cancer Therapy. *Mol. Cell Pharmacol* **2009**, *1*, 29−43.
(28) Scagliotti, G. V.; Novello, S.; von Pawel, J. The emerging role of MET/HGF inhibitors in oncology. *Cancer Treat Rev.* **2013**, *39*, 793−801.
(29) Shojaei, F. Anti-angiogenesis therapy in cancer: current challenges and future perspectives. *Cancer Lett.* **2012**, *320*, 130−7.
(30) Davar, D.; Beumer, J. H.; Hamieh, L.; Tawbi, H. Role of PARP inhibitors in cancer biology and therapy. *Curr. Med. Chem.* **2012**, *19*, 3907−21.
(31) Billard, C. BH3 mimetics: status of the field and new developments. *Mol. Cancer Ther* **2013**, *12*, 1691−700.
(32) Lessene, G.; Czabotar, P. E.; Colman, P. M. BCL-2 family antagonists for cancer therapy. *Nat. Rev. Drug Discovery* **2008**, *7*, 989−1000.
(33) Bellail, A. C.; Qi, L.; Mulligan, P.; Chhabra, V.; Hao, C. TRAIL agonists on clinical trials for cancer therapy: the promises and the challenges. *Rev. Recent Clin Trials* **2009**, *4*, 34−41.
(34) Ashkenazi, A.; Herbst, R. S. To kill a tumor cell: the potential of proapoptotic receptor agonists. *J. Clin. Invest.* **2008**, *118*, 1979−90.
(35) Pelicano, H.; Martin, D. S.; Xu, R. H.; Huang, P. Glycolysis inhibition for anticancer treatment. *Oncogene* **2006**, *25*, 4633−4646.
(36) McAfee, Q.; Zhang, Z.; Samanta, A.; Levi, S. M.; Ma, X. H.; Piao, S.; Lynch, J. P.; Uehara, T.; Sepulveda, A. R.; Davis, L. E.; Winkler, J. D.; Amaravadi, R. K. Autophagy inhibitor Lys05 has single-agent antitumor activity and reproduces the phenotype of a genetic autophagy deficiency. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 8253−8.
(37) Chen, K.; Huang, Y. H.; Chen, J. L. Understanding and targeting cancer stem cells: therapeutic implications and challenges. *Acta Pharmacol. Sin.* **2013**, *34*, 732−40.
(38) Ball, S. G.; Shuttleworth, C. A.; Kielty, C. M. Platelet-derived growth factor receptors regulate mesenchymal stem cell fate: implications for neovascularization. *Expert Opin. Biol. Ther.* **2010**, *10*, 57−71.
(39) Gottesman, M. M. Mechanisms of cancer drug resistance. *Annu. Rev. Med.* **2002**, *53*, 615−27.
(40) Varmus, H. The new era in cancer research. *Science* **2006**, *312*, 1162−5.
(41) Shackleton, M.; Quintana, E.; Fearon, E. R.; Morrison, S. J. Heterogeneity in cancer: cancer stem cells versus clonal evolution. *Cell* **2009**, *138*, 822−9.
(42) Bonnet, D.; Dick, J. E. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat. Med.* **1997**, *3*, 730−737.
(43) Reya, T.; Morrison, S. J.; Clarke, M. F.; Weissman, I. L. Stem cells, cancer, and cancer stem cells. *Nature* **2001**, *414*, 105−11.
(44) Al-Hajj, M.; Wicha, M. S.; Benito-Hernandez, A.; Morrison, S. J.; Clarke, M. F. Prospective identification of tumorigenic breast cancer cells. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 3983−8.
(45) Li, X.; Lewis, M. T.; Huang, J.; Gutierrez, C.; Osborne, C. K.; Wu, M. F.; Hilsenbeck, S. G.; Pavlick, A.; Zhang, X.; Chamness, G. C.; Wong, H.; Rosen, J.; Chang, J. C. Intrinsic resistance of tumorigenic breast cancer cells to chemotherapy. *J. Natl. Cancer Inst.* **2008**, *100*, 672−9.
(46) Dalerba, P.; Dylla, S. J.; Park, I. K.; Liu, R.; Wang, X.; Cho, R. W.; Hoey, T.; Gurney, A.; Huang, E. H.; Simeone, D. M.; Shelton, A. A.; Parmiani, G.; Castelli, C.; Clarke, M. F. Phenotypic characterization of human colorectal cancer stem cells. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 10158−63.
(47) O'Brien, C. A.; Pollett, A.; Gallinger, S.; Dick, J. E. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. *Nature* **2007**, *445*, 106−10.
(48) Ricci-Vitiani, L.; Lombardi, D. G.; Pilozzi, E.; Biffoni, M.; Todaro, M.; Peschle, C.; De Maria, R. Identification and expansion of human colon-cancer-initiating cells. *Nature* **2007**, *445*, 111−5.
(49) Fang, D.; Nguyen, T. K.; Leishear, K.; Finko, R.; Kulp, A. N.; Hotz, S.; Van Belle, P. A.; Xu, X.; Elder, D. E.; Herlyn, M. A tumorigenic subpopulation with stem cell properties in melanomas. *Cancer Res.* **2005**, *65*, 9328−37.
(50) Bao, S.; Wu, Q.; McLendon, R. E.; Hao, Y.; Shi, Q.; Hjelmeland, A. B.; Dewhirst, M. W.; Bigner, D. D.; Rich, J. N. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature* **2006**, *444*, 756−60.
(51) Singh, S. K.; Hawkins, C.; Clarke, I. D.; Squire, J. A.; Bayani, J.; Hide, T.; Henkelman, R. M.; Cusimano, M. D.; Dirks, P. B. Identification of human brain tumour initiating cells. *Nature* **2004**, *432*, 396−401.
(52) Taylor, M. D.; Poppleton, H.; Fuller, C.; Su, X.; Liu, Y.; Jensen, P.; Magdaleno, S.; Dalton, J.; Calabrese, C.; Board, J.; Macdonald, T.; Rutka, J.; Guha, A.; Gajjar, A.; Curran, T.; Gilbertson, R. J. Radial glia cells are candidate stem cells of ependymoma. *Cancer Cell* **2005**, *8*, 323−35.
(53) Bozic, I.; Reiter, J. G.; Allen, B.; Antal, T.; Chatterjee, K.; Shah, P.; Moon, Y. S.; Yaqubie, A.; Kelly, N.; Le, D. T.; Lipson, E. J.; Chapman, P. B.; Diaz, L. A., Jr.; Vogelstein, B.; Nowak, M. A. Evolutionary dynamics of cancer in response to targeted combination therapy. *Elife* **2013**, *2*, e00747.
(54) Hannon, G. J. RNA interference. *Nature* **2002**, *418*, 244−51.
(55) Hannon, G. J.; Rossi, J. J. Unlocking the potential of the human genome with RNA interference. *Nature* **2004**, *431*, 371−8.
(56) McNeer, N. A.; Chin, J. Y.; Schleifman, E. B.; Fields, R. J.; Glazer, P. M.; Saltzman, W. M. Nanoparticles deliver triplex-forming PNAs for site-specific genomic recombination in CD34+ human hematopoietic progenitors. *Mol. Ther.* **2011**, *19*, 172−80.
(57) Frampton, G. M.; Fichtenholtz, A.; Otto, G. A.; Wang, K.; Downing, S. R.; He, J.; Schnall-Levin, M.; White, J.; Sanford, E. M.; An, P.; Sun, J.; Juhn, F.; Brennan, K.; Iwanik, K.; Maillet, A.; Buell, J.; White, E.; Zhao, M.; Balasubramanian, S.; Terzic, S.; Richards, T.; Banning, V.; Garcia, L.; Mahoney, K.; Zwirko, Z.; Donahue, A.; Beltran, H.; Mosquera, J. M.; Rubin, M. A.; Dogan, S.; Hedvat, C. V.; Berger, M. F.; Pusztai, L.; Lechner, M.; Boshoff, C.; Jarosz, M.; Vietz, C.; Parker, A.; Miller, V. A.; Ross, J. S.; Curran, J.; Cronin, M. T.; Stephens, P. J.; Lipson, D.; Yelensky, R. Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. *Nat. Biotechnol.* **2013**, *31*, 1023−31.
(58) Wagle, N.; Berger, M. F.; Davis, M. J.; Blumenstiel, B.; Defelice, M.; Pochanard, P.; Ducar, M.; Van Hummelen, P.; Macconaill, L. E.; Hahn, W. C.; Meyerson, M.; Gabriel, S. B.; Garraway, L. A. High-throughput detection of actionable genomic alterations in clinical tumor samples by targeted, massively parallel sequencing. *Cancer Discovery* **2012**, *2*, 82−93.
(59) Pritchard, C. C.; Salipante, S. J.; Koehler, K.; Smith, C.; Scroggins, S.; Wood, B.; Wu, D.; Lee, M. K.; Dintzis, S.; Adey, A.; Liu, Y.; Eaton, K. D.; Martins, R.; Stricker, K.; Margolin, K. A.; Hoffman, N.; Churpek, J. E.; Tait, J. F.; King, M. C.; Walsh, T. Validation and implementation of targeted capture and sequencing for the detection of actionable mutation, copy number variation, and gene rearrangement in clinical cancer specimens. *J. Mol. Diagn.* **2014**, *16*, 56−67.
(60) Ledford, H. "Master protocol" aims to revamp cancer trials. *Nature* **2013**, *498*, 146−147.
(61) Collins, J. Cancer Pharmacology. In *Abeloff's Clinical Oncology*, 5th ed.; Niederhuber, J. E.; Armitage, J. O.; Doroshow, J. H.; Kastan, M. B.; Tepper, J. E., Eds. Elsevier Health Sciences: Maryland Park, MO, 2013; pp 434−484.
(62) Harris, T.; Maltzahn, G.; Bhatia, S. N. Multifunctional Nanoparticles for Cancer Therapy. In *Nanotechnology for Cancer Therapy*; Amiji, M., Ed.; Taylor & Francis: Boca Raton, FL, 2006; pp 59−75.
(63) Sultana, S.; Khan, M. R.; Kumar, M.; Kumar, S.; Ali, M. Nanoparticles-mediated drug delivery approaches for cancer targeting: a review. *J. Drug Target* **2013**, *21*, 107−25.
(64) Kamaly, N.; Xiao, Z.; Valencia, P. M.; Radovic-Moreno, A. F.; Farokhzad, O. C. Targeted polymeric therapeutic nanoparticles: design, development and clinical translation. *Chem. Soc. Rev.* **2012**, *41*, 2971−3010.
(65) Ferrucci, J. T. Advances in abdominal MR imaging. *Radiographics* **1998**, *18*, 1569−86.

DOI: 10.1021/ab500084g
ACS Biomater. Sci. Eng. 2015, 1, 64−78

(66) Libutti, S. K.; Paciotti, G. F.; Byrnes, A. A.; Alexander, H. R., Jr.; Gannon, W. E.; Walker, M.; Seidel, G. D.; Yuldasheva, N.; Tamarkin, L. Phase I and pharmacokinetic studies of CYT-6091, a novel PEGylated colloidal gold-rhTNF nanomedicine. *Clin. Cancer Res.* **2010**, *16*, 6139−49.
(67) Barenholz, Y. Doxil(R)−the first FDA-approved nano-drug: lessons learned. *J. Controlled Release* **2012**, *160*, 117−34.
(68) Azim, H. A., Jr.; Awada, A. Clinical development of new formulations of cytotoxics in solid tumors. *Curr. Opin Oncol* **2012**, *24*, 325−31.
(69) Hawkins, M. J.; Soon-Shiong, P.; Desai, N. Protein nanoparticles as drug carriers in clinical medicine. *Adv. Drug Delivery Rev.* **2008**, *60*, 876−885.
(70) Ledet, G.; Mandal, T. K. Nanomedicine: Emerging therapeutics for the 21st century. *U.S. Pharm* **2012**, *37*, 7−11.
(71) O'Brien, M. E.; Socinski, M. A.; Popovich, A. Y.; Bondarenko, I. N.; Tomova, A.; Bilynsky, B. T.; Hotko, Y. S.; Ganul, V. L.; Kostinsky, I. Y.; Eisenfeld, A. J.; Sandalic, L.; Oldham, F. B.; Bandstra, B.; Sandler, A. B.; Singer, J. W. Randomized phase III trial comparing single-agent paclitaxel Poliglumex (CT-2103, PPX) with single-agent gemcitabine or vinorelbine for the treatment of PS 2 patients with chemotherapy-naive advanced non-small cell lung cancer. *J. Thorac. Oncol.* **2008**, *3*, 728−34.
(72) Dinndorf, P. A.; Gootenberg, J.; Cohen, M. H.; Keegan, P.; Pazdur, R. FDA drug approval summary: pegaspargase (oncaspar) for the first-line treatment of children with acute lymphoblastic leukemia (ALL). *Oncologist* **2007**, *12*, 991−8.
(73) Sanna, V.; Pala, N.; Sechi, M. Targeted therapy using nanotechnology: focus on cancer. *Int. J. Nanomedicine* **2014**, *9*, 467−83.
(74) Theuer, C. P.; Leigh, B. R.; Multani, P. S.; Allen, R. S.; Liang, B. C. Radioimmunotherapy of non-Hodgkin's lymphoma: clinical development of the Zevalin regimen. *Biotechnol. Annu. Rev.* **2004**, *10*, 265−95.
(75) Folkman, J.; Long, D. M. The use of silicone rubber as a carrier for prolonged drug therapy. *J. Surg Res.* **1964**, *4*, 139−142.
(76) Langer, R. New methods of drug delivery. *Science* **1990**, *249*, 1527−33.
(77) Goldberg, M.; Langer, R.; Jia, X. Nanostructured materials for applications in drug delivery and tissue engineering. *J. Biomater Sci. Polym. Ed* **2007**, *18*, 241−68.
(78) Chaubal, M. Polylactides/glycolides-excipients for injectable drug delivery and beyond. *Drug Delivery Technology* **2002**, *2*, 34−36.
(79) Whitehead, K. A.; Langer, R.; Anderson, D. G. Knocking down barriers: advances in siRNA delivery. *Nat. Rev. Drug Discovery* **2009**, *8*, 129−38.
(80) Cheng, J.; Teply, B. A.; Sherifi, I.; Sung, J.; Luther, G.; Gu, F. X.; Levy-Nissenbaum, E.; Radovic-Moreno, A. F.; Langer, R.; Farokhzad, O. C. Formulation of functionalized PLGA-PEG nanoparticles for in vivo targeted drug delivery. *Biomaterials* **2007**, *28*, 869−76.
(81) Panyam, J.; Labhasetwar, V. Biodegradable nanoparticles for drug and gene delivery to cells and tissue. *Adv. Drug Deliv Rev.* **2003**, *55*, 329−47.
(82) Buchwald, P.; Bodor, N. Octanol-water partition: searching for predictive models. *Curr. Med. Chem.* **1998**, *5*, 353−80.
(83) Park, T. G. Degradation of poly(lactic-co-glycolic acid) microspheres: effect of copolymer composition. *Biomaterials* **1995**, *16*, 1123−30.
(84) Panyam, J.; Labhasetwar, V. Biodegradable nanoparticles for drug and gene delivery to cells and tissue. *Adv. Drug Delivery Rev.* **2012**, *64*, 61−71.
(85) Jokerst, J. V.; Lobovkina, T.; Zare, R. N.; Gambhir, S. S. Nanoparticle PEGylation for imaging and therapy. *Nanomedicine (Lond)* **2011**, *6*, 715−28.
(86) Deng, Y.; Saucier-Sawyer, J. K.; Hoimes, C. J.; Zhang, J.; Seo, Y. E.; Andrejecsk, J. W.; Saltzman, W. M. The effect of hyperbranched polyglycerol coatings on drug delivery using degradable polymer nanoparticles. *Biomaterials* **2014**, *35*, 6595−602.
(87) Kay, M. A. State-of-the-art gene-based therapies: the road ahead. *Nat. Rev. Genet* **2011**, *12*, 316−28.
(88) Urnov, F. D.; Miller, J. C.; Lee, Y. L.; Beausejour, C. M.; Rock, J. M.; Augustus, S.; Jamieson, A. C.; Porteus, M. H.; Gregory, P. D.; Holmes, M. C. Highly efficient endogenous human gene correction using designed zinc-finger nucleases. *Nature* **2005**, *435*, 646−651.
(89) Hwang, W. Y.; Fu, Y. F.; Reyon, D.; Maeder, M. L.; Tsai, S. Q.; Sander, J. D.; Peterson, R. T.; Yeh, J. R. J.; Joung, J. K. Efficient genome editing in zebrafish using a CRISPR-Cas system. *Nat. Biotechnol.* **2013**, *31*, 227−229.
(90) Mali, P.; Yang, L.; Esvelt, K. M.; Aach, J.; Guell, M.; DiCarlo, J. E.; Norville, J. E.; Church, G. M. RNA-guided human genome engineering via Cas9. *Science* **2013**, *339*, 823−6.
(91) Bordignon, C.; Notarangelo, L. D.; Nobili, N.; Ferrari, G.; Casorati, G.; Panina, P.; Mazzolari, E.; Maggioni, D.; Rossi, C.; Servida, P.; Ugazio, A. G.; Mavilio, F. Gene therapy in peripheral blood lymphocytes and bone marrow for ADA- immunodeficient patients. *Science* **1995**, *270*, 470−5.
(92) Hacein-Bey-Abina, S.; Le Deist, F.; Carlier, F.; Bouneaud, C.; Hue, C.; De Villartay, J. P.; Thrasher, A. J.; Wulffraat, N.; Sorensen, R.; Dupuis-Girod, S.; Fischer, A.; Davies, E. G.; Kuis, W.; Leiva, L.; Cavazzana-Calvo, M. Sustained correction of X-linked severe combined immunodeficiency by ex vivo gene therapy. *N Engl J. Med.* **2002**, *346*, 1185−93.
(93) Cavazzana-Calvo, M.; Hacein-Bey, S.; de Saint Basile, G.; Gross, F.; Yvon, E.; Nusbaum, P.; Selz, F.; Hue, C.; Certain, S.; Casanova, J. L.; Bousso, P.; Deist, F. L.; Fischer, A. Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease. *Science* **2000**, *288*, 669−72.
(94) Puumalainen, A. M.; Vapalahti, M.; Agrawal, R. S.; Kossila, M.; Laukkanen, J.; Lehtolainen, P.; Viita, H.; Paljarvi, L.; Vanninen, R.; Yla-Herttuala, S. beta-galactosidase gene transfer to human malignant glioma in vivo using replication-deficient retroviruses and adenoviruses. *Hum. Gene Ther.* **1998**, *9*, 1769−1774.
(95) Wirth, T.; Parker, N.; Yla-Herttuala, S. History of gene therapy. *Gene* **2013**, *525*, 162−9.
(96) Ginn, S. L.; Alexander, I. E.; Edelstein, M. L.; Abedi, M. R.; Wixon, J. Gene therapy clinical trials worldwide to 2012 - an update. *J. Gene Med.* **2013**, *15*, 65−77.
(97) Pearson, S.; Jia, H.; Kandachi, K. China approves first gene therapy. *Nat. Biotechnol.* **2004**, *22*, 3−4.
(98) Young, S. When Will Gene Therapy Come to the U.S.? *MIT Technology Review* **2013**. Published Online: September 30, 2013. http://www.technologyreview.com/news/519071/when-will-gene-therapy-come-to-the-us/ (accessed December 9, **2013**).
(99) Grupp, S. A.; Kalos, M.; Barrett, D.; Aplenc, R.; Porter, D. L.; Rheingold, S. R.; Teachey, D. T.; Chew, A.; Hauck, B.; Wright, J. F.; Milone, M. C.; Levine, B. L.; June, C. H. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. *N Engl J. Med.* **2013**, *368*, 1509−18.
(100) Kalos, M.; Levine, B. L.; Porter, D. L.; Katz, S.; Grupp, S. A.; Bagg, A.; June, C. H. T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia. *Sci. Transl Med.* **2011**, *3*, 95ra73.
(101) Kalos, M.; Nazimuddin, F.; Finklestein, J. M.; Gupta, M.; Kulikovskaya, I.; Ambrose, D. E.; Gill, S.; Lacey, S. F.; Zheng, Z.; Melenhorst, J. J.; Levine, B. L.; Frey, N. V.; Grupp, S. A.; Porter, D. L.; Carl H. June, M. Long-Term Functional Persistence, B Cell Aplasia and Anti-Leukemia Efficacy In Refractory B Cell Malignancies Following T Cell Immunotherapy Using CAR-Redirected T Cells Targeting CD19. In *American Society of Hematology Annual Meeting*; New Orleans, Dec 7−10, 2013 ; American Society of Hematology: Washington, D.C., 2013.
(102) Stolberg, S. G. The biotech death of Jesse Gelsinger. *N.Y. Times Mag.* **1999**, 136−140, 149−150.
(103) Hacein-Bey-Abina, S.; Garrigue, A.; Wang, G. P.; Soulier, J.; Lim, A.; Morillon, E.; Clappier, E.; Caccavelli, L.; Delabesse, E.; Beldjord, K.; Asnafi, V.; MacIntyre, E.; Dal Cortivo, L.; Radford, I.; Brousse, N.; Sigaux, F.; Moshous, D.; Hauer, J.; Borkhardt, A.;

DOI: 10.1021/ab500084g
ACS Biomater. Sci. Eng. 2015, 1, 64−78

Belohradsky, B. H.; Wintergerst, U.; Velez, M. C.; Leiva, L.; Sorensen, R.; Wulffraat, N.; Blanche, S.; Bushman, F. D.; Fischer, A.; Cavazzana-Calvo, M. Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1. *J. Clin. Invest.* **2008**, *118*, 3132–42.

(104) Howe, S. J.; Mansour, M. R.; Schwarzwaelder, K.; Bartholomae, C.; Hubank, M.; Kempski, H.; Brugman, M. H.; Pike-Overzet, K.; Chatters, S. J.; de Ridder, D.; Gilmour, K. C.; Adams, S.; Thornhill, S. I.; Parsley, K. L.; Staal, F. J.; Gale, R. E.; Linch, D. C.; Bayford, J.; Brown, L.; Quaye, M.; Kinnon, C.; Ancliff, P.; Webb, D. K.; Schmidt, M.; von Kalle, C.; Gaspar, H. B.; Thrasher, A. J. Insertional mutagenesis combined with acquired somatic mutations causes leukemogenesis following gene therapy of SCID-X1 patients. *J. Clin. Invest.* **2008**, *118*, 3143–50.

(105) Liu, C.; Zhang, N. Nanoparticles in Gene Therapy: Principles, Prospects, and Challenges. In *Progress in Molecular Biology and Translational Science*, Antonio, V., Ed.; Academic Press: New York, 2011; Vol. *104*, pp 509–562.

(106) Gascón, A. R.; Pozo-Rodríguez, A. d.; Solinís, M. A. Non-Viral Delivery Systems in Gene Therapy. In *Gene Therapy—Tools and Potential Applications*; Molina, F. M., Ed. InTech: Rijeka, Croatia, 2013; pp 3–33.

(107) Kabanov, A. V. Polymer genomics: An insight into pharmacology and toxicology of nanomedicines. *Adv. Drug Delivery Rev.* **2006**, *58*, 1597–1621.

(108) Zhou, J.; Patel, T. R.; Fu, M.; Bertram, J. P.; Saltzman, W. M. Octa-functional PLGA nanoparticles for targeted and efficient siRNA delivery to tumors. *Biomaterials* **2012**, *33*, 583–91.

(109) Woodrow, K. A.; Cu, Y.; Booth, C. J.; Saucier-Sawyer, J. K.; Wood, M. J.; Saltzman, W. M. Intravaginal gene silencing using biodegradable polymer nanoparticles densely loaded with small-interfering RNA. *Nat. Mater.* **2009**, *8*, 526–533.

(110) Pathak, A.; Patnaik, S.; Gupta, K. C. Recent trends in non-viral vector-mediated gene delivery. *Biotechnol. J.* **2009**, *4*, 1559–72.

(111) Luo, D.; Saltzman, W. M. Synthetic DNA delivery systems. *Nat. Biotechnol.* **2000**, *18*, 33–7.

(112) Zhou, J.; Liu, J.; Cheng, C. J.; Patel, T. R.; Weller, C. E.; Piepmeier, J. M.; Jiang, Z.; Saltzman, W. M. Biodegradable poly(amine-co-ester) terpolymers for targeted gene delivery. *Nat. Mater.* **2012**, *11*, 82–90.

(113) Patil, S. D.; Rhodes, D. G.; Burgess, D. J. DNA-based therapeutics and DNA delivery systems: a comprehensive review. *AAPS J.* **2005**, *7*, E61–77.

(114) Hofland, H. E.; Shephard, L.; Sullivan, S. M. Formation of stable cationic lipid/DNA complexes for gene transfer. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 7305–9.

(115) Moghimi, S. M.; Symonds, P.; Murray, J. C.; Hunter, A. C.; Debska, G.; Szewczyk, A. A two-stage poly(ethylenimine)-mediated cytotoxicity: implications for gene transfer/therapy. *Mol. Ther.* **2005**, *11*, 990–5.

(116) Nel, A. E.; Madler, L.; Velegol, D.; Xia, T.; Hoek, E. M. V.; Somasundaran, P.; Klaessig, F.; Castranova, V.; Thompson, M. Understanding biophysicochemical interactions at the nano-bio interface. *Nat. Mater.* **2009**, *8*, 543–557.

(117) Baeuerle, P. A.; Huttner, W. B. Chlorate–a potent inhibitor of protein sulfation in intact cells. *Biochem. Biophys. Res. Commun.* **1986**, *141*, 870–7.

(118) Mounkes, L. C.; Zhong, W.; Cipres-Palacin, G.; Heath, T. D.; Debs, R. J. Proteoglycans mediate cationic liposome-DNA complex-based gene delivery in vitro and in vivo. *J. Biol. Chem.* **1998**, *273*, 26164–70.

(119) Zhang, S.; Li, J.; Lykotrafitis, G.; Bao, G.; Suresh, S. Size-Dependent Endocytosis of Nanoparticles. *Adv. Mater.* **2009**, *21*, 419–424.

(120) Rejman, J.; Oberle, V.; Zuhorn, I. S.; Hoekstra, D. Size-dependent internalization of particles via the pathways of clathrin- and caveolae-mediated endocytosis. *Biochem. J.* **2004**, *377*, 159–69.

(121) Endocytosis and the Endosomal Membrane System. In *Cell Biology*, 2nd ed.; Pollard, T. D., Earnshaw, W. C., Lippincott-Schwartz, J., Eds.; Elsevier Health Sciences: Maryland Heights, MO, 2007; pp 391–407.

(122) Sahay, G.; Alakhova, D. Y.; Kabanov, A. V. Endocytosis of nanomedicines. *J. Controlled Release* **2010**, *145*, 182–95.

(123) Mosesson, Y.; Mills, G. B.; Yarden, Y. Derailed endocytosis: an emerging feature of cancer. *Nat. Rev. Cancer* **2008**, *8*, 835–50.

(124) Pichon, C.; Billiet, L.; Midoux, P. Chemical vectors for gene delivery: uptake and intracellular trafficking. *Curr. Opin. Biotechnol.* **2010**, *21*, 640–5.

(125) Madani, F.; Lindberg, S.; Langel, U.; Futaki, S.; Graslund, A. Mechanisms of cellular uptake of cell-penetrating peptides. *J. Biophys.* **2011**, *2011*, 414729.

(126) Nakase, I.; Niwa, M.; Takeuchi, T.; Sonomura, K.; Kawabata, N.; Koike, Y.; Takehashi, M.; Tanaka, S.; Ueda, K.; Simpson, J. C.; Jones, A. T.; Sugiura, Y.; Futaki, S. Cellular uptake of arginine-rich peptides: roles for macropinocytosis and actin rearrangement. *Mol. Ther.* **2004**, *10*, 1011–22.

(127) Nakase, I.; Takeuchi, T.; Tanaka, G.; Futaki, S. Methodological and cellular aspects that govern the internalization mechanisms of arginine-rich cell-penetrating peptides. *Adv. Drug Deliv Rev.* **2008**, *60*, 598–607.

(128) Richard, J. P.; Melikov, K.; Brooks, H.; Prevot, P.; Lebleu, B.; Chernomordik, L. V. Cellular uptake of unconjugated TAT peptide involves clathrin-dependent endocytosis and heparan sulfate receptors. *J. Biol. Chem.* **2005**, *280*, 15300–6.

(129) Rothberg, K. G.; Ying, Y. S.; Kolhouse, J. F.; Kamen, B. A.; Anderson, R. G. W. The Glycophospholipid-Linked Folate Receptor Internalizes Folate without Entering the Clathrin-Coated Pit Endocytic Pathway. *J. Cell Biol.* **1990**, *110*, 637–649.

(130) Tsuji, T.; Yoshitomi, H.; Usukura, J. Endocytic mechanism of transferrin-conjugated nanoparticles and the effects of their size and ligand number on the efficiency of drug delivery. *Microscopy* **2013**, *62*, 341–52.

(131) Cheng, C. J.; Saltzman, W. M. Enhanced siRNA delivery into cells by exploiting the synergy between targeting ligands and cell-penetrating peptides. *Biomaterials* **2011**, *32*, 6194–203.

(132) Medina-Kauwe, L. K.; Xie, J.; Hamm-Alvarez, S. Intracellular trafficking of nonviral vectors. *Gene Ther.* **2005**, *12*, 1734–51.

(133) Wagner, E.; Plank, C.; Zatloukal, K.; Cotten, M.; Birnstiel, M. L. Influenza virus hemagglutinin HA-2 N-terminal fusogenic peptides augment gene transfer by transferrin-polylysine-DNA complexes: toward a synthetic virus-like gene-transfer vehicle. *Proc. Natl. Acad. Sci. U. S. A.* **1992**, *89*, 7934–8.

(134) Pack, D. W.; Hoffman, A. S.; Pun, S.; Stayton, P. S. Design and development of polymers for gene delivery. *Nat. Rev. Drug Discovery* **2005**, *4*, 581–93.

(135) Sonawane, N. D.; Szoka, F. C., Jr.; Verkman, A. S. Chloride accumulation and swelling in endosomes enhances DNA transfer by polyamine-DNA polyplexes. *J. Biol. Chem.* **2003**, *278*, 44826–31.

(136) Cheng, J.; Zeidan, R.; Mishra, S.; Liu, A.; Pun, S. H.; Kulkarni, R. P.; Jensen, G. S.; Bellocq, N. C.; Davis, M. E. Structure-function correlation of chloroquine and analogues as transgene expression enhancers in nonviral gene delivery. *J. Med. Chem.* **2006**, *49*, 6522–31.

(137) Suh, J.; Wirtz, D.; Hanes, J. Efficient active transport of gene nanocarriers to the cell nucleus. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 3878–82.

(138) Vaughan, E. E.; Geiger, R. C.; Miller, A. M.; Loh-Marley, P. L.; Suzuki, T.; Miyata, N.; Dean, D. A. Microtubule acetylation through HDAC6 inhibition results in increased transfection efficiency. *Mol. Ther.* **2008**, *16*, 1841–7.

(139) Brunner, S.; Sauer, T.; Carotta, S.; Cotten, M.; Saltik, M.; Wagner, E. Cell cycle dependence of gene transfer by lipoplex, polyplex and recombinant adenovirus. *Gene Ther.* **2000**, *7*, 401–7.

(140) Branden, L. J.; Mohamed, A. J.; Smith, C. I. E. A peptide nucleic acid-nuclear localization signal fusion that mediates nuclear transport of DNA. *Nat. Biotechnol.* **1999**, *17*, 784–787.

(141) Vandenbroucke, R. E.; Lucas, B.; Demeester, J.; De Smedt, S. C.; Sanders, N. N. Nuclear accumulation of plasmid DNA can be

DOI: 10.1021/ab500084g
ACS Biomater. Sci. Eng. 2015, 1, 64–78

enhanced by non-selective gating of the nuclear pore. *Nucleic Acids Res.* **2007**, *35*, e86.
(142) Chen, Z. Y.; He, C. Y.; Ehrhardt, A.; Kay, M. A. Minicircle DNA vectors devoid of bacterial DNA result in persistent and high-level transgene expression in vivo. *Mol. Ther.* **2003**, *8*, 495−500.
(143) Sui, H.; Zhou, M.; Chen, Q.; Lane, H. C.; Imamichi, T. siRNA enhances DNA-mediated interferon lambda-1 response through crosstalk between RIG-I and IFI16 signalling pathway. *Nucleic Acids Res.* **2014**, *42*, 583−98.
(144) Tan, Y.; Li, S.; Pitt, B. R.; Huang, L. The inhibitory role of CpG immunostimulatory motifs in cationic lipid vector-mediated transgene expression in vivo. *Hum. Gene Ther.* **1999**, *10*, 2153−61.
(145) Wang, Z.; Kuhr, C. S.; Allen, J. M.; Blankinship, M.; Gregorevic, P.; Chamberlain, J. S.; Tapscott, S. J.; Storb, R. Sustained AAV-mediated dystrophin expression in a canine model of Duchenne muscular dystrophy with a brief course of immunosuppression. *Mol. Ther.* **2007**, *15*, 1160−6.
(146) Dileo, J.; Banerjee, R.; Whitmore, M.; Nayak, J. V.; Falo, L. D., Jr.; Huang, L. Lipid-protamine-DNA-mediated antigen delivery to antigen-presenting cells results in enhanced anti-tumor immune responses. *Mol. Ther.* **2003**, *7*, 640−8.
(147) Whitmore, M. M.; Li, S.; Falo, L., Jr.; Huang, L. Systemic administration of LPD prepared with CpG oligonucleotides inhibits the growth of established pulmonary metastases by stimulating innate and acquired antitumor immune responses. *Cancer Immunol Immunother* **2001**, *50*, 503−14.
(148) Diwan, M.; Elamanchili, P.; Cao, M.; Samuel, J. Dose sparing of CpG oligodeoxynucleotide vaccine adjuvants by nanoparticle delivery. *Curr. Drug Deliv* **2004**, *1*, 405−12.
(149) Alexis, F.; Pridgen, E.; Molnar, L. K.; Farokhzad, O. C. Factors affecting the clearance and biodistribution of polymeric nanoparticles. *Mol. Pharmaceutics* **2008**, *5*, 505−15.
(150) Dobrovolskaia, M. A.; McNeil, S. E. Immunological properties of engineered nanomaterials. *Nat. Nanotechnol.* **2007**, *2*, 469−78.
(151) Goppert, T. M.; Muller, R. H. Protein adsorption patterns on poloxamer- and poloxamine-stabilized solid lipid nanoparticles (SLN). *Eur. J. Pharm. Biopharm* **2005**, *60*, 361−72.
(152) Torchilin, V. P. Multifunctional nanocarriers. *Adv. Drug Delivery Rev.* **2012**, *64*, 302−315.
(153) Rodriguez, P. L.; Harada, T.; Christian, D. A.; Pantano, D. A.; Tsai, R. K.; Discher, D. E. Minimal "Self" peptides that inhibit phagocytic clearance and enhance delivery of nanoparticles. *Science* **2013**, *339*, 971−5.
(154) Danhier, F.; Feron, O.; Préat, V. To exploit the tumor microenvironment: Passive and active tumor targeting of nanocarriers for anti-cancer drug delivery. *J. Controlled Release* **2010**, *148*, 135−146.
(155) Fang, J.; Sawa, T.; Maeda, H. Factors and Mechanism of "EPR" Effect and the Enhanced Antitumor Effects of Macromolecular Drugs Including SMANCS. In *Polymer Drugs in the Clinical Stage*; Maeda, H.; Kabanov, A.; Kataoka, K.; Okano, T., Eds.; Springer: New York, 2003; Vol. *519*, pp 29−49.
(156) Fahmy, T. M.; Fong, P. M.; Goyal, A.; Saltzman, W. M. Targeted for drug delivery. *Mater. Today* **2005**, *8*, 18−26.
(157) Peer, D.; Karp, J. M.; Hong, S.; FarokHzad, O. C.; Margalit, R.; Langer, R. Nanocarriers as an emerging platform for cancer therapy. *Nat. Nanotechnol.* **2007**, *2*, 751−760.
(158) Davis, M. E.; Chen, Z.; Shin, D. M. Nanoparticle therapeutics: an emerging treatment modality for cancer. *Nat. Rev. Drug Discovery* **2008**, *7*, 771−782.
(159) Pridgen, E. M.; Alexis, F.; Kuo, T. T.; Levy-Nissenbaum, E.; Karnik, R.; Blumberg, R. S.; Langer, R.; Farokhzad, O. C. Transepithelial Transport of Fc-Targeted Nanoparticles by the Neonatal Fc Receptor for Oral Delivery. *Sci. Transl. Med.* **2013**, *5*, 213ra167.
(160) Teesalu, T.; Sugahara, K. N.; Kotamraju, V. R.; Ruoslahti, E. C-end rule peptides mediate neuropilin-1-dependent cell, vascular, and tissue penetration. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 16157−62.
(161) Sugahara, K. N.; Teesalu, T.; Karmali, P. P.; Kotamraju, V. R.; Agemy, L.; Girard, O. M.; Hanahan, D.; Mattrey, R. F.; Ruoslahti, E. Tissue-penetrating delivery of compounds and nanoparticles into tumors. *Cancer Cell* **2009**, *16*, 510−20.
(162) Schroeder, A.; Heller, D. A.; Winslow, M. M.; Dahlman, J. E.; Pratt, G. W.; Langer, R.; Jacks, T.; Anderson, D. G. Treating metastatic cancer with nanotechnology. *Nat. Rev. Cancer* **2012**, *12*, 39−50.
(163) Cheng, C. J.; Tietjen, G. T.; Saucier-Sawyer, J. K.; Saltzman, W. M. A holistic approach to targeting disease with polymer nanoparticles. *Nat. Rev. Drug Discovery* **2015**, 10.1038/nrd4503.
(164) Danhier, F.; Feron, O.; Preat, V. To exploit the tumor microenvironment: Passive and active tumor targeting of nanocarriers for anti-cancer drug delivery. *J. Controlled Release* **2010**, *148*, 135−46.
(165) Heitz, F.; Morris, M. C.; Divita, G. Twenty years of cell-penetrating peptides: from molecular mechanisms to therapeutics. *Br. J. Pharmacol.* **2009**, *157*, 195−206.
(166) Pinto, A. C.; Moreira, J. o. N.; Simões, S. r. Combination Chemotherapy in Cancer: Principles, Evaluation and Drug Delivery Strategies. In *Current Cancer Treatment—Novel Beyond Conventional Approaches*; Ozdemir, O., Ed.; InTech: Rijeka, Croatia, 2011; Chapter 29, pp 693−714; http://www.intechopen.com/books/current-cancer-treatment-novel-beyond-conventional-approaches/combination-chemotherapy-in-cancer-principles-evaluation-and-drug-delivery-strategies.
(167) Hu, C.-M. J.; Zhang, L. Nanoparticle-based combination therapy toward overcoming drug resistance in cancer. *Biochem. Pharmacol.* **2012**, *83*, 1104−1111.
(168) Sengupta, S.; Eavarone, D.; Capila, I.; Zhao, G.; Watson, N.; Kiziltepe, T.; Sasisekharan, R. Temporal targeting of tumour cells and neovasculature with a nanoscale delivery system. *Nature* **2005**, *436*, 568−72.
(169) Wang, H.; Zhao, P.; Su, W.; Wang, S.; Liao, Z.; Niu, R.; Chang, J. PLGA/polymeric liposome for targeted drug and gene co-delivery. *Biomaterials* **2010**, *31*, 8741−8.
(170) Wang, Y.; Gao, S.; Ye, W. H.; Yoon, H. S.; Yang, Y. Y. Co-delivery of drugs and DNA from cationic core-shell nanoparticles self-assembled from a biodegradable copolymer. *Nat. Mater.* **2006**, *5*, 791−6.
(171) Kaneshiro, T. L.; Lu, Z. R. Targeted intracellular codelivery of chemotherapeutics and nucleic acid with a well-defined dendrimer-based nanoglobular carrier. *Biomaterials* **2009**, *30*, 5660−6.
(172) Hu, C. M.; Aryal, S.; Zhang, L. Nanoparticle-assisted combination therapies for effective cancer treatment. *Ther Deliv* **2010**, *1*, 323−34.
(173) Han, L.; Huang, R.; Li, J.; Liu, S.; Huang, S.; Jiang, C. Plasmid pORF-hTRAIL and doxorubicin co-delivery targeting to tumor using peptide-conjugated polyamidoamine dendrimer. *Biomaterials* **2011**, *32*, 1242−52.
(174) Liu, S.; Guo, Y.; Huang, R.; Li, J.; Huang, S.; Kuang, Y.; Han, L.; Jiang, C. Gene and doxorubicin co-delivery system for targeting therapy of glioma. *Biomaterials* **2012**, *33*, 4907−16.
(175) Chen, Y.; Bathula, S. R.; Li, J.; Huang, L. Multifunctional nanoparticles delivering small interfering RNA and doxorubicin overcome drug resistance in cancer. *J. Biol. Chem.* **2010**, *285*, 22639−50.
(176) Tardi, P.; Johnstone, S.; Harasym, N.; Xie, S.; Harasym, T.; Zisman, N.; Harvie, P.; Bermudes, D.; Mayer, L. In vivo maintenance of synergistic cytarabine:daunorubicin ratios greatly enhances therapeutic efficacy. *Leuk Res.* **2009**, *33*, 129−39.
(177) Harasym, T. O.; Tardi, P. G.; Harasym, N. L.; Harvie, P.; Johnstone, S. A.; Mayer, L. D. Increased preclinical efficacy of irinotecan and floxuridine coencapsulated inside liposomes is associated with tumor delivery of synergistic drug ratios. *Oncol Res.* **2007**, *16*, 361−74.
(178) Tardi, P. G.; Dos Santos, N.; Harasym, T. O.; Johnstone, S. A.; Zisman, N.; Tsang, A. W.; Bermudes, D. G.; Mayer, L. D. Drug ratio-dependent antitumor activity of irinotecan and cisplatin combinations in vitro and in vivo. *Mol. Cancer Ther* **2009**, *8*, 2266−75.
(179) Wong, M. Y.; Chiu, G. N. Liposome formulation of co-encapsulated vincristine and quercetin enhanced antitumor activity in

DOI: 10.1021/ab500084g
ACS Biomater. Sci. Eng. 2015, 1, 64−78

a trastuzumab-insensitive breast tumor xenograft model. *Nanomedicine* **2011**, *7*, 834−40.
(180) Sun, T. M.; Du, J. Z.; Yao, Y. D.; Mao, C. Q.; Dou, S.; Huang, S. Y.; Zhang, P. Z.; Leong, K. W.; Song, E. W.; Wang, J. Simultaneous delivery of siRNA and paclitaxel via a "two-in-one" micelleplex promotes synergistic tumor suppression. *ACS Nano* **2011**, *5*, 1483−94.
(181) Ediriwickrema, A.; Zhou, J.; Deng, Y.; Saltzman, W. M. Multilayered nanoparticles for combination gene and drug delivery to tumors. *Biomaterials* **2014**, *35*, 9343−54.
(182) Lee, I.-H.; An, S.; Yu, M. K.; Kwon, H.-K.; Im, S.-H.; Jon, S. Targeted chemoimmunotherapy using drug-loaded aptamer−dendrimer bioconjugates. *J. Controlled Release* **2011**, *155*, 435−441.
(183) Clementi, C.; Miller, K.; Mero, A.; Satchi-Fainaro, R.; Pasut, G. Dendritic poly(ethylene glycol) bearing paclitaxel and alendronate for targeting bone neoplasms. *Mol. Pharmaceutics* **2011**, *8*, 1063−72.
(184) Chou, T. C.; Talalay, P. Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. *Adv. Enzyme Regul* **1984**, *22*, 27−55.
(185) Chou, T. C. Theoretical basis, experimental design, and computerized simulation of synergism and antagonism in drug combination studies. *Pharmacol Rev.* **2006**, *58*, 621−81.
(186) Chou, T.-C.; Martin, N. *CompuSyn for Drug Combinations: PC Software and User's Guide: A Computer Program for Quantitation of Synergism and Antagonism in Drug Combinations, and the Determination of IC50 and ED50 and LD50 Values*; ComboSyn Inc.: Paramus, NJ, 2005.
(187) Prabhakar, U.; Blakey, D. C.; Maeda, H.; Jain, R. K.; Sevick-Muraca, E. M.; Zamboni, W.; Farokhzad, O. C.; Barry, S. T.; Gabizon, A.; Grodzinski, P. Challenges and key considerations of the enhanced permeability and retention effect (EPR) for nanomedicine drug delivery in oncology. *Cancer Res.* **2013**, *73*, 2412.
(188) Mak, I. W. Y.; Evaniew, N.; Ghert, M. Lost in translation: animal models and clinical trials in cancer treatment. *Am. J. Transl. Res.* **2014**, *6*, 114−118.
(189) Maeda, H.; Nakamura, H.; Fang, J. The EPR effect for macromolecular drug delivery to solid tumors: Improvement of tumor uptake, lowering of systemic toxicity, and distinct tumor imaging in vivo. *Adv. Drug Delivery Rev.* **2013**, *65*, 71−79.
(190) Kratz, F.; Warnecke, A. Finding the optimal balance: Challenges of improving conventional cancer chemotherapy using suitable combinations with nano-sized drug delivery systems. *J. Controlled Release* **2012**, *164*, 221−235.
(191) Tzeng, S. Y.; Guerrero-Cázares, H.; Martinez, E. E.; Sunshine, J. C.; Quiñones-Hinojosa, A.; Green, J. J. Non-viral gene delivery nanoparticles based on poly(β-amino esters) for treatment of glioblastoma. *Biomaterials* **2011**, *32*, 5402−5410.
(192) Zhou, J.; Patel, T. R.; Sirianni, R. W.; Strohbehn, G.; Zheng, M.-Q.; Duong, N.; Schafbauer, T.; Huttner, A. J.; Huang, Y.; Carson, R. E.; Zhang, Y.; Sullivan, D. J.; Piepmeier, J. M.; Saltzman, W. M. Highly penetrative, drug-loaded nanocarriers improve treatment of glioblastoma. *Proc. Natl. Acad. Sci. U.S.A.* **2013**, *110*, 11751−11756.
(193) de la Fuente, M.; Langer, R.; Alonso, M. Nanotechnology Approaches for Cancer Immunotherapy and Immunomodulation. In *Nano-Oncologicals*; Alonso, M. J.; Garcia-Fuentes, M., Eds.; Springer: New York, 2014; pp 215−242.

DOI: 10.1021/ab500084g
ACS Biomater. Sci. Eng. 2015, 1, 64−78