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Nanoscale Drug Delivery Platforms Overcome Platinum-Based Resistance in Cancer Cells Due to Abnormal Membrane Protein Trafficking

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Abstract

The development of cellular resistance to platinum-based chemotherapies is often associated with reduced intracellular platinum concentrations. In some models, this reduction is due to abnormal membrane protein trafficking, resulting in reduced uptake by transporters at the cell surface. Given the central role of platinum drugs in the clinic, it is critical to overcome cisplatin resistance by bypassing the plasma membrane barrier to significantly increase the intracellular cisplatin concentration enough to inhibit the proliferation of cisplatin-resistant cells. Therefore, rational design of appropriate nanoscale drug delivery platforms (nDDPs) loaded with cisplatin or other platinum analogs as payloads is a possible strategy to solve this problem. This review will focus on the known mechanism of membrane trafficking in cisplatin-resistant cells, and the development and employment of nDDPs to improve cell uptake of cisplatin.

Keywords

cancer; cisplatin; drug resistance; nanoscale drug delivery platforms; membrane trafficking; nanotechnology; chemotherapy; abnormal membrane proteins

Platinum (Pt)-based chemotherapeutic drugs, principally cisplatin (*cis*-[PtCl₂(NH₃)₂]) and carboplatin ([Pt(*O,O'*-cdbc)(NH₃)₂], cdbc = cyclobutane-1,1-dicarboxylate), are widely employed in the clinic to treat malignancies such as cancer of the testis, lung, ovary, breast, bladder, head and neck, colon and rectum.^{1–3} Clinically, the chemotherapeutic effect of platinum-based drugs presents a satisfactory response when tumors are first exposed to the drugs.⁴ However, after repeated treatments, most malignancies sooner or later become resistant to even unrelated anticancer agents, in spite of different chemical structures or

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different mechanisms of intracellular activity.⁵ The exception to this is testicular cancer, for which platinum therapy provides an approximately 99% cure rate. Thus, intrinsic and/or acquired resistance, as well as the formidable side effects of accumulating platinum in normal tissues, often hamper Pt-based treatment of cancer.^{6, 7} Movement of chemotherapeutic agents through the cellular lipid bilayer membrane was first thought to occur predominantly by passive diffusion.^{8, 9} However, emerging evidence in the literature indicates that active processes are more likely the major determinant of cellular uptake of cisplatin.^{6, 10} Evidence suggests that various membrane proteins collectively regulate the uptake and efflux of drugs. The reduction of platinum accumulation as a pivotal factor influencing the effectiveness of tumor chemotherapy is therefore mediated by down-regulation of these facilitative transporters, and alteration in membrane protein trafficking.^{11–13} Understanding the role of abnormal membrane proteins in the development of platinum drug resistance can serve as a basis for selecting drug targets and promoting drug development.

Many studies have been published concerning the role of active transport of platinum drugs across biological membranes.^{14–16} However, in order to achieve successful and effective drug delivery in cases of resistant cancer, new therapeutic strategies still need to be developed.^{17–19} Newer, more targeted agents have not displaced shotgun therapeutics such as cisplatin. In recent years, nanotechnology, through an amalgamation of chemistry, engineering, biology, and medicine has provided potential solutions to some of the daunting challenges associated with cancer therapy. Furthermore, although the feasibility and efficacy of reversing drug resistance have been studied in the clinic, ways to use nanotechnology to circumvent the resistant phenotype have not been clarified or fully explored. This review examines the reduced drug accumulation that occurs in resistant cancer cells caused by abnormal membrane transporter expression and unusual protein-related metabolic modulation, and introduces nanotechnology formulations and current nanomedical approaches to address platinum-based resistance, with a specific focus on the effort to overcome abnormal membrane protein trafficking and increase cellular uptake of chemotherapeutic agents.

Abnormal Membrane Proteins Play Pivotal Roles in Platinum-based Resistance

Modulation of Membrane Transporters in Resistant Cells

Membrane transporters are a group of integral membrane proteins that facilitate the movement of a variety of endogenous and exogenous substrates across cellular and organelle membranes, including the movement of ions, small molecules and macromolecules. An increasing number of membrane transporters have been identified as contributing to cancer resistance. These transporters govern the movement of drugs and their secondary metabolites, thereby determining their pharmacodynamics and adverse drug reactions. Changes in several transporters, such as in the ATP-binding cassette (ABC) transporters, solute carriers (SLCs) and ATPase membrane protein superfamilies have been implicated as determinants of the pharmacology of cisplatin, oxaliplatin, carboplatin and related investigational compounds.^{11, 20, 21} Changes in membrane transporters affect the accumulation of platinum drugs in resistant cells or tissues by increasing drug efflux or decreasing drug uptake, by metabolic modifications, or by detoxification.^{22, 23}

Of these transporter-related resistance mechanisms, overexpression of ABC transport molecules is generally considered the most frequent. ABC transporters are transmembrane proteins that use the energy of ATP hydrolysis to shuttle various substrates against the concentration gradient outward or into intracellular organelles. To date, there are 48 known

human transporters in the ABC family, classified into seven subfamilies A through G. At least thirteen of them have been recognized as drug transporters when drugs share physiochemical characteristics with certain endogenous substrates,^{21, 24, 25} and three, ABCB1 (P-glycoprotein), ABCC1 (MRP, or multidrug resistance-associated protein) and ABCG2 (BCRP, or breast cancer resistance protein) are broad-spectrum multidrug efflux pumps.²⁴

The solute carrier (SLC) family of transporters is another superfamily of membrane proteins that mediates the cellular uptake of anticancer agents, including SLC19A1 (RFC1) and SLC1B1 (SLC21A6). SLC transporters play a critical role in multiple cellular physiological processes and traffic specific substrates such as amino acids, oligopeptides, sugars, monocarboxylic acids, organic cations, anions, phosphates, nucleosides, metals and vitamins. SLCs also mediate drug absorption, distribution, metabolism and elimination, particularly in the case of uptake of hydrophilic anticancer drugs that cannot rely solely on passive diffusion, including cisplatin, carboplatin and oxaliplatin.^{26, 27} Structurally, members of the SLC19 family (the reduced folate carrier (RFC) family), SLC28 and 29 families (Concentrative and Equilibrative Nucleoside Transporter proteins (CNT and ENT, respectively)), SLC7A and 3A families (amino acid transporters) and SLC31A (the copper transporter family (CTR)) are associated with uptake of anticancer drugs.^{10, 20} CTR1 has been identified as a mediator that increases drug accumulation and cytotoxic properties.²⁸ For instance, CTR1-deficient mouse embryonic fibroblasts have been shown to demonstrate reduced influx of cisplatin, carboplatin and oxaliplatin.²⁹ In addition, studies have indicated that platinum accumulation is partly mediated by different energy-dependent cellular proteins that use ATP hydrolysis as an energy source. In resistant cancer cells, ATP levels tend to be depleted, leading to metabolic dysfunction and decreased drug accumulation.²⁸

Modulation of Membrane Content and Potential in Resistant Cells

The plasma membrane itself also plays a role in drug resistance, especially through abnormal membrane protein trafficking. A number of studies have pointed to abnormal membranes as contributing to resistance in cancer cells. These abnormalities include higher membrane potentials, abnormal fluidity of the plasma membrane, and changes in competency. Altered membrane protein trafficking results in a lowered level of transporters at the cell surface, therefore reducing the potential capacity of cells to facilitate the uptake of drugs (and nutrients).

Previous studies by us found that the biophysical status of cellular membranes was associated with cisplatin resistance. Compared to sensitive cells, resistant cells had higher plasma membrane potentials³⁰ and mitochondrial membrane potentials³¹, which would be dependent on differences in the structure or function of fatty acid composition, resistance-related membrane protein expression, ion conductivity or metabolic regulation.³² One study indicated that the change of lipid content in cisplatin-resistant cells could mediate the modulation of membrane fluidity, which was determined by cholesterol, total lipids and phospholipid content.³³ The study pointed out that compared to sensitive cells, the cholesterol and cholesterol ether content was significantly higher, while diacylglycerol and triacylglycerol content was apparently lower in the resistant cells. These differences provide potential opportunities for drugs designed to selectively target resistant tumor cells. For instance, the orphan drug dichloroacetate (DCA) reverses the Warburg effect by inhibiting pyruvate dehydrogenase kinase (PDK), providing a mitochondrial target to influence the unique cellular metabolism of cancer cells, and promote their apoptosis.^{34, 35} Mitaplatin, a platinum (IV) complex containing cisplatin and two DCA molecules bound as ligands that are released when the complex is reduced, alters the mitochondrial membrane potential and selectively kills cancer cells by targeting both nuclear DNA via cisplatin and mitochondria via DCA.³⁶ In cancer sensitive cells, oxidative phosphorylation is inhibited to force cancer

cells to rely on cytoplasmic glycolysis to produce energy. This metabolic shift induces apoptosis resistance. The enhanced lipophilicity of mitaplatin increases its ability to cross the plasma membrane, thus be further employed to overcome tumor resistance by modulating abnormal glycolysis and by rendering resistant cancer cells more vulnerable to hyperpolarized mitochondrial membrane potentials (Figure 1).³¹ Subsequently, other platinum(II) and platinum(IV) complexes containing DCA incorporated as a ligand have been reported.³⁷

Modulation of Intracellular pH in Resistant Cells

In addition, an acidic pH-activated mechanism to overcome efflux-dependent resistance has been explored.^{38–41} This has been driven by observations that cells resistant to cisplatin have acidified intracellular compartments. Furthermore, the influence of pH on the cytotoxicity of cisplatin in mouse mammary tumor cells can be exploited, as tumor cells are more sensitive to cisplatin when cultured in pH 6.0 medium rather than physiologic pH.⁴² Given this, the expectation is that an acid-labile linker would release its payload at a greater rate inside the more acidic cisplatin-resistant cells. By way of example, Kievit *et al.* demonstrated that doxorubicin tethered to iron oxide NPs by an acid-labile hydrazone linkage was released to a greater extent at acidic pH.³⁹

Modulation of Cell Cycle in Resistant Cells

The cell life cycle is the sequence of events that occur during DNA replication and cell division, which is divided into four successive phases: G₁, S (synthesis), G₂ (collectively known as interphase) and M (mitosis). During G₁, S and G₂ cells accumulate nutrients needed for mitosis. After mitosis, cells enter a state of quiescence called the G₀ phase, and stop dividing temporarily.^{43, 44} Cell cycle arrest is coordinated with the production of membrane phospholipids, the major cellular constituents required for the assembly of biological membranes. A doubling of membrane phospholipids is required for cell proliferation. Previous studies have demonstrated that phospholipids accumulate when cells enter S phase⁴⁵ and are synthesized in the G₂/M phase⁴⁶, which are controlled by a series of cell cycle regulators.^{47, 48} The cell cycle can be disturbed or delayed by various molecular events, including the intertwined actions of cyclin-dependent kinases (CDKs)⁴⁹ and specific proteolytic mechanisms,⁵⁰ as well as chemotherapeutic agents.^{51, 52} Cisplatin is well known to arrest cells at G₂,⁵³ a process mediated by checkpoint kinases⁵⁴ and the miRNAs that control them.⁵⁵ In cells that have acquired multidrug resistance, cell cycle distribution and cell cycle arrest is often altered as a result of this cycle-specific toxin. For example, cisplatin-resistant hepatocellular carcinoma cells have been shown to spend more time in the G₂/M and S phases (allowing them to spend greater time recognizing and repairing DNA damage).⁵⁶ Interfering with cell cycle arrest, by inhibiting or down-regulating checkpoint kinases, can re-sensitize cisplatin-resistant cells by forcing the cells to continue through the G₂ checkpoint into mitosis, enforcing apoptosis.^{55, 57} However, gene silencing technologies are limited in their efficiency, and small molecules face challenges associated with pharmacokinetics and unwanted side-effects.

As such, NPs-mediated interference with the cell cycle state has received attention. In fact, bare liposomes not loaded with drug have been shown to arrest cells in G₀/G₁ phase and induce apoptosis, though obviously the delivery of a drug by liposomes results in altered cellular responses^{58–60}. Roa *et.al*⁶¹ reported that glucose-capped gold nanoparticles accelerate cells through the G₀/G₁ phase, and arrest them in G₂M (much like cisplatin). Increasing evidence has been reported that metal-based nanomaterials such as iron NPs,⁶² silver NPs^{63–65}, albumin NPs,^{66, 67} ENREF 60 ZnO NPs,⁶⁸ and Au NPs^{61, 69} can affect the cell cycle in different phases. While modification of the cell cycle state of cells may alter cell fate by sensitizing chemotherapy, further study of the mechanisms of interaction

between nanoparticles and phospholipid cell membranes is required, as drugs that arrest cells can inhibit each other's efficacy.⁷⁰

Successful Applications of Nanotechnology as Therapy for Drug-Resistant Cancer

Nanoscale Drug Delivery Platforms that Target Membrane Transporters

Drugs are often internalized by diffusion across the cellular membrane or by transport-facilitated processes. The drug efflux pumps (such as P-glycoprotein (P-gp; ABCB1) on the cell membrane can recognize free drug molecules, capture, and efflux them when they attempt to cross the membrane.

Nanoscale drug delivery platforms (nDDPs, not to be confused with the oft-used acronym for cisplatin, DDP) based on biodegradable, biocompatible, and FDA-approved components, are taken up by endocytosis, preventing the drugs from being recognized by efflux pumps. The drugs are covalently bound to the nDDP, which results in a higher intracellular accumulation unaffected by transport processes.^{71–73} This increase is achieved partly because of circumvention of membrane-crossing events, and partly because each nDDP nanoparticle can deliver many drug molecules (the analogy being that of a Trojan horse).

Because of the side effects of platinum-based chemotherapeutic drugs, efforts to design targeted and/or controlled-release drug delivery systems is ongoing, with various modifications and accommodations for multiple types of drug payloads. At a chemical level, controlled release is achieved by installing linkers between the nanoparticle and drug containing functional groups that are susceptible to either enzymatic (e.g. esterase) or non-enzymatic (e.g. hydrolysis) cleavage.

The main objective of nDDPs is to localize the therapeutic agent at its site of action for maximal effect without resulting in a toxic distribution of the agent at non-target sites. After careful consideration of their size, toxicity, absorbance, distribution and elimination, most nanostructure platforms derive their effectiveness from adequate delivery systems, including polymers, liposomes, micelles, dendrimers, nanoshells, and nanotubes, as well as magnetic or metal nanoparticles.^{74–76} Some of them are promising applications or becoming realities in healthcare. For instance, it has been reported that poly (ethylene glycol)-conjugated (PEGylated) multi-walled carbon nanotubes (MWCNTs) act as drug efflux modulators. They accumulate in resistant cancer cells as well as in sensitive cancer cells without damaging the plasma membrane, indicating that they are efficient drug carriers able to overcome drug resistance.⁷⁷ This is likely achieved because the nanoparticle itself is either not recognized by, or is too large to be extruded by, multidrug resistance efflux pumps. Moreover, ligand-mediated interaction between nanoparticles and the surface of resistant cancer cells is one of the most popular strategies. Properly designed nanoparticles can focus on active targets at specific targeting sites with therapeutic payloads, taking advantage of markers on the membranes of resistant cancer cells, reducing the dispersal of the drug and enhancing its therapeutic potential.^{78, 79}

Defective endocytosis causes less intracellular accumulation of drugs such as cisplatin. Metallofullerene nanoparticles have been successfully designed to repair receptor-mediated endocytosis in resistant cells, resulting in more efficient formation of cisplatin-DNA adducts to sensitize the resistant cells both *in vitro* and *in vivo*.⁸⁰ In addition, active nanocarrier endocytosis has been accomplished by other ligands such as transferrin,⁸⁰ epidermal growth factor receptor (EGFR), peptides^{81, 82} and siRNA⁸³ via receptor-mediated endocytosis.

Other Modification Strategies Related to Abnormal Membrane Protein Trafficking

nDDPs that Modify the Phospholipids of Resistant Cells—Alterations in the composition of the cell membranes of resistant tumor cells have been observed^{84–86}. For example, a study based on virtual screening found a novel phospholipid named phosphatidylinositol-(1,2-dioctanoyl)-sodium salt, identified with transmembrane P-gp transportation inhibitory activity. Further tests showed that the phosphatidylinositol derivative increased the bioactivity of drugs in several tumor cell lines, due to P-gp inhibition.⁸⁷ Another study indicated that MCF-7 cisplatin-resistant cells accumulated more 3,3'-diocadecyloxacarbocyanine perchlorate (DiO) dye from dye-loaded liposomes than sensitive cells.⁸⁸

Among different kinds of drug carriers, such as polymeric micelles, niosomes, liposomes, microspheres, immunoglobulins, peptides and small proteins, liposomes are considered as suitable lipophilic carriers due to their natural lipid components. Liposomes are nano-size artificial vesicles composed of one or more phospholipid-enriched bilayers containing mixed lipid chains that are employed to attach to unhealthy tissue.^{89, 90} Liposomes afford a unique opportunity to deliver drugs due to their attractive composition, including fluidity, permeability, stability and structure, which makes them biocompatible and biodegradable. On the other hand, liposomes sometimes alter the pharmacokinetic parameters and dynamic interactions between tumor cells and encapsulated drugs: strongly lipophilic drugs are entrapped almost completely in the lipid bilayer, strongly hydrophilic drugs are located exclusively in the aqueous compartment, and drugs with intermediate logP easily partition between the lipid and aqueous phases, both in the bilayer and in the aqueous core. Furthermore, liposomes can easily be loaded with different drugs for combination chemotherapy. Ye *et al.* showed that cationic liposome-mediated inducible nitric oxide synthase (iNOS) gene therapy is effective with low dose cisplatin treatment in lung cancer. Systemic delivery of the liposome-pVAX-iNOS complex enhanced cisplatin-mediated suppression of tumors by inhibition of cell proliferation, invasion, migration and promotion of cell apoptosis both *in vitro* and *in vivo*.⁹¹ Because lipid-based nanoparticles have the advantage of minimum toxicity for *in vivo* applications, their potential success in the clinic has been apparent. Lipoplatin, a liposomal encapsulation of cisplatin into tumor-targeted 110 nm nanoparticles (Figure 2A), shown to be effective in non-small-cell lung cancer (NSCLC) both in Phase II and III trials, combines a reduction in the toxicity associated with antitumor activity similar to the free drug.^{92, 93} Lipoplatin infusion in tumor cells exhibited 10–50 times higher activity than in the adjacent normal specimens, with less general toxicity and nephrotoxicity, no significant weight gain reduction, and fewer renal and liver impairments than cisplatin administration.^{93, 94} The direct fusion of lipoplatin with the membrane allows for a therapeutic effect even after the development of cisplatin resistance (Figure 2B).⁹⁵

nDDPs that Modify Cellular Metabolism—An alternative to traditional ways to treat cancer resistance is to decrease intracellular ATP levels by inhibiting mitochondrial function, which can significantly reduce the activity of drug efflux pumps. The pluronic block copolymer (P85) is an important and promising example of a modifying agent for P-gp, the best-known and most thoroughly studied multidrug resistance membrane transporter, which was discovered in 1986.^{96, 97} Membrane fluidization by P85 treatment inhibits the P-gp ATPase drug efflux system and interferes with metabolic processes. Therefore, both energy depletion and increased permeability and fluidization of a broad spectrum of drugs are critical factors contributing to the activity of the block copolymer for reversion of multidrug resistance (MDR).^{98, 99}

As an alternative approach to the use of biological nanoparticles, anticancer peptide therapy focuses on the development of therapeutic peptides to kill resistant cells. A novel peptide,

CT20p, derived from a helical unit of the pro-apoptotic protein Bax, is an example of a peptide that is an effective killer both *in vitro* and in a murine breast cancer tumor model. Boohaker and colleagues found that CT20p is amphiphilic. It can be encapsulated in polymeric nanoparticles, modifying tumor metabolism by causing an increase in mitochondrial membrane potential.⁸² Another group focused on the small ubiquitin-like modifier 1 (SUMO1) peptidase SENP1, which reduces hypoxia and enhances chemosensitivity as a potential therapeutic target for drug-resistant testicular germ cell tumors.¹⁰⁰ Garg *et al.* also reported that PEGylated liposomes modified with a fibronectin-mimetic peptide to target metastatic colon cancer cells inhibited tumor growth, reduced tumor metastasis, and stimulated drug internalization.¹⁰¹ By targeting metabolism in resistant tumor cells, nanotechnology exhibits significant antitumor efficacy by inducing apoptosis in both sensitive and resistant cancer cells.

nDDPs that Regulate Protein Trafficking and Degradation—Autophagy begins with the formation of double-membrane vesicles (autophagosomes), which then fuse with lysosomes, where the sequestered contents undergo degradation and recycling, eliminating misfolded proteins and damaged organelles^{102, 103}. The critically important process of autophagy, which is a mechanism of cell survival in the presence of genomic injury, oxidant stress, nutrient deprivation, hypoxia, inflammation and viral/bacterial infection, has been recently recognized as important for conferring resistance to cancer treatment. Moreover, it was found that autophagy protects tumors from drug-treated apoptosis and aids survival and recovery with chemotherapeutic drug treatment. Modulation of autophagy dysfunction was found to re-sensitize resistant cancer cells to anticancer therapy.^{104–106} Unlike cisplatin, which mainly causes cell death by inducing apoptosis, other platinum compounds have been shown to kill cells via autophagy.¹⁰⁷

Fullerene C₆₀ (a spherical carbon structure) is a chemotherapeutic sensitizer that causes authentic autophagy at non-cytotoxic concentrations.¹⁰⁸ These nanoparticles have been reported to induce autophagy and sensitize resistant cells to chemotherapy when combined with platinum drugs, killing both drug-sensitive and -resistant cancer cells, a novel therapeutic approach to circumvent drug resistance through modifying intracellular metabolism.¹⁰⁹

Recently Developed Pt-tether nDDPs to Treat Drug-Resistant Cancer with Abnormal Membrane Protein Trafficking

A number of nanoparticles have recently been produced to conjugate with platinum. The process of designing Pt-tether nanoparticles includes tuning their shape and size in order to avoid disturbing the abnormal membrane protein in resistant cells. Surface modifications are also made, including various coatings and charges to increase hydrophilicity, which can alter the pharmacokinetics of platinum-based drugs. As it is difficult to entrap cisplatin in polymeric sustained-release nanoparticles (due to its small cross-section), Dhar *et al.* generated a platinum(IV) complex (*c,t,c*-[Pt(NH₃)₂(O₂CCH₂CH₂COOH)₂Cl₂]) as a prodrug that can be intracellularly processed into cisplatin. This release of cisplatin is achieved by reduction of the platinum(IV) complex by endogenous reductants, and loss of the axial ligands. The prodrug has increased hydrophobicity and offers a position (pendant carboxylic acids) on the axial ligands for conjugation to a nanocarrier for efficient delivery, for example by reaction with terminal amines (Figure 3A).^{110–113}

Based on this chemistry, a series of Pt (IV)-tethered nanoparticles has been produced including single-walled carbon nanotubes,¹¹⁴ gold nanoparticles,¹¹⁵ PLGA-PEG nanoparticles,^{110, 116} peptides,¹¹⁷ and aptamers,¹¹¹ providing good examples of both active platinum (II) derivative development and nDDPs. These nanoparticles provide platinum-

based drugs with true drug carriers (Figure 3A). These studies also shed light on the impact of the complex environment of platinum on drug efficacy. Additionally, various studies have reported that several kinds of Pt(IV)-based nanoparticles have shown very promising efficacy *in vivo*, with long blood circulation time and thereby high accumulation in tumors, with low systemic toxicity and better tolerance.^{111, 117–120}

Meanwhile, researchers are working to advance the ability of nDDPs to carry platinum drugs into resistant cells, especially with nanoparticle-Pt linkers. One popular strategy is to link a nanoparticle via a pH-sensitive coordination bond for endosomal release. Comenge J *et al.*¹²¹ used gold nanoparticles to detoxify cisplatin with pH-sensitive linkers, without affecting the therapeutic and imaging benefits. A novel rational engineering of cisplatin nanoparticles by polyethylene glycol (PEG)-functionalized poly-isobutylene-maleic acid (PEG-PIMA) copolymer can coordinate with a [*cis*-diammineplatinum(II)] moiety through the pendant carboxylate ligands in a similar fashion to the carboxylate ligands of oxaliplatin. In effect, the NP acts as a bidentate ligand (Figure 3B). This complex self-assembles into a nanoparticle, releasing cisplatin in a pH-dependent manner.

Another strategy often used is to incorporate cisplatin into the hollow interior of nanoparticles. Single-walled carbon nanotubes (SWNTs), a “long boat” delivery system, offer abundant space to encapsulate cisplatin. It was reported that cisplatin-bearing SWNTs increased anticancer efficiency 4–6 times that of cisplatin alone, causing high concentrations locally in cells of tumor xenograft tissue.¹²² An assessment of a series of platinum (IV) complexes based on cisplatin with increasing lipophilicity were assessed by Johnstone *et al.*, showing that the most lipophilic platinum complex displayed the highest level of encapsulation in PLGA-PEG-COOH nanoparticles.¹²³ Lian *et al.* used highly biocompatible hollow Prussian blue (HPB) nanoparticles with a hollow interior and a microporous framework to absorb cisplatin non-covalently, and these were demonstrated to exert cytotoxicity in cell culture.¹²⁴ Mesoporous materials containing pores with diameters between 2 to 50 nm have become popular due to their large surface area, high core volume, and tunable nanoscale pores. The matrix pore architecture makes them suitable for hosting a broad variety of compounds, and they achieve localized intracellular release of the platinum drugs to minimize the influence of abnormal membrane proteins.^{83, 125} For example, mesoporous silica materials loaded with cisplatin and transplatin demonstrated cellular internalization and synergistic cell killing (the nanoparticles themselves display cytotoxicity).¹²⁵ Mesoporous silica nanoparticles conjugated with folic acid have been shown to enter cells via folate receptor (FR)-mediated endocytosis.¹²⁶ When loaded with cisplatin, these targeted mesoporous silica nanoparticles only showed cytotoxicity towards cells expressing FR. While this targeting concept is attractive, relying on endocytic processing for targeting resistant cells may be limiting in cisplatin-resistant cells given their depressed endocytic rate.

Although novel chemotherapeutic nDDPs have been established everyday, several problems still need careful investigation. Researchers expect nanomedicine to resolve the problem of how to get enough of the right drug to the right place without causing side effects (from either the drug or the NPs), immune responses or inducing resistance. A micelle-encapsulated hydrophobic platinum(II) nanomedicine displayed excellent tumor to tissue ratios and 6 times higher cellular accumulation compared with the free platinum compound, given a good example of not only outstanding pharmacokinetics and tumor selectivity, but also specifically high cytotoxicity against tumor cells.¹²⁷ Harnessing the immune system capacity in order to induce antitumor response remains an important challenge. How to utilize immune response in prognosis and therapy remains unknown despite its high prevalence. A 15 kDa variable domains of *camelid* heavy-chain-only antibodies, called Nanobodies, exhibited the advantage for cancer therapy that has been reported by many

researchers.^{128–130} In other cases, bio-nanoparticles, such as antibody, peptides, etc., showed their ability to induce immune response and apoptosis.¹³¹ In recent years, several nanoscale drug carriers are currently in clinical trials.¹³² For instance, cisplatin results in severe kidney toxicity, requiring patients to drink large amounts of water during treatment. However, that is not the case in NanoCarrier (Nanoplatin) trials, as the carrier's size allows it to move into and accumulate in the pancreatic tumor, instead of accumulating in the kidney.^{133–135} A 30 nm polymer to transport chemotherapeutic drugs is currently undergoing phase II clinical trials with advanced or metastatic pancreatic cancer, doubling survival time from five months to more than twelve.^{132, 134, 136–138}

Further Challenges and Prospects for Therapeutic Strategies to Combat Drug-Resistant Cancer

Cisplatin resistance still remains a major challenge to successful treatment of cancer. Nanotechnology is a field that has developed rapidly and provides a promising approach to chemotherapy. In addition, the properties of nDDPs, including stable and strong fluorescence *etc.*, also give promising opportunities to evaluate the sensitivity of imaging systems for chemotherapy.^{80, 139, 140} For example, Xue *et al.* developed a self-indicating drug delivery system that visualized spatiotemporal drug release via tunable aggregation-induced emission. When doxorubicin escaped from lysosome, the drug delivery system located and lightened the cytoplasm.¹⁴¹ Although exciting approaches have been reported in the recent years, applications of nanotechnology to cancer treatment appear to overcome some of the limitations of traditional chemotherapy, giving hope that solutions to the problems of drug resistance can be found. To develop this approach, further study is needed in the following areas:

1. Abnormal membrane proteins as potential drug targets
2. The interaction and relationship between abnormal membrane proteins and tumor metabolism as well as the extracellular environment
3. The pharmacokinetics (including absorbance, distribution, metabolism and excretion) of nanoscale drug delivery systems, especially active delivery of functionalized nanocarriers
4. Higher sensitivity imaging techniques with molecular specificity (as platinum-based drugs are difficult to trace)
5. The safety and toxicity of nanoparticles, as well as immune response

To achieve tangible therapeutic benefits from the above information, the mechanisms that cause abnormal membrane protein trafficking to develop into cisplatin resistance need to be exploited. Based on research reported so far, it can be expected that nDDPs that target abnormal membrane proteins may represent a useful approach to improving the clinical outcomes of existing platinum-based anticancer drugs.

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VOCABULARY

nDDPs	nanoscale drug delivery platforms
Pt	platinum
ABC	ATP-binding cassette
SLCs	solute carriers
MRP	Multidrug resistance-associated protein
BCRP	Breast cancer resistance protein
RFC	Reduced folate carrier
CNT	Concentrative nucleoside transporter
ENT	Equilibrative nucleoside transporter
CTR	copper transporter
DCA	dichloroacetate
CDKs	Cyclin-dependent kinases
PDK	pyruvate dehydrogenase
MDR	Multidrug resistance
EGFR	Epidermal growth factor receptor
FDA	Food and Drug Administration
PGELYated	poly (ethylene glycol)-conjugated
P-gp	P-glycoprotein
SWNTs	Single walled carbon nanotubes
MWNTs	Multi-walled carbon nanotubes
iNOS	Inducible nitric oxide synthase
NSCLC	Non-small-cell lung cancer
FR	Folate receptor

References and Notes

1. McWhinney SR, Goldberg RM, McLeod HL. Platinum neurotoxicity pharmacogenetics. *Mol. Cancer Ther.* 2009; 8:6–10.
2. Cepeda V, Fuertes MA, Castilla J, Alonso C, Quevedo C, Perez JM. Biochemical mechanisms of cisplatin cytotoxicity. *Anticancer Agents Med. Chem.* 2007; 7:3–18. [PubMed: 17266502]
3. Paris I, Cappellini GC, Malaguti P, Bassanelli M, Marchetti P. Pharmacogenomics and chemotherapy. *Recenti. Prog. Med.* 2010; 101:277–282. [PubMed: 20842953]
4. Gottlieb JA, Drewinko B. Review of the current clinical status of platinum coordination complexes in cancer chemotherapy. *Cancer Chemother. Rep.* 1975; 59:621–628. [PubMed: 1106840]
5. Shen DW, Pouliot LM, Hall MD, Gottesman MM. Cisplatin resistance: a cellular self-defense mechanism resulting from multiple epigenetic and genetic changes. *Pharmacol. Rev.* 2012; 64:706–721. [PubMed: 22659329]
6. Hall MD, Okabe M, Shen DW, Liang XJ, Gottesman MM. The role of cellular accumulation in determining sensitivity to platinum-based chemotherapy. *Annu Rev Pharmacol. Toxicol.* 2008; 48:495–535. [PubMed: 17937596]
7. Koberle B, Tomicic MT, Usanova S, Kaina B. Cisplatin resistance: preclinical findings and clinical implications. *Biochim. Biophys. Acta.* 2010; 1806:172–182. [PubMed: 20647037]

8. Pelletier H, Millot JM, Chauffert B, Manfait M, Genne P, Martin F. Mechanisms of resistance of confluent human and rat colon cancer cells to anthracyclines: alteration of drug passive diffusion. *Cancer Res.* 1990; 50:6626–6631. [PubMed: 2208125]
9. Binks SP, Dobrota M. Kinetics and mechanism of uptake of platinum-based pharmaceuticals by the rat small intestine. *Biochem. Pharmacol.* 1990; 40:1329–1336. [PubMed: 2206139]
10. Huang Y. Pharmacogenetics/genomics of membrane transporters in cancer chemotherapy. *Cancer Metastasis Rev.* 2007; 26:183–201. [PubMed: 17323126]
11. Ohmichi M, Hayakawa J, Tasaka K, Kurachi H, Murata Y. Mechanisms of platinum drug resistance. *Trends Pharmacol. Sci.* 2005; 26:113–116. [PubMed: 15749154]
12. Wernyj RP, Morin PJ. Molecular mechanisms of platinum resistance: still searching for the Achilles' heel. *Drug Resist. Updat.* 2004; 7:227–232. [PubMed: 15533760]
13. Koga H, Kotoh S, Nakashima M, Yokomizo A, Tanaka M, Naito S. Accumulation of intracellular platinum is correlated with intrinsic cisplatin resistance in human bladder cancer cell lines. *Int. J. Oncol.* 2000; 16:1003–1007. [PubMed: 10762637]
14. Stordal B, Hamon M, McEneaney V, Roche S, Gillet JP, O'Leary JJ, Gottesman M, Clynes M. Resistance to paclitaxel in a cisplatin-resistant ovarian cancer cell line is mediated by P-glycoprotein. *PLoS One.* 2012; 7:e40717. [PubMed: 22792399]
15. Kishimoto S, Kawazoe Y, Ikeno M, Saitoh M, Nakano Y, Nishi Y, Fukushima S, Takeuchi Y. Role of Na⁺, K⁺-ATPase alpha1 subunit in the intracellular accumulation of cisplatin. *Cancer Chemother. Pharmacol.* 2006; 57:84–90. [PubMed: 16044341]
16. Deeley RG, Westlake C, Cole SP. Transmembrane transport of endo- and xenobiotics by mammalian ATP-binding cassette multidrug resistance proteins. *Physiol. Rev.* 2006; 86:849–899. [PubMed: 16816140]
17. Song IS, Savaraj N, Siddik ZH, Liu P, Wei Y, Wu CJ, Kuo MT. Role of human copper transporter Ctr1 in the transport of platinum-based antitumor agents in cisplatin-sensitive and cisplatin-resistant cells. *Mol. Cancer Ther.* 2004; 3:1543–1549. [PubMed: 15634647]
18. Samimi G, Varki NM, Wilczynski S, Safaei R, Alberts DS, Howell SB. Increase in expression of the copper transporter ATP7A during platinum drug-based treatment is associated with poor survival in ovarian cancer patients. *Clin. Cancer Res.* 2003; 9:5853–5859. [PubMed: 14676106]
19. More SS, Akil O, Ianculescu AG, Geier EG, Lustig LR, Giacomini KM. Role of the copper transporter, CTR1, in platinum-induced ototoxicity. *J. Neurosci.* 2010; 30:9500–9509. [PubMed: 20631178]
20. Huang Y, Sadee W. Membrane transporters and channels in chemoresistance and -sensitivity of tumor cells. *Cancer Lett.* 2006; 239:168–182. [PubMed: 16169662]
21. Ejendal KF, Hrycyna CA. Multidrug resistance and cancer: the role of the human ABC transporter ABCG2. *Curr. Protein Pept. Sci.* 2002; 3:503–511. [PubMed: 12369998]
22. Kis O, Robillard K, Chan GN, Bendayan R. The complexities of antiretroviral drug-drug interactions: role of ABC and SLC transporters. *Trends Pharmacol. Sci.* 2010; 31:22–35. [PubMed: 20004485]
23. Glavinas H, Krajcsi P, Cserepes J, Sarkadi B. The role of ABC transporters in drug resistance, metabolism and toxicity. *Curr. Drug Deliv.* 2004; 1:27–42. [PubMed: 16305368]
24. Gottesman MM, Fojo T, Bates SE. Multidrug resistance in cancer: role of ATP-dependent transporters. *Nat. Rev. Cancer.* 2002; 2:48–58. [PubMed: 11902585]
25. Fukuda Y, Schuetz JD. ABC transporters and their role in nucleoside and nucleotide drug resistance. *Biochem. Pharmacol.* 2012; 83:1073–1083. [PubMed: 22285911]
26. Fujino H, Saito T, Ogawa S, Kojima J. Transporter-mediated influx and efflux mechanisms of pitavastatin, a new inhibitor of HMG-CoA reductase. *J. Pharm. Pharmacol.* 2005; 57:1305–1311. [PubMed: 16259759]
27. Marin JJ, Briz O, Monte MJ, Blazquez AG, Macias RI. Genetic variants in genes involved in mechanisms of chemoresistance to anticancer drugs. *Curr. Cancer Drug Targets.* 2012; 12:402–438. [PubMed: 22229248]
28. Larson CA, Blair BG, Safaei R, Howell SB. The role of the mammalian copper transporter 1 in the cellular accumulation of platinum-based drugs. *Mol. Pharmacol.* 2009; 75:324–330. [PubMed: 18996970]

29. Fink D, Nebel S, Aebi S, Nehme A, Howell S. Loss of DNA mismatch repair due to knockout of MSH2 or PMS2 results in resistance to cisplatin and carboplatin. *Int. J. Oncol.* 1997; 11:539–542. [PubMed: 21528244]
30. Liang XJ, Yin JJ, Zhou JW, Wang PC, Taylor B, Cardarelli C, Kozar M, Forte R, Aszalos A, Gottesman MM. Changes in biophysical parameters of plasma membranes influence cisplatin resistance of sensitive and resistant epidermal carcinoma cells. *Exp. Cell Res.* 2004; 293:283–291. [PubMed: 14729466]
31. Xue X, You S, Zhang Q, Wu Y, Zou GZ, Wang PC, Zhao YL, Xu Y, Jia L, Zhang X, Liang XJ. Mitaplatin increases sensitivity of tumor cells to cisplatin by inducing mitochondrial dysfunction. *Mol. Pharm.* 2012; 9:634–644. [PubMed: 22289032]
32. Liang XJ, Taylor B, Cardarelli C, Yin JJ, Annereau JP, Garfield S, Wincovitch S, Szakacs G, Gottesman MM, Aszalos A. Different roles for K⁺ channels in cisplatin-resistant cell lines argue against a critical role for these channels in cisplatin resistance. *Anticancer Res.* 2005; 25:4113–4122. [PubMed: 16309205]
33. Todor IN, Lukyanova NY, Chekhun VF. The lipid content of cisplatin- and doxorubicin-resistant MCF-7 human breast cancer cells. *Exp. Oncol.* 2012; 34:97–100. [PubMed: 23013760]
34. Michelakis ED, Webster L, Mackey JR. Dichloroacetate (DCA) as a potential metabolic-targeting therapy for cancer. *Br. J. Cancer.* 2008; 99:989–994. [PubMed: 18766181]
35. Stacpoole PW. The pharmacology of dichloroacetate. *Metabolism.* 1989; 38:1124–1144. [PubMed: 2554095]
36. Dhar S, Lippard SJ. Mitaplatin, a potent fusion of cisplatin and the orphan drug dichloroacetate. *Proc. Natl. Acad. Sci. U S A.* 2009; 106:22199–22204. [PubMed: 20007777]
37. Xiao H, Yan L, Zhang Y, Qi R, Li W, Wang R, Liu S, Huang Y, Li Y, Jing X. A dual-targeting hybrid platinum(IV) prodrug for enhancing efficacy. *Chem. Commun. (Camb).* 2012; 48:10730–10732. [PubMed: 23012698]
38. Yadav S, van Vlerken LE, Little SR, Amiji MM. Evaluations of combination MDR-1 gene silencing and paclitaxel administration in biodegradable polymeric nanoparticle formulations to overcome multidrug resistance in cancer cells. *Cancer Chemother. Pharmacol.* 2009; 63:711–722. [PubMed: 18618115]
39. Kievit FM, Wang FY, Fang C, Mok H, Wang K, Silber JR, Ellenbogen RG, Zhang M. Doxorubicin loaded iron oxide nanoparticles overcome multidrug resistance in cancer in vitro. *J. Control Release.* 2011; 152:76–83. [PubMed: 21277920]
40. Huang Z, Huang Y. The change of intracellular pH is involved in the cisplatin-resistance of human lung adenocarcinoma A549/DDP cells. *Cancer. Invest.* 2005; 23:26–32. [PubMed: 15779865]
41. Murakami T, Shibuya I, Ise T, Chen ZS, Akiyama S, Nakagawa M, Izumi H, Nakamura T, Matsuo K, Yamada Y, Kohno K. Elevated expression of vacuolar proton pump genes and cellular PH in cisplatin resistance. *Int. J. Cancer.* 2001; 93:869–874. [PubMed: 11519050]
42. Laurencot CM, Kennedy KA. Influence of pH on the cytotoxicity of cisplatin in EMT6 mouse mammary tumor cells. *Oncol. Res.* 1995; 7:371–379. [PubMed: 8747600]
43. Yanishevsky RM, Stein GH. Regulation of the cell cycle in eukaryotic cells. *Int. Rev. Cytol.* 1981; 69:223–259. [PubMed: 7012067]
44. Robinson AC, Collins JF, Donachie WD. Prokaryotic and eukaryotic cell-cycle proteins. *Nature.* 1987; 328:766. [PubMed: 3306399]
45. Peeper DS, van der Eb AJ, Zantema A. The G1/S cell-cycle checkpoint in eukaryotic cells. *Biochim. Biophys. Acta.* 1994; 1198:215–230. [PubMed: 7819276]
46. Lin W, Arthur G. Phospholipids are synthesized in the G2/M phase of the cell cycle. *Int. J. Biochem. Cell Biol.* 2007; 39:597–605. [PubMed: 17113814]
47. Jackowski S. Coordination of membrane phospholipid synthesis with the cell cycle. *J. Biol. Chem.* 1994; 269:3858–3867. [PubMed: 8106431]
48. Hunter T. Regulation of the eukaryotic cell cycle. Introduction. *Ciba. Found Symp.* 1992; 170:1–6. [PubMed: 1483342]
49. Nigg EA. Cyclin-dependent protein kinases: key regulators of the eukaryotic cell cycle. *Bioessays.* 1995; 17:471–480. [PubMed: 7575488]

50. Hoyt MA. Eliminating all obstacles: regulated proteolysis in the eukaryotic cell cycle. *Cell*. 1997; 91:149–151. [PubMed: 9346231]
51. Owa T, Yoshino H, Yoshimatsu K, Nagasu T. Cell cycle regulation in the G1 phase: a promising target for the development of new chemotherapeutic anticancer agents. *Curr. Med. Chem*. 2001; 8:1487–1503. [PubMed: 11562278]
52. Shen H, Perez RE, Davaadelger B, Maki CG. Two 4N cell-cycle arrests contribute to cisplatin-resistance. *PLoS One*. 2013; 8:e59848. [PubMed: 23560058]
53. Eastman, A. The mechanism of action of cisplatin: from adducts to apoptosis. In: Lippert, B., editor. *Cisplatin: chemistry and biochemistry of a leading anticancer drug*. Switzerland: Verlag Helvetica Chimica Acta; 1999. p. 111-134.
54. Shah MA, Schwartz GK. Cell cycle-mediated drug resistance: an emerging concept in cancer therapy. *Clin. Cancer Res*. 2001; 7:2168–2181. [PubMed: 11489790]
55. Pouliot LM, Chen YC, Bai J, Guha R, Martin SE, Gottesman MM, Hall MD. Cisplatin sensitivity mediated by WEE1 and CHK1 is mediated by miR-155 and the miR-15 family. *Cancer Res*. 2012; 72:5945–5955. [PubMed: 22942255]
56. Ling X, Wen L, Zhou Y. Role of mitochondrial translocation of telomerase in hepatocellular carcinoma cells with multidrug resistance. *Int. J. Med. Sci*. 2012; 9:545–554. [PubMed: 22991493]
57. Hirai H, Iwasawa Y, Okada M, Arai T, Nishibata T, Kobayashi M, Kimura T, Kaneko N, Ohtani J, Yamanaka K, et al. Small-molecule inhibition of Wee1 kinase by MK-1775 selectively sensitizes p53-deficient tumor cells to DNA-damaging agents. *Mol. Cancer Ther*. 2009; 8:2992–3000. [PubMed: 19887545]
58. Komizu Y, Ueoka H, Goto K, Ueoka R. Remarkable inhibitory effects of hybrid liposomes on growth of human colon cancer cells through induction of cell cycle arrest along with apoptosis. *Int. J. Nanomedicine*. 2011; 6:1913–1920. [PubMed: 21931486]
59. Yang XQ, Zhang ZM, Wang D, Wang G, Zeng LL, Yang ZZ. nm23-H1-siRNA enhances the chemosensitivity to liposome-encapsulated paclitaxel in lung adenocarcinoma cells in vitro. *Zhonghua Zhong Liu Za Zhi*. 2011; 33:405–409. [PubMed: 21875478]
60. Ruan F, Xie XK, Liu SY. Inhibitory effects of survivin antisense oligonucleotide on drug-resistant human ovarian cancer cell COC1/DDP. *Ai Zheng*. 2004; 23:896–899. [PubMed: 15301710]
61. Roa W, Zhang X, Guo L, Shaw A, Hu X, Xiong Y, Gulavita S, Patel S, Sun X, Chen J, et al. Gold nanoparticle sensitize radiotherapy of prostate cancer cells by regulation of the cell cycle. *Nanotechnology*. 2009; 20:375101. [PubMed: 19706948]
62. Lange TS, Kim KK, Singh RK, Strongin RM, McCourt CK, Brard L. Iron(III)-salophene: an organometallic compound with selective cytotoxic and anti-proliferative properties in platinum-resistant ovarian cancer cells. *PLoS One*. 2008; 3:e2303. [PubMed: 18509533]
63. Eom HJ, Choi J. p38 MAPK activation, DNA damage, cell cycle arrest and apoptosis as mechanisms of toxicity of silver nanoparticles in Jurkat T cells. *Environ. Sci. Technol*. 2010; 44:8337–8342. [PubMed: 20932003]
64. Asharani PV, Sethu S, Lim HK, Balaji G, Valiyaveetil S, Hande MP. Differential regulation of intracellular factors mediating cell cycle, DNA repair and inflammation following exposure to silver nanoparticles in human cells. *Genome Integr*. 2012; 3:2. [PubMed: 22321936]
65. Wang CH, Shih WC, Chang HC, Kuo YY, Hung WC, Ong TG, Li WS. Preparation and characterization of amino-linked heterocyclic carbene palladium, gold, and silver complexes and their use as anticancer agents that act by triggering apoptotic cell death. *J. Med. Chem*. 2011; 54:5245–5249. [PubMed: 21671598]
66. Casaluze F, Sgambato A, Maione P, Ciardiello F, Gridelli C. Emerging mitotic inhibitors for non-small cell carcinoma. *Expert Opin. Emerg. Drugs*. 2013; 18:97–107. [PubMed: 23448156]
67. Cui M, Naczynski DJ, Zevon M, Griffith CK, Sheihet L, Poventud-Fuentes I, Chen S, Roth CM, Moghe PV. Multifunctional Albumin Nanoparticles As Combination Drug Carriers for Intra-Tumoral Chemotherapy. *Adv. Healthc. Mater*. 2013 Epub ahead of print.
68. Sasidharan A, Chandran P, Menon D, Raman S, Nair S, Koyakutty M. Rapid dissolution of ZnO nanocrystals in acidic cancer microenvironment leading to preferential apoptosis. *Nanoscale*. 2011; 3:3657–3669. [PubMed: 21826307]

69. Coronello M, Marcon G, Carotti S, Caciagli B, Mini E, Mazzei T, Orioli P, Messori L. Cytotoxicity, DNA damage, and cell cycle perturbations induced by two representative gold(III) complexes in human leukemic cells with different cisplatin sensitivity. *Oncol. Res.* 2000; 12:361–370. [PubMed: 11697815]
70. Blagosklonny MV, Robey R, Bates S, Fojo T. Pretreatment with DNA-damaging agents permits selective killing of checkpoint-deficient cells by microtubule-active drugs. *J. Clin. Invest.* 2000; 105:533–539. [PubMed: 10683383]
71. Shi J, Votruba AR, Farokhzad OC, Langer R. Nanotechnology in drug delivery and tissue engineering: from discovery to applications. *Nano Lett.* 2010; 10:3223–3230. [PubMed: 20726522]
72. Talevi A, Gantner ME, Ruiz ME. Applications of Nanosystems to Anticancer Drug Therapy (Part I. Nanogels, Nanospheres, Nanocapsules). *Recent Pat. Anticancer Drug Discov.* 2012 Epub ahead of print.
73. Ruiz ME, Gantner ME, Talevi A. Applications of Nanosystems to Anticancer Drug Therapy (Part II. Dendrimers, Micelles, Lipid-based Nanosystems). *Recent Pat. Anticancer Drug Discov.* 2013 Epub ahead of print.
74. Jabir NR, Tabrez S, Ashraf GM, Shakil S, Damanhour GA, Kamal MA. Nanotechnology-based approaches in anticancer research. *Int. J. Nanomedicine.* 2012; 7:4391–4408. [PubMed: 22927757]
75. Goncalves AS, Macedo AS, Souto EB. Therapeutic nanosystems for oncology nanomedicine. *Clin. Transl. Oncol.* 2012; 14:883–890. [PubMed: 22855194]
76. Kratz F, Warnecke A. Finding the optimal balance: Challenges of improving conventional cancer chemotherapy using suitable combinations with nano-sized drug delivery systems. *J. Control Release.* 2012; 164:221–235. [PubMed: 22705248]
77. Cheng J, Meziani MJ, Sun YP, Cheng SH. Poly(ethylene glycol)-conjugated multi-walled carbon nanotubes as an efficient drug carrier for overcoming multidrug resistance. *Toxicol. Appl. Pharmacol.* 2011; 250:184–193. [PubMed: 20970441]
78. Haley B, Frenkel E. Nanoparticles for drug delivery in cancer treatment. *Urol. Oncol.* 2008; 26:57–64. [PubMed: 18190833]
79. Arayne MS, Sultana N. Review: nanoparticles in drug delivery for the treatment of cancer. *Pak. J. Pharm. Sci.* 2006; 19:258–268. [PubMed: 16935836]
80. Liang XJ, Meng H, Wang Y, He H, Meng J, Lu J, Wang PC, Zhao Y, Gao X, Sun B, Chen C, Xing G, Shen D, Gottesman MM, Wu Y, Yin JJ, Jia L. Metallofullerene nanoparticles circumvent tumor resistance to cisplatin by reactivating endocytosis. *Proc. Natl. Acad. Sci. U S A.* 2010; 107:7449–7454. [PubMed: 20368438]
81. Singhal SS, Yadav S, Singhal J, Sahu M, Sehrawat A, Awasthi S. Diminished drug transport and augmented radiation sensitivity caused by loss of RLIP76. *FEBS Lett.* 2008; 582:3408–3414. [PubMed: 18789326]
82. Boohaker RJ, Zhang G, Lee MW, Nemec KN, Santra S, Perez JM, Khaled AR. Rational Development of a Cytotoxic Peptide To Trigger Cell Death. *Mol. Pharm.* 2012 Epub ahead of print.
83. Taratula O, Garbuzenko OB, Chen AM, Minko T. Innovative strategy for treatment of lung cancer: targeted nanotechnology-based inhalation co-delivery of anticancer drugs and siRNA. *J. Drug Target.* 2011; 19:900–914. [PubMed: 21981718]
84. Nosko MM, Pivnyuk VM, Solyanik GI, Kulik GI, Todor IN, Momot VY, Melnikov OR, Ponomareva OV, Chekhun VF. Biodistribution analysis of cisplatin in liposomal form in animals with cisplatin-resistant and cisplatin-sensitive carcinoma. *Exp. Oncol.* 2010; 32:40–43. [PubMed: 20355294]
85. dos Santos Giuberti C, de Oliveira Reis EC, Ribeiro Rocha TG, Leite EA, Lacerda RG, Ramaldes GA, de Oliveira MC. Study of the pilot production process of long-circulating and pH-sensitive liposomes containing cisplatin. *J. Liposome Res.* 2011; 21:60–69. [PubMed: 20429813]
86. Zalba S, Garrido MJ. Liposomes, a promising strategy for clinical application of platinum derivatives. *Expert Opin. Drug Deliv.* 2013; 10:829–844. [PubMed: 23470129]

87. Lucas X, Simon S, Schubert R, Gunther S. Discovery of the inhibitory effect of a phosphatidylinositol derivative on P-glycoprotein by virtual screening followed by in vitro cellular studies. *PLoS One*. 2013; 8:e60679. [PubMed: 23593281]
88. Yefimova SL, Kurilchenko IY, Tkacheva TN, Rozhkov VA, Sorokin AV, Lukianova NY, Bezdenezhnykh NA, Malyukin YV, Chekhun VF. Comparative study of dye-loaded liposome accumulation in sensitive and resistant human breast cancer cells. *Exp. Oncol*. 2012; 34:101–106. [PubMed: 23013761]
89. Chen M, Chen J, Hou T, Fang Y, Sun W, Hu R, Cai B. Effect of phospholipid composition on pharmaceutical properties and anti-tumor activity of stealth liposomes containing brucine. *Zhongguo Zhong Yao Za Zhi*. 2011; 36:864–867. [PubMed: 21761723]
90. Krieger ML, Eckstein N, Schneider V, Koch M, Royer HD, Jaehde U, Bendas G. Overcoming cisplatin resistance of ovarian cancer cells by targeted liposomes in vitro. *Int. J. Pharm*. 2010; 389:10–17. [PubMed: 20060458]
91. Ye S, Yang W, Wang Y, Ou W, Ma Q, Yu C, Ren J, Zhong G, Shi H, Yuan Z, et al. Cationic liposome-mediated nitric oxide synthase gene therapy enhances the antitumor effects of cisplatin in lung cancer. *Int. J. Mol. Med*. 2013; 31:33–42. [PubMed: 23128378]
92. Farhat FS, Temraz S, Kattan J, Ibrahim K, Bitar N, Haddad N, Jalloul R, Hatoum HA, Nsouli G, Shamseddine AI. A phase II study of lipoplatin (liposomal cisplatin)/vinorelbine combination in HER-2/neu-negative metastatic breast cancer. *Clin. Breast Cancer*. 2011; 11:384–389. [PubMed: 21993011]
93. Canta A, Chiorazzi A, Carozzi V, Meregalli C, Oggioni N, Sala B, Crippa L, Avezza F, Forestieri D, Rotella G, Zucchetti M, Cavaletti G. In vivo comparative study of the cytotoxicity of a liposomal formulation of cisplatin (lipoplatin). *Cancer Chemother. Pharmacol*. 2011; 68:1001–1008. [PubMed: 21327681]
94. Devarajan P, Tarabishi R, Mishra J, Ma Q, Kourvetaris A, Vougiouka M, Boulikas T. Low renal toxicity of lipoplatin compared to cisplatin in animals. *Anticancer Res*. 2004; 24:2193–2200. [PubMed: 15330160]
95. Arienti C, Tesei A, Ravaioli A, Ratta M, Carloni S, Mangianti S, Ulivi P, Nicoletti S, Amadori D, Zoli W. Activity of lipoplatin in tumor and in normal cells in vitro. *Anticancer Drugs*. 2008; 19:983–990. [PubMed: 18827563]
96. Chen CJ, Chin JE, Ueda K, Clark DP, Pastan I, Gottesman MM, Roninson IB. Internal duplication and homology with bacterial transport proteins in the *mdr1* (P-glycoprotein) gene from multidrug-resistant human cells. *Cell*. 1986; 47:381–389. [PubMed: 2876781]
97. Thiebaut F, Tsuruo T, Hamada H, Gottesman MM, Pastan I, Willingham MC. Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. *Proc. Natl. Acad. Sci. U S A*. 1987; 84:7735–7738. [PubMed: 2444983]
98. Batrakova EV, Li S, Vinogradov SV, Alakhov VY, Miller DW, Kabanov AV. Mechanism of pluronic effect on P-glycoprotein efflux system in blood-brain barrier: contributions of energy depletion and membrane fluidization. *J. Pharmacol. Exp. Ther*. 2001; 299:483–493. [PubMed: 11602658]
99. Batrakova EV, Li S, Li Y, Alakhov VY, Kabanov AV. Effect of pluronic P85 on ATPase activity of drug efflux transporters. *Pharm. Res*. 2004; 21:2226–2233. [PubMed: 15648254]
100. Wu YC, Ling TY, Lu SH, Kuo HC, Ho HN, Yeh SD, Shen CN, Huang YH. Chemotherapeutic sensitivity of testicular germ cell tumors under hypoxic conditions is negatively regulated by SENP1-controlled sumoylation of OCT4. *Cancer Res*. 2012; 72:4963–4973. [PubMed: 23002208]
101. Garg A, Tisdale AW, Haidari E, Kokkoli E. Targeting colon cancer cells using PEGylated liposomes modified with a fibronectin-mimetic peptide. *Int. J. Pharm*. 2009; 366:201–210. [PubMed: 18835580]
102. Codogno P, Meijer AJ. Autophagy and signaling: their role in cell survival and cell death. *Cell Death Differ*. 2005; 12 Suppl 2:1509–1518. [PubMed: 16247498]
103. Levine B, Yuan J. Autophagy in cell death: an innocent convict? *J. Clin. Invest*. 2005; 115:2679–2688. [PubMed: 16200202]

104. Ren JH, He WS, Nong L, Zhu QY, Hu K, Zhang RG, Huang LL, Zhu F, Wu G. Acquired cisplatin resistance in human lung adenocarcinoma cells is associated with enhanced autophagy. *Cancer Biother Radiopharm.* 2010; 25:75–80. [PubMed: 20187799]
105. Sirichanchuen B, Pengsuparp T, Chanvorachote P. Long-term cisplatin exposure impairs autophagy and causes cisplatin resistance in human lung cancer cells. *Mol. Cell Biochem.* 2012; 364:11–18. [PubMed: 22278384]
106. Chen S, Rehman SK, Zhang W, Wen A, Yao L, Zhang J. Autophagy is a therapeutic target in anticancer drug resistance. *Biochim. Biophys. Acta.* 2010; 1806:220–229. [PubMed: 20637264]
107. Bednarski PJ, Korpis K, Westendorf AF, Perfahl S, Grunert R. Effects of light-activated diazido-Pt(IV) complexes on cancer cells in vitro. *Philos. Trans. A Math. Phys. Eng. Sci.* 2013; 371:20120118.
108. Zhang Q, Yang W, Man N, Zheng F, Shen Y, Sun K, Li Y, Wen LP. Autophagy-mediated chemosensitization in cancer cells by fullerene C60 nanocrystal. *Autophagy.* 2009; 5:1107–1117. [PubMed: 19786831]
109. Wei P, Zhang L, Lu Y, Man N, Wen L. C60(Nd) nanoparticles enhance chemotherapeutic susceptibility of cancer cells by modulation of autophagy. *Nanotechnology.* 2010; 21:495101. [PubMed: 21071824]
110. Dhar S, Gu FX, Langer R, Farokhzad OC, Lippard SJ. Targeted delivery of cisplatin to prostate cancer cells by aptamer functionalized Pt(IV) prodrug-PLGA-PEG nanoparticles. *Proc. Natl. Acad. Sci. U S A.* 2008; 105:17356–17361. [PubMed: 18978032]
111. Dhar S, Kolishetti N, Lippard SJ, Farokhzad OC. Targeted delivery of a cisplatin prodrug for safer and more effective prostate cancer therapy in vivo. *Proc. Natl. Acad. Sci. U S A.* 2011; 108:1850–1855. [PubMed: 21233423]
112. Wilson JJ, Lippard SJ. Synthesis, characterization, and cytotoxicity of platinum(IV) carbamate complexes. *Inorg. Chem.* 2011; 50:3103–31015. [PubMed: 21361279]
113. Wilson JJ, Lippard SJ. Modulation of Ligand Fluorescence by the Pt(II)/Pt(IV) Redox Couple. *Inorganica. Chim. Acta.* 2012; 389:77–84. [PubMed: 22837584]
114. Dhar S, Liu Z, Thomale J, Dai H, Lippard SJ. Targeted single-wall carbon nanotube-mediated Pt(IV) prodrug delivery using folate as a homing device. *J. Am. Chem. Soc.* 2008; 130:11467–11476. [PubMed: 18661990]
115. Dhar S, Daniel WL, Giljohann DA, Mirkin CA, Lippard SJ. Polyvalent oligonucleotide gold nanoparticle conjugates as delivery vehicles for platinum(IV) warheads. *J. Am. Chem. Soc.* 2009; 131:14652–14653. [PubMed: 19778015]
116. Graf N, Bielenberg DR, Kolishetti N, Muus C, Banyard J, Farokhzad OC, Lippard SJ. $\alpha(V)\beta(3)$ integrin-targeted PLGA-PEG nanoparticles for enhanced anti-tumor efficacy of a Pt(IV) prodrug. *ACS Nano.* 2012; 6:4530–4539. [PubMed: 22584163]
117. Mukhopadhyay S, Barnes CM, Haskel A, Short SM, Barnes KR, Lippard SJ. Conjugated platinum(IV)-peptide complexes for targeting angiogenic tumor vasculature. *Bioconjug. Chem.* 2008; 19:39–49. [PubMed: 17845003]
118. Yang J, Sun X, Mao W, Sui M, Tang J, Shen Y. Conjugate of Pt(IV)-histone deacetylase inhibitor as a prodrug for cancer chemotherapy. *Mol. Pharm.* 2012; 9:2793–2800. [PubMed: 22953987]
119. Yang J, Liu W, Sui M, Tang J, Shen Y. Platinum (IV)-coordinate polymers as intracellular reduction-responsive backbone-type conjugates for cancer drug delivery. *Biomaterials.* 2011; 32:9136–9143. [PubMed: 21889206]
120. Wang R, Hu X, Wu S, Xiao H, Cai H, Xie Z, Huang Y, Jing X. Biological characterization of folate-decorated biodegradable polymer-platinum(II) complex micelles. *Mol. Pharm.* 2012; 9:3200–3208. [PubMed: 22954154]
121. Comenge J, Sotelo C, Romero F, Gallego O, Barnadas A, Parada TG, Dominguez F, Puentes VF. Detoxifying antitumoral drugs via nanoconjugation: the case of gold nanoparticles and cisplatin. *PLoS One.* 2012; 7:e47562. [PubMed: 23082177]
122. Ajima K, Murakami T, Mizoguchi Y, Tsuchida K, Ichihashi T, Iijima S, Yudasaka M. Enhancement of in vivo anticancer effects of cisplatin by incorporation inside single-wall carbon nanohorns. *ACS Nano.* 2008; 2:2057–2064. [PubMed: 19206452]

123. Johnstone TC, Lippard SJ. The Effect of Ligand Lipophilicity on the Nanoparticle Encapsulation of Pt(IV) Prodrugs. *Inorg. Chem.* 2013 Epub ahead of Print.
124. Lian HY, Hu M, Liu CH, Yamauchi Y, Wu KC. Highly biocompatible, hollow coordination polymer nanoparticles as cisplatin carriers for efficient intracellular drug delivery. *Chem. Commun. (Camb)*. 2012; 48:5151–5153. [PubMed: 22514015]
125. Tao Z, Toms B, Goodisman J, Asefa T. Mesoporous silica microparticles enhance the cytotoxicity of anticancer platinum drugs. *ACS Nano*. 2010; 4:789–794. [PubMed: 20131868]
126. Morelli C, Maris P, Sisci D, Perrotta E, Brunelli E, Perrotta I, Panno ML, Tagarelli A, Versace C, Casula MF, Testa F, Ando S, Nagy JB, Pasqua L. PEG-templated mesoporous silica nanoparticles exclusively target cancer cells. *Nanoscale*. 2011; 3:3198–3207. [PubMed: 21725561]
127. Jadhav VB, Jun YJ, Song JH, Park MK, Oh JH, Chae SW, Kim IS, Choi SJ, Lee HJ, Sohn YS. A novel micelle-encapsulated platinum(II) anticancer agent. *J. Control Release*. 2010; 147:144–150. [PubMed: 20654662]
128. Shoaee-Hassani A, Mortazavi-Tabatabaei SA, Sharif S, Madadi S, Rezaei-Khaligh H, Verdi J. Recombinant lambda bacteriophage displaying nanobody towards third domain of HER-2 epitope inhibits proliferation of breast carcinoma SKBR-3 cell line. *Arch. Immunol. Ther. Exp. (Warsz)*. 2013; 61:75–83. [PubMed: 23224340]
129. Tijink BM, Laeremans T, Budde M, Stigter-van Walsum M, Dreier T, de Haard HJ, Leemans CR, van Dongen GA. Improved tumor targeting of anti-epidermal growth factor receptor Nanobodies through albumin binding: taking advantage of modular Nanobody technology. *Mol. Cancer Ther.* 2008; 7:2288–2297. [PubMed: 18723476]
130. Oliveira S, Heukers R, Sornkom J, Kok RJ, van Bergen En Henegouwen PM. Targeting tumors with nanobodies for cancer imaging and therapy. *J. Control Release*. 2013 Epub ahead of print.
131. Chudecka-Glaz A, Rzepka-Gorska I. Evaluation of serum levels of sCD30L ligand in patients with ovarian cancer in terms of selected clinico-pathological factors. *Contemp. Oncol. (Pozn)*. 2012; 16:520–525. [PubMed: 23788939]
132. Bourzac K. Nanotechnology: Carrying drugs. *Nature*. 2012; 491:58–60.
133. Endo K, Ueno T, Kondo S, Wakisaka N, Muroto S, Ito M, Kataoka K, Kato Y, Yoshizaki T. Tumor-targeted chemotherapy with the nanopolymer-based drug NC-6004 for oral squamous cell carcinoma. *Cancer Sci*. 2013; 104:369–374. [PubMed: 23216802]
134. Baba M, Matsumoto Y, Kashio A, Cabral H, Nishiyama N, Kataoka K, Yamasoba T. Micellization of cisplatin (NC-6004) reduces its ototoxicity in guinea pigs. *J. Control Release*. 2012; 157:112–117. [PubMed: 21807044]
135. Uchino H, Matsumura Y, Negishi T, Koizumi F, Hayashi T, Honda T, Nishiyama N, Kataoka K, Naito S, Kakizoe T. Cisplatin-incorporating polymeric micelles (NC-6004) can reduce nephrotoxicity and neurotoxicity of cisplatin in rats. *Br. J. Cancer*. 2005; 93:678–687. [PubMed: 16222314]
136. Takahashi R, Ishiwatari T. Preparation of helical gold nanowires on surfactant tubules. *Chem. Commun. (Camb)*. 2004:1406140–1406147.
137. Nishiyama N, Kataoka K. [Development of polymeric micelles for the delivery of antitumor agents]. *Gan To Kagaku Ryoho*. 2009; 36:357–361. [PubMed: 19295258]
138. Murakami M, Cabral H, Matsumoto Y, Wu S, Kano MR, Yamori T, Nishiyama N, Kataoka K. Improving drug potency and efficacy by nanocarrier-mediated subcellular targeting. *Sci. Transl. Med*. 2011; 3 64ra2.
139. Huo S, Ma H, Huang K, Liu J, Wei T, Jin S, Zhang J, He S, Liang XJ. Superior penetration and retention behavior of 50 nm gold nanoparticles in tumors. *Cancer Res*. 2013; 73:319–330. [PubMed: 23074284]
140. Zhao Y, Zhang X, Xue X, Li Z, Chen F, Li S, Kumar A, Zou G, Liang XJ. High throughput detection of human neutrophil peptides from serum, saliva, and tear by anthrax lethal factor-modified nanoparticles. *ACS Appl. Mater. Interfaces*. 2013; 5:8267–8272. [PubMed: 23965149]
141. Xue X, Zhao Y, Dai L, Zhang X, Hao X, Zhang C, Huo S, Liu J, Liu C, Kumar A, Chen WQ, Zou G, Liang XJ. Spatiotemporal Drug Release Visualized through a Drug Delivery System with Tunable Aggregation-Induced Emission. *Adv. Mater*. 2013 Epub ahead of print.

142. Boulikas T. Clinical overview on Lipoplatin: a successful liposomal formulation of cisplatin. *Expert Opin. Investig. Drugs*. 2009; 18:1197–1218.
143. Stathopoulos GP, Boulikas T. Lipoplatin formulation review article. *J. Drug Deliv*. 2012; 2012:581363. [PubMed: 21904682]

Figure 1.

Mitaplatin circumvents cancer resistance to cisplatin by targeting mitochondria. In cancer cells, oxidative phosphorylation is inhibited, and cancer cells rely on cytoplasmic glycolysis to produce energy. This metabolic shift induces apoptosis resistance. After crossing the membrane, mitaplatin targets mitochondria, inhibits the activity of mitochondrial PDK, and leads to activation of PDH, which promotes the influx of acetyl-CoA into mitochondria and increases the Krebs cycle. With triggering hyperpolarized $\Delta\psi_m$ in resistant cells, mitaplatin also results in reduced glucose utilization. Similar to cisplatin, mitaplatin also enters the nuclei and targets DNA to form 1,2-intrastrand d (GpG) cross-links.³¹ (Adapted from Xue et al., Mitaplatin increases sensitivity of tumor cells to cisplatin by inducing mitochondrial dysfunction. *Mol Pharm* 2012, 9, 634-44, Figure 7)

Figure 2.

Depiction of a lipoplatin nanoparticle. **A.** Cisplatin molecules are depicted as blue spheres surrounded by the lipid bilayer with hair-like PEGylated lipids protruding from the outer surface. **B.** Penetration of lipoplatin nanoparticles through the cell membrane of tumor cells. Lipoplatin nanoparticles once inside the tumor cell mass can fuse with the cell membrane because of the presence of the fusogenic lipid DPPG in their lipid bilayer; an alternative mechanism proposed is that lipoplatin is taken up by endocytosis by tumor cells. These processes occurring at the cell membrane level are promoted by the lipid shell of the

nanoparticles (disguised as nutrients).^{142, 143} These images are from CRNS research/
SAGASCIENCE/Caillaud Francois.

Figure 3.

Examples of the chemical strategies used to tether cisplatin and cisplatin-like moieties to nanoparticle constructs. **A.** The platinum(IV) complex *cis,trans,cis*-[PtCl₂(OH)₂(NH₃)₂] contains cisplatin in the equatorial plane along with two hydroxido ligands in the axial positions. Reaction with succinic acid produces *cis,trans,cis*-[PtCl₂(OH)(O₂CCH₂CH₂CO₂H)(NH₃)₂]. The terminal carboxylic acid can then be conjugated to amine-functionalized nanoparticles (NPs) by amide coupling. In a biological environment, the tethered NP-platinum(IV) complex can be reduced by biological reductants such as glutathione or ascorbate. This results in the release of cisplatin, a platinum(II) complex, and

the axial ligands (one of which is the NP). As such, provided that ligand exchange reactions at the platinum(IV) center do not take place, this conjugation method results in NPs that release cisplatin. **B.** In this instance, cisplatin is tethered to the NP via its 'leaving groups'. To achieve this chemistry, cisplatin is reacted with silver nitrate. While silver nitrate is soluble, silver chloride is highly insoluble – as silver precipitates with chloride exchanged from cisplatin, a 'diaqua' species is produced. Aqua ligands are relatively unstable, and this aquation step of cisplatin (by loss of chlorido ligands) in cells is considered to be a necessary intermediate reaction before cisplatin reacts with DNA. Once the 'reactive' diaqua species is produced, it is reacted with carboxylic acid functionalized NPs. The NP carboxylate groups coordinate with platinum(II) complex in place of the aqua ligands. In this sense the NP acts as a very large bidentate ligand, analogous to the leaving groups of the platinum(II) drug carboplatin. Release of the platinum complex from the NP in a relatively acidic environment in theory releases the highly reactive diaqua form of cisplatin.