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RETHINKING ALKYLATING(-LIKE) AGENTS FOR SOLID TUMOR MANAGEMENT

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Abstract

Although old molecules, alkylating agents and platinum derivatives are still widely used in the treatment of various solid tumors. However, systemic toxicity and cellular resistance mechanisms impede their efficacy. Innovative strategies, including local administration, optimization of treatment schedule/dosage, synergistic combinations, and the encapsulation of bioactive molecules in smart, multifunctional drug delivery systems, have shown promising results in potentiating anticancer activity while circumventing such hurdles. Furthermore, questioning of the old paradigm according to which nuclear DNA is the critical target of their anticancer activity has shed light on subcellular alternative and neglected targets that obviously participate in the mediation of cytotoxicity or resistance. Thus, rethinking of the use of these pivotal antineoplastic agents appears critical to improve clinical outcomes in the management of solid tumors.

Highlights

Alkylating agents and platinum derivatives remain key in the management of solid tumors.

Conventional clinical practice is dramatically impeded by cellular and molecular resistance mechanisms to alkylating agents and platinum derivatives.

The design of alternative strategies that aim at rethinking the use of these old drugs is of paramount importance to give them a new impetus in oncology.

Synergistic combinations and optimization of the treatment schedule/ dosage by metronomic chemotherapy have shown positive outcomes in cancer patients.

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Local drug delivery and targeting of critical organelles in cancer cells through the investigation of new routes of administration and the encapsulation of bioactive molecules inside smart, multifunctional drug delivery systems constitute promising strategies to potentiate the therapeutic index of alkylating agents and platinum derivatives while circumventing drug resistance.

Strategic Paths Towards Anticancer Therapy

Oncology mainly focuses on patient symptoms, treating hallmarks acquired by normal cells that gradually progress to a neoplastic state, instead of fighting against a still-unknown causal entity responsible for cancer occurrence and progression [1]. Global strategies, namely chemotherapy and radiotherapy (RT), still comprise the mainstay of the treatment of solid tumors by addressing specific mechanisms involved in tumorigenesis. More targeted therapies (see Glossary), such as antiangiogenesis strategies, have been developed with various degrees of success depending on the patient pathogenesis [2,3]. A better insight into the diverse underlying processes, including causes, triggered cellular and molecular pathways, and potential related targets, would definitely help in the development of relevant and effective anticancer treatments.

In contrast to the empiricism from animal models that gave rise to alkylating agents or to the rational design emanating from the targeting of pathways altered in tumors, we suggest that rethinking the use of conventional anticancer drugs could make it possible to exploit their full potential. This alternative approach relies on the optimization of an already marketed bioactive drug capable of reaching its target in effective concentrations to exert its anticancer activity while limiting adverse side effects. In this context, alkylating agents are old molecules still widely used in the frontline treatment of various solid tumors. Among them, platinum derivatives do not alkylate but rather complex with their nucleophilic targets. Although historically affiliated to alkylating agents, they should therefore rather be referred to as 'alkylating-like' agents. Half of cancer patients experience platinum-based drug therapy [4,5]. Thus, the clinical relevance of platinum compounds is key in daily practice. **Cisplatin** is the oldest platinum drug approved by the FDA. Although alternative platinum derivatives have been developed to improve its therapeutic index, cisplatin remains the leader molecule of platinum complexes and one of the most compelling anticancer drugs, with a pivotal role in the management of solid tumors [6,7]. Therefore, cisplatin will be addressed as a prototypic platinum-based anticancer agent to exemplify paradigms, mechanisms, limitations, and new directions that fall under a broader understanding of the future of alkylating agents and platinum compounds in the clinic.

In the following, we provide an up-to-date review of the rationale and conventional use of alkylating agents and platinum derivatives in clinical practice. Then, we focus on optimization, synergies, and innovative alternatives that pave the way for rethinking how to potentiate their anticancer efficacy, laying down future challenges for these old molecules in the treatment of solid tumors, with the ultimate view of personalized medicine.

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Rationale and Conventional Use of Alkylating Agents and Platinum Derivatives in Clinical Practice

After the attack on Bari Harbor in 1943 revealed the effects of mustard gas on bone marrow depletion and the first therapeutic outcomes in lymphoma, alkylating agents gradually became a gold standard as first-line treatment for various cancer indications. The DrugBank database reports all FDA-approved alkylating agents and affiliated compounds in worldwide use, their initial indications, delivery type, and administration route (Table 1) [8]. Other alkylating agents (e.g., mitolactol, which has been granted orphan drug designation from the FDA for the treatment of invasive carcinoma of the uterine cervix and as adjuvant therapy in the treatment of primary brain tumors) and platinum complexes [lobaplatin for inoperable metastatic breast cancer, chronic myelogenous leukemia, and small cell lung cancer in China, heptaplatin for gastric cancer in Korea, and nedaplatin for non-small cell lung cancer, esophageal cancer, and head and neck cancer and miriplatin for hepatocellular carcinoma in Japan] are also currently in use in humans [5].

Mechanism

Anticancer agents are traditionally classified in chemical families according to their mode of action. Intercalating and alkylating agents are reported to directly interact with DNA by inter- or intrastrand crosslinking. However, the mechanism of action of intercalating agents that form formaldehyde-based covalent bonds with DNA bases as shown through the example of anthracycline antibiotics on Figure 1A strictly differs from that of alkylating agents [9]. Alkylators allow the transfer of an alkyl group from one molecule to another under physiological conditions. Such nucleophilic substitutions occur by an S_N1 or S_N2 mechanism depending on the kinetics of the reaction and result in covalent binding to an organic macromolecule as depicted in Figure 1B in the case of temozolomide [10]. Since exposure to alkylating agents leads to chromosomal aberrations in dividing cells, DNA represents the key target site for alkylation in cells. This hypothesis is further supported by its high molar mass, which makes DNA the major nucleophilic substrate for alkylation within the organism, far ahead of RNA and proteins. Alkylation mainly occurs during S phase, while DNA is replicating; the two strands are separated, making nucleophilic substrates easily reachable. A blockage in G2 phase was also reported [11]. Alkylating agents are more likely to bind to exposed nucleophilic sites in the grooves of the DNA double helix: guanine (positions N_7, O_6, N_2, and N_3), adenine (N_3 and N_7), and cytosine (N_3) bases. The resulting adducts prevent strands from uncoiling and separating, making DNA replication and downstream RNA transcription impossible where the alkylation occurred. Platinum complexes are stabilized by various ligands that can be substituted by nucleophilic substrates to form a strong coordination bound with the central platinum atom. In this respect, platinum compounds were historically considered as alkylating agents although they do not interact with biological macromolecules through an alkyl group but rather by complexation. Cisplatin, whose mechanism of action is illustrated in Figure 1C, enabled a dramatic improvement in the prognosis of germinal cancer cells and is still used as a gold standard in the treatment of various solid tumors [6,7]. Contrary to alterations caused by monofunctional alkylating agents such as nitrosoureas, inter- or intracatenary bridges induced by platinum derivatives between the two DNA strands are extremely difficult to repair.

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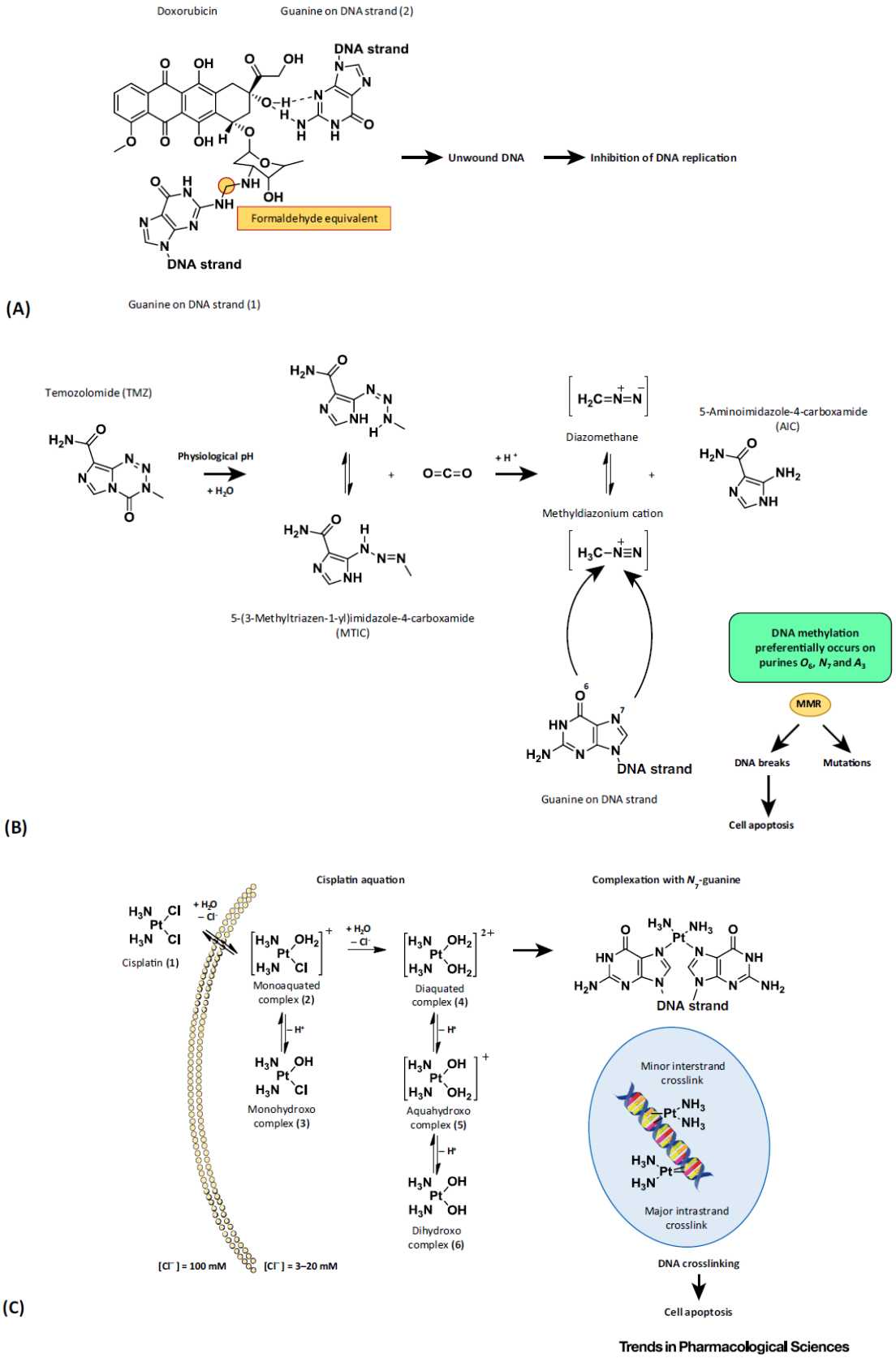

Figure 1. Various Mechanisms of Action of DNA-Targeting Agents. (A) Intercalation of doxorubicin between DNA strands. Doxorubicin forms with guanine a covalent bond (formaldehyde equivalent) on one DNA strand and hydrogen bonds on the

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opposite strand to stabilize the structure. Consequently, DNA stays unwound and replication becomes impossible. Interactions with DNA preferentially occur with neighboring GC base pairs [9]. (B) DNA methylation by temozolomide. Temozolomide acts as a prodrug spontaneously hydrolyzed at physiological pH to its active metabolite MTIC, subsequently converted to AIC and methyldiazonium [10]. This highly reactive cation methylates purine bases, preferentially O_6 and N_7 guanines and to a lesser extent A_3 adenine, thus inhibiting DNA replication. Excision of O_6methylguanine adducts by MMR enzymes may induce either mutations continuously recovered along replications or DNA single- or double-strand breaks responsible for cell apoptosis [43]. (C) DNA complexation with cisplatin. Cisplatin (1) requires the substitution of at least one chloride group by water for its activation, a process called aquation. This hydrolysis automatically occurs once cisplatin is internalized because of the low intracellular chloride concentration. The reactivity of Pt(II) complexes – (4) > (2), (5) > (1), (3) >> (6) – is determined by the ability of every ligand to be substituted by a nucleophile [4]. Active Pt(II) species complex with nucleophilic intracellular ligands: N_7 sites on purine DNA or RNA bases, mainly guanine and to a lesser extent adenine, and nucleophilic sites on several proteins [7,12]. Guanine intrastrand crosslinking with cisplatin impedes DNA replication and transcription [6,11].

Glossary

Alkyl group: a univalent group derived from alkanes by the removal of a hydrogen atom from any carbon atom, an alkane being an acyclic branched or unbranched hydrocarbon having the general formula C_nH_{n+2}.

cis-Diamminedichloroplatinum(II) (cisplatin): a metallic coordination complex with a central platinum atom in a divalent state, two labile chlorine groups, and two stable amine ligands located in a *cis* configuration. Its ability to inhibit DNA synthesis on *Escherichia coli* bacterial culture was serendipitously discovered in 1965 by Rosenberg and led to its FDA approval as an antineoplastic agent in 1978.

Dose deposition: quantifies the concentration of energy absorbed in a tissue following exposure to ionizing radiation. Basically, the absorption of X-rays of a given frequency increases with the atomic number Z of the penetrated material, which explains the radiosensitizing properties of platinum derivatives.

Drug delivery system (DDS): a formulation or device that carries a therapeutic compound throughout the body and improves its efficacy while limiting systemic toxicity by controlling the location, time, and rate of drug release.

Gliadel®: 3.85%-carmustine-loaded polymeric wafers that enable controlled and sustained drug release. Although controversial, their implantation in the resection bed in operable newly diagnosed glioblastoma patients was approved in 2002 by the FDA as first-line treatment.

Glutathione (GSH): with a concentration of 0.5–10 mM, this tripeptide is the most abundant thiol in the cell.

Heat-shock proteins (HSPs): produced by living organisms in response to a stress such as temperature or exposure to heavy metals; overexpressed in cisplatin-resistant cells. HSPs prevent protein impairment.

Maximum tolerated dose (MTD): evaluated in Phase I clinical trials, the MTD is the highest dose of a drug or a treatment that does not induce unacceptable side effects.

Metallothioneins: proteins comprising large amounts of sulfur-rich amino acids – namely, cysteine. Exhibiting high affinity to metals, they play a key role in drug detoxification.

microRNA (miRNA): regulatory endogenous noncoding RNAs produced by the genome.

O6-Methylguanine-DNA methyltransferase (MGMT): enzyme involved in the repair of methylated DNA adducts. p53: tumor suppressor notably involved in cell cycle regulation and apoptotic cell death, its mutation is a common feature in human cancer cells.

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Poloxamers: amphiphilic block copolymers able to self-assemble into micelles.

Targeted therapies: therapeutic strategies that use drugs or other substances to recognize particular entities associated with hallmarks of cancer cells while sparing normal cells. Some targeted therapies work by blocking the action of cancer-specific genes, proteins, or environmental cues that contribute to cancer growth and survival.

Temozolomide: small, orally available lipophilic molecule of great interest in the treatment of malignant gliomas due to its ability to cross the blood–brain barrier.

Theranostics: merger between the words 'therapy' and 'diagnosis'.

Resistance

Intrinsic or acquired resistance to alkylating agents and platinum derivatives is considered a multifactorial phenomenon. In the case of cisplatin, it involves avoidance (e.g., drug exclusion from the cell [12–14] or from the nucleus [15]), prevention or escape (e.g., drug inactivation [4,6,15–17], resistance to apoptosis [11,13,14,18–20]), and repair (e.g., DNA repair [6,13,15,16,21–24]) mechanisms. Multiple intrinsic regulators that may also be modulated by extracellular triggers represent key (in)activators of these pleiotropic processes, as exemplified by mTOR in autophagy or microRNAs (miRNAs), and could be identified as relevant predictive biomarkers of patient response to a treatment with the perspective of providing more accurate and personalized chemotherapeutic regimens [24]. Figure 2A (Key Figure) illustrates the main cellular mechanisms that mediate resistance to cisplatin. The development of alternative platinum derivatives with a milder toxicity profile and able to alter all cells whatever their stage in the cell cycle, including stem cells located in the tumor margins that are insensitive to radio- or chemotherapy, is of particular interest to circumvent drug resistance [6].

Radiosensitization

RT constitutes a key strategy in the treatment of several solid tumors, including glioma, lung, breast, head and neck, uterine cervix, rectal, vulvar, and prostate cancers. The radiation beam causes direct DNA damage but also indirectly impacts cell death through the formation of highly reactive oxygen species (ROS). Modulation of the tumor response to RT can be achieved by resorting to various antineoplastic agents and has been extensively investigated in alkylating agents and platinum-based strategies with the aim of amplifying the differential effects between tumor and normal cells [25–28]. Due to their ability to form DNA adducts leading to double-strand breaks and the heavy platinum atom that locally enhances the effect of external-beam radiation, respectively, alkylating agents and platinum compounds are particularly used in combination with RT as effective radiosensitizing chemotherapy [25,27–29]. Clinical studies have shown further evidence of the superior efficacy of concomitant chemoradiotherapy in various solid tumors compared with RT alone [25,26,30]. This synergistic effect depicted in Figure 2B can be explained by more accurate locoregional control of the pathology with reduced or at least contained tumor cell proliferation that would otherwise quickly entail radioresistance, resulting in a better prognosis. Paradoxically, radioresistance can also occur from disruption of blood supply to the altered tissue after surgery

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Table1. FDA-Approved Alkylating Agents and Affiliated Compounds for Anticancer Therapy[a]

Drug	Approval year	Indication	Delivery type	Route
Nitrogen mustards				
Mechlorethamine	1949	Lung cancer Leukemia Lymphoma	Single	IV injection Intracavitary Intrapericardial
Chlorambucil	1957	Leukemia Lymphoma	Single	Oral
Cyclophosphamide	1959	Lymphoma Multiple myeloma Leukemia Brain cancer Ovarian cancer Retinoblastoma Breast cancer	Single or in combination	Oral IV injection
Uracil mustard	1962	Leukemia Lymphoma	Single	Oral
Melphalan	1964	Multiple myeloma Ovarian cancer	Combination	IV injection Oral
Estramustine phosphate sodium	1981	Prostate cancer	Combination	Oral
Ifosfamide	1988	Testicular cancer	Combination	IV injection
Bendamustine hydrochloride	2008	Lymphoma Leukemia	Single	IV injection
Nitrosoureas				
Lomustine (CCNU)	1976	Brain cancer Lymphoma	Single or in combination	Oral
Carmustine (BCNU)	1977	Brain cancer Lymphoma Multiple myeloma	Single or in combination	IV injection
Streptozocin	1982	Pancreatic cancer	Single	IV injection
Carmustine wafers (Gliadel®)	1996	Brain cancer	Single or in combination	Intracranial implantation
Platinum complexes				
Cisplatin	1978	Testicular cancer Ovarian cancer Bladder cancer	Single or in combination	IV injection
Carboplatin	1989	Ovarian cancer	Single or in combination	IV injection
Oxaliplatin	2004	Colon cancer Colorectal cancer	Combination	IV injection
Others				
Busulfan	1954	Leukemia	Combination	Oral IV injection
Thiotepa	1959	Breast cancer Ovarian cancer Bladder cancer	Single	IV injection Intravesical instillation
Pipobroman	1966	Leukemia	Single	Oral
Procarbazine hydrochloride	1969	Lymphoma	Combination	Oral

Table1. (continued)

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Drug	Approval year	Indication	Delivery type	Route
Mitomycin C	1974	Stomach cancer Pancreatic cancer Bladder cancer	Single or in combination	IV injection
Dacarbazine	1975	Melanoma Lymphoma	Single or in combination	IV injection
Altretamine	1990	Ovarian cancer	Single	Oral
Temozolomide	2005	Brain cancer	Single or in combination	Oral
Trabectedin	2015	Soft tissue sarcoma	Single	IV injection

[a]Marketing authorization and clinical practice guidelines are likely to evolve over time and depending on the country. IV, intravenous.

and chemotherapy, leading to hypoxic foci. Tumor radiosensitivity can then be modulated by chemical radiosensitizers that simultaneously enhance the therapeutic benefit of RT locally and exert their own cytotoxic effects [31,32]. The time schedule between chemotherapy and RT is a key point for their effective combination owing to dose- and time-dependent cytotoxicity of the drug, leading to synergism or at least to an additive effect on tumor cells [30]. Polychemotherapy (i.e., the combination of several drugs) offers another way to achieve synergism in anticancer treatment.

Polychemotherapy

Alkylating agents as well as platinum compounds are commonly used concurrently with other antineoplastic agents, including targeted drugs and antibodies, in the management of solid tumors. The combination of drugs that exert their anticancer activity through various mechanisms of action induces cell damage and metabolic dysfunction by altering several molecular targets and signaling pathways involved in tumorigenesis [14,33]. This option is therefore commonly considered in clinical practice to potentiate drug efficacy and reverse acquired drug resistance like that in ovarian, biliary tract, lung, breast, and prostate cancers that primary respond to a platinum-based treatment but ultimately relapse. For instance, the standard treatment for patients with advanced colorectal cancer that comprises the combination of 5fluorouracil, leucovorin, and oxaliplatin exhibited potentiation of the anticancer activity of oxaliplatin with fluoropyrimidines resulting in a significant improvement in overall survival. The design of complementary targeted drugs since the 2000s has further reinforced this trend [34,35]. In parallel, in the mid-1970s a breakthrough in the treatment of men with metastatic testicular cancer arose from a combinatory regimen based on cisplatin supplemented with bleomycin and vinblastine, leading to an increase in complete response rates from 5% to 60%. Substitution of vinblastine with etoposide further enabled the achievement of up-to-80% cure rates [6]. Additional adjunctive drugs can also be considered to modulate platinum activity or toxicity [36–41].

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Optimization of the Use of Alkylating Agents and Platinum Derivatives

Although dramatically limited by resistance mechanisms and lack of specificity associated with high systemic toxicity, alkylating agents and platinum derivatives remain pivotal in the management of solid tumors. Promising alternatives to their conventional use in clinical practice will be addressed in the following part that paves the way for reflection on the optimization of their use in anticancer therapy and suggests that the time may have come to bring these old molecules back on the stage again.

Key Figure

Strategies to Overcome Cellular Resistance and Enhance the Therapeutic Index of a Drug: The Example of Cisplatin

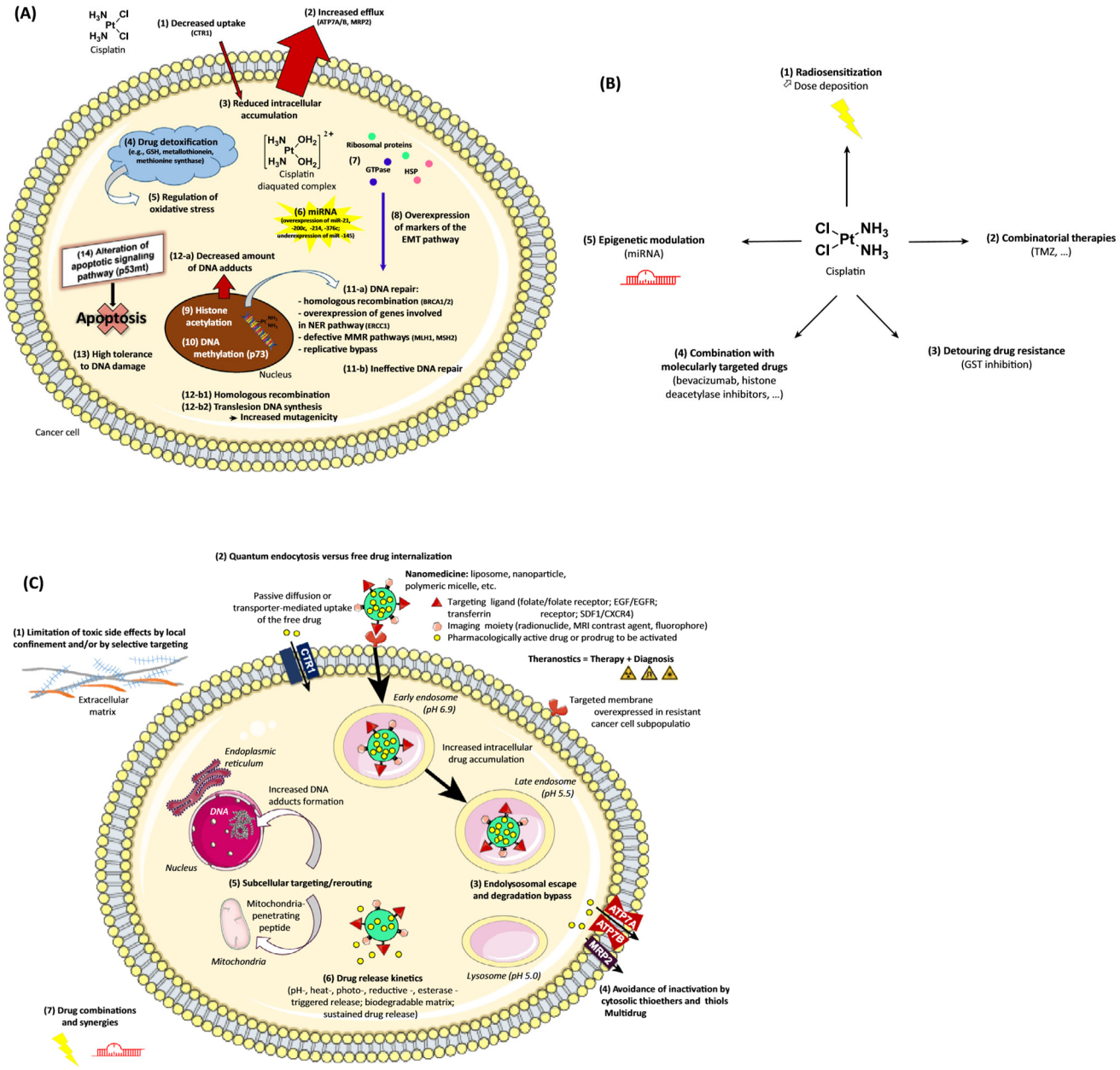

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Figure 2. (A) Cellular resistance mechanisms to cisplatin. (1) Impaired influx through altered transporter-mediated uptake (CTR1) [13,14] or, conversely, (2) active efflux outside the cell (ATP7A/B, MRP2) [6] is responsible for (3) reduced total intracellular accumulation of the drug [12]. Cisplatin efflux from the nucleus back to the cytoplasm also reduces drug distribution to nuclear DNA [15]. (4) The abundance in the cytosol of thiol- and thioether-containing amino acids and proteins for which cisplatin exhibits high affinity is responsible for detoxification processes that lead to drug sequestration and inactivation [4,16,17,73]. Furthermore, glutathione (GSH) may quench Pt-DNA monoadducts before their conversion into cytotoxic DNA crosslinks and (5) reduce cisplatin-induced oxidative stress in cells [15,16]. To overcome drug cytotoxicity, tumor cells trigger an overall abnormal phenotype by silencing or activating multiple genes, notably involved in modulation of the expression of (6) miRNA and (7) GTPases, ribosomal proteins, and **heat-shock proteins (HSPs)**, in (8) overexpression of markers of the epithelial-to-mesenchymal transition (EMT) pathway, and in (9) histone acetylation, (10) aberrant DNA methylation, (11) DNA damage repair, and (14) apoptotic signaling pathways [13]. (11-a) DNA crosslinking recognition and repair mechanisms allow (12-a) a decreased amount of DNA adducts, whereas (11-b) ineffective DNA repair leads to (12-b1) homologous recombination or (12-b2) translesion DNA synthesis that further results in genome instability and the recurrence of aggressive and resistant tumor cells [16]. Among other indications, cisplatin provided a breakthrough in the management of testis cancer attributed to intrinsic cellular hypersensitivity together with reduced ability to repair DNA adducts through the nucleotide excision repair (NER) pathway [6]. Other molecular pathways are also involved in the efficacy/toxicity of platinum-based regimens [13,15,17,24]. (13) Enhanced tolerance to DNA damage and (14) alteration of the apoptotic signaling pathway, especially p53 mutation (p53mt), result in cell escape from apoptosis and acquired resistance [6,15]. (B) Innovative synergies capable of detouring drug resistance mechanisms. (1) Cisplatin is conventionally used in combination with radiotherapy (RT) in the treatment of various solid tumors as it enhances dose deposition [25,28,29]. RT can increase the cellular uptake of cisplatin and promote the activation of toxic Pt(II) complexes. Conversely, cisplatin may stop the cell cycle and inhibit the molecular repair machinery that tackles radiation-induced DNA damage [28]. (2) Interestingly, cisplatin was reported to decrease the activity of O_6-methylguanineDNA methyltransferase (MGMT), whose expression counters temozolomide efficacy in glioma treatment [76]. (3) An alternative to reverse resistance-induced detoxification processes comprises taking advantage of the elevated levels of GSH in resistant cancer cells to specifically damage them [6,78] or to inhibit GSHS-transferase (GST) [103]. (4) Combination with histone deacetylase inhibitors prevents histones from binding to DNA, leaving it more accessible to alkylation/complexation [104]. (5) Sensitivity can also be restored through epigenetic modulations involving miRNAs for permissive or synergistic effects [82–85]. (C) Advantages of cisplatin nanovectorization over traditional regimens. Multifunctional nanocarriers are developed and evaluated towards optimized drug delivery to tumor cells for (1) locoregional confinement in specific environments and/or for selective targeting of receptors in relation to administration routes and modalities[87,94,95].They can also be engineered using radionuclides, MRI contrast agents, or fluorophores to assess the patient response in real time and adjust the treatment [116,118]. (2) Whereas free molecules individually enter the intracellular space by passive diffusion through the membrane or by transporter mediation, endocytosis of nanosized drug delivery systems (DDSs) enables internalization of the drug in a quantum form [109]. Nanocarriers have been synthesized to bypass (3) endolysosomal degradation, (4) detoxification processes, and drug elimination by multidrug resistance efflux [94]. Furthermore, tailored nanosystems can mediate (5) the rerouting or subcellular trafficking of the drug to target specific organelles [96]. The high intracellular platinum accumulation favored by the endocytic process is associated with increased formation of DNA adducts and markedly enhanced antitumor activity whatever the resistance status of the cells [69,91,97,103,104]. The versatility of structure of smart DDSs also allows (6) sustained drug release mediated by environmental triggers in the intracellular compartment or in the extracellular space [98,116]. (7) Drug combinations and synergies with alternative approaches such as adjuvant RT or modulation of the expression of resistance signals through miRNA agonist or antagonist strategies may reinforce the cytotoxicity of nanovectorized cisplatin.

Drug Administration and Dosage

Chemotherapy is often limited by systemic injection, which causes drug dilution within the organism and is responsible for severe side effects, especially on highly proliferative cells. The high systemic toxicity of conventional anticancer agents can be overcome by using a more suited route of administration depending on the tumor type. In the case of operable patients with glioblastoma, an alternative to temozolomide relies on the implantation of carmustine-loaded wafers (Gliadel®) in the resection cavity at the end of surgery [42,43]. In such an aqueous environment, the anhydride bonds of the biodegradable polymeric

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matrix are hydrolyzed, allowing controlled and sustained release of the drug, which can diffuse within the surrounding parenchyma over several weeks. After degradation, the active metabolite can alkylate DNA, crosslink with RNA, and entail protein carbamylation, ultimately leading to cell apoptosis [43]. Although Gliadel[1] exhibited high therapeutic efficacy in animal models, clinical translation is limited by side effects and poor diffusion within the damaged parenchyma, since the concentration gradient may not be great enough to allow carmustine to penetrate deep into the brain tissue and be distributed through the tumor margins [44–47]. Although alkylating agents have provided therapeutic efficacy and improved patient outcomes in the management of brain cancer, alternative strategies are required to reach therapeutic doses in close vicinity to the tumor burden and maximize their anticancer activity.

In this context, the locoregional administration of chemotherapy directly into the brain enables both bypassing of the blood–brain barrier that prevents most macromolecules and therapeutic drugs from reaching the central nervous system and a local increase of drug concentration. Convection-enhanced delivery (CED) comprises infusion of the drug at high concentration directly into the brain or the tumor via intraparenchymal microcatheters [44]. A constant hydrostatic positive-pressure gradient is established by an infusion pump that forces convection of the therapeutic solution at a rate of 0.1–10 ml min^{1}. Thus, by exploiting bulk flow through the interstitial spaces of the brain, CED achieves a homogeneous elliptical to spherical distribution of molecules of various molar masses over large distances compared with suboptimal therapeutic doses reached by classical infusion from a concentration gradient [45,48–50]. Because they cannot easily cross the blood–brain barrier, platinum derivatives do not reach brain tumors in optimal therapeutic concentrations when administered intravenously [51]. In animal models, CED was shown to dramatically increase the concentration of cisplatin and carboplatin in the brain tumor relative to traditional administration routes while reducing systemic toxicity [52]. Although safety and feasibility have been demonstrated in Phase I clinical trials, translation to the clinic has failed so far because of surgical complications [53,54]. In addition, increased interstitial fluid pressure in brain tumors and leakage into the cerebrospinal fluid drastically reduce the drug concentration at the targeted site and can even induce neurotoxicity [55,56]. Thus, technical advances are expected to fill the gap between the view of CED as a promising strategy to deliver therapeutic agents in situ to large and clinically relevant brain volumes and the current state of an invasive technique in which continuous or repeated administration is at risk due to infection, hemorrhage, or neurologic disorders related to catheter positioning inside the brain parenchyma [45,55,57]. In case of localized disease, other clinically relevant routes of administration have been investigated, such as intraperitoneal chemotherapy for primary or recurrent ovarian cancer [58,59].

The use of drugs at their **maximum tolerated dose (MTD)** requires intermittent drug-free periods between two cycles of chemotherapy that should allow the patient to recover from acute toxicities. However, tumor cells can regenerate during that resting time, and selected clones may develop resistance to the treatment [60,61]. As a result, the traditional rationale according to which higher doses are necessary for tumor eradication is slowly shifting to the concept that 'less is more', which favors stabilization of the disease over time for maintenance of quality of life. As hyperfractionated RT suggests that a continuous low-dose schedule may be more efficient in killing highly proliferative cells than standard RT by avoiding tumor cell repair, metronomic chemotherapy comprises the chronic and equally spaced administration of drugs at a low dose (one-tenth to one-third of the MTD) without extended rest periods [33,62]. Whereas drug administration by intermittent bolus generally results in high peak plasma concentrations that are further responsible for severe toxicity, 'dose-dense' strategies have shown encouraging results with evidence of

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disease stabilization and improved outcomes associated with a low toxicity profile in patients with solid tumors [61,63–65]. Interestingly, the frequent low-dose administration of traditional drugs makes them able to target dividing vascular endothelial cells, thus demonstrating additional antiangiogenic potential, while the stimulation of the anticancer immune response may further contribute to force tumor dormancy [33,60,62,64]. Furthermore, metronomic chemotherapy results in more convenient treatment administration and promotes the maintenance of patients' quality of life [65]. Economic reasons can also favor oral metronomic chemotherapy as a minimal-cost but still compelling alternative to the current standard of care, particularly in developing countries [66,67]. Metronomic regimens based on alkylating agents or platinum derivatives have shown a therapeutic benefit in patients with solid tumors [60,65,68]. However, large-scale studies and controlled randomized trials that compare conventional MTD with the same metronomic administration regimen are required to define the optimal drug dosage and schedule.

Alternative and Neglected Targets

Since chromosomal aberrations in dividing cells were an outstanding feature of mustard gas intoxication, most hypotheses postulated that nuclear DNA was the most critical pharmacological target of alkylating agents and platinum derivatives [13,14]. However, in the case of platinum-based treatments, the level of Pt-DNA adducts does not necessarily correlate with either intracellular drug accumulation or cytotoxicity, suggesting that other cellular or molecular components must be involved with various degrees of specificity and severity in anticancer activity [4,16,25,69,70]. Growing evidence notably suggests a role for mitochondria in cisplatin anticancer activity [13,17,71,72]. Mitochondria are involved in the apoptotic pathway through the release of cytochrome c into the cytosol and subsequent activation of caspases 8 and 9, thus constituting a critical target for cytotoxic drugs. Rerouting of chlorambucil through engineered mitochondria-penetrating peptides (MPPs) that are able to cross the dense and highly hydrophobic membranes of mitochondria exhibited dramatic potentiation of its anticancer activity in various cancer cell lines by promoting apoptosis and evading deactivation processes that commonly occur in the cytosol [73]. Interestingly, the development of a cisplatin analog from MPPs showed that mtDNA damage was sufficient to induce cytotoxicity and promote apoptotic cell death without impairing nuclear DNA or entailing cell cycle arrest [74]. Therefore, mitochondria-specific targeting should be reconsidered for the implementation of innovative and efficient anticancer strategies. Furthermore, since platinum complexes show high affinity for nucleophilic sites, various studies have investigated their ability to trigger interactions at the molecular level by binding to various intracellular non-DNA components that constitute many potential targets of cytotoxicity or resistance. This rationale is schematized in Figure 3 with the example of cisplatin, whose participation in DNA adducts accounts for only about 10% of the total amount of cisplatin covalently bound to biomolecules in cells [4,13,17]. Therefore, the proper significance of the multifactorial mechanisms that mediate cytotoxicity in a highly concerted way at both the cellular and the molecular level should be reconsidered with the perspective of giving traditional drugs a new impetus [5,75].

Innovative Synergies

The combination of alkylating agents or platinum derivatives with relevant therapeutic strategies capable of promoting a synergistic effect and therefore potentiating anticancer activity is of paramount interest in the treatment of solid tumors, as illustrated in Figure 2B with the example of cisplatin. Inhibition of

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abundant thiol- and thioether-containing amino acids and proteins for which platinum complexes exhibit high affinity can hamper drug detoxification processes [4]. Based on *in vitro* assays that demonstrated enhanced cell sensitivity to DNA damage and apoptosis in glioblastoma cell lines exposed to buthionine sulfoximine (BSO) beforehand, significant inhibition of tumor growth was achieved in animal groups treated with BSO in combination with either temozolomide or cisplatin compared with animal groups treated with each of these drugs independently. Thanks to a putative synergistic effect, even low doses of anticancer agents were sufficient to achieve substantial outcomes while preventing severe side effects. According to these promising results, the authors suggest that the combination of **glutathione (GSH)** inhibitors with alkylating agents or platinum complexes may improve the clinical outcome in brain cancer patients [76,77]. Conversely, advantage can be taken of the elevated levels of GSH in resistant cancer cells to specifically damage them [6,78]. Biomimicking molecules can also be synthesized to supersede their bioanalogs in the organism. Methylation of the gene promoter of **O_6-methylguanine-DNA methyltransferase (MGMT)**, a common feature in glioblastoma patients, is of good prognosis since it improves cell sensitivity to temozolomide and results in an increase in median survival [21–23]. O_6-Benzylguanine, a structural analog of O_6-methylguanine, is able to divert and irreversibly inactivate the MGMT enzyme, preventing it from repairing DNA adducts induced by temozolomide. Such synergistic combination is expected to lead to the restoration of tumor sensitivity and to maximize drug cytotoxicity. Despite promising preliminary results, the efficacy of this strategy was limited in clinical practice by severe side effects attributed to the inactivation of the MGMT enzyme also in normal tissues [79,80]. Epigenetic modulations that may alter the DNA repair machinery can also play a role in circumventing drug resistance [6,13,24]. DNA demethylating agents are able to reverse hypermethylation of genes involved in the DNA mismatch repair (MMR) pathway, whose alteration participates in cell resistance to platinum compounds and is indicative of a bad prognosis for patients with ovarian carcinoma. A Phase II clinical trial in patients with platinum-resistant ovarian carcinoma supported impairment of gene methylation by low-dose decitabine administration and subsequent alteration of the MMR pathway to restore sensitivity to carboplatin, resulting in high response rates and extended progression-free survival [81].

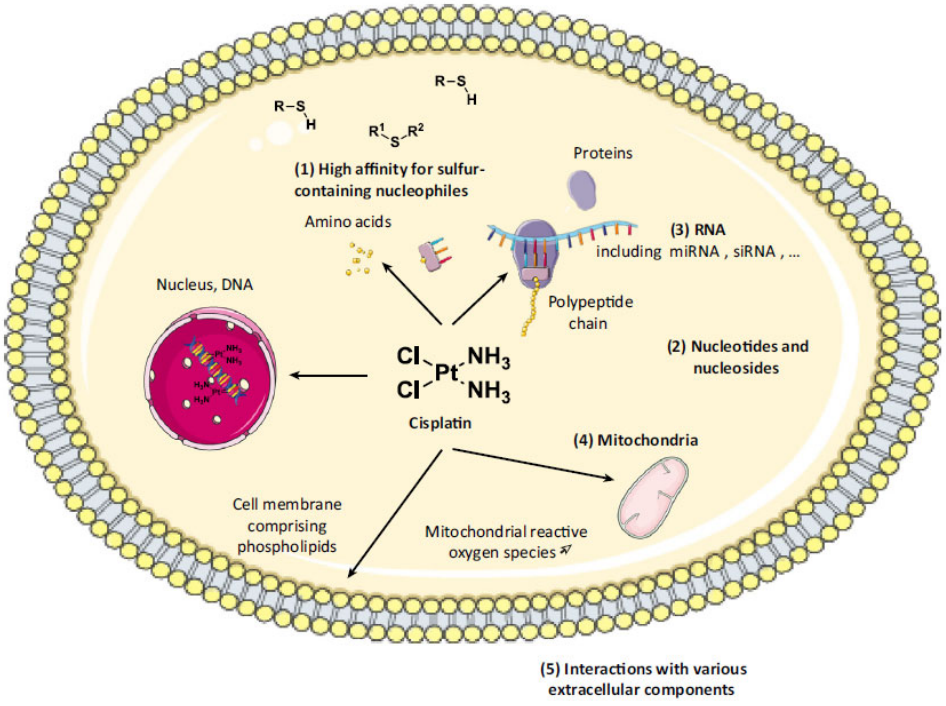

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Figure 3. Cisplatin Alternative and Neglected Targets. Cisplatin binds to various intracellular non-DNA components that constitute potential targets or factors in efficacy or resistance. (1) Cisplatin's interactions with proteins account for most adducts in cells due to the high reactivity of thiol and thioether protein constitutive residues and their abundance in the cytosol [4]. (2) Nucleotides and nucleosides are characterized by lower steric hindrance compared with their analogs involved in DNA, which may result in easier interactions with Pt(II) complexes. (3) Kinetic considerations show faster and higher complexation rates of cisplatin with RNA than DNA in vitro, although resulting in less stable adducts. Crosslinking with mRNA was reported to inhibit translation in vitro. Interactions with noncoding RNA may impair downstream cellular and molecular processes [4]. (4) Positively charged Pt(II) activated species were reported to accumulate in negatively charged mitochondria due to electrostatic interactions. There, cisplatin produces a significantly greater amount of adducts with mitochondrial DNA (mtDNA) than with nuclear DNA, subsequently impairing the response and clinical outcome in cancer patients [16,72,119]. Furthermore, since resistance to cisplatin is partly linked to extensive repair of Pt-DNA adducts by the nucleotide excision repair (NER) machinery, rerouting the drug towards mitochondria whose DNA lacks such repair mechanisms may overcome resistance and enhance therapeutic efficacy [96]. (5) Although the amount of Pt(II) complexes with hemoglobin that persists following oxaliplatin-based treatment was correlated with an increased risk of disease progression in patients with colorectal cancer, the impact of cisplatin's interactions with extracellular components has not yet been reported [120].

The expression of a panel of genes involved in cell sensitivity or resistance mechanisms and the molecular pathways below may also be modulated to reverse drug resistance and achieve a synergistic effect through miRNAs that play key roles in cellular development but also in oncogenesis, cancer progression, and drug resistance [82–85].

Development of Smart Nanocarriers

Nanotechnologies may offer tremendous opportunities in the field of medicine due to their size and versatility of structure, as described with the example of cisplatin in Figure 2C. Various **drug delivery systems (DDSs)** have been engineered to locally deliver their bioactive cargo, thus concentrating drug efficacy at the tumor site while preventing systemic toxicity [86]. DDSs have been described to passively target tumor cells through the enhanced permeability and retention effect (EPR) [87,88]. Although controversial, this paradigm has given rise to the development of various DDSs, including for vectorization of platinum derivatives [70,88–93]. Interestingly, active targeting can be achieved by functionalizing nanocarriers with various ligands that specifically bind to receptors overexpressed on the surface of cancer cells, such as folate or epidermal growth factor [87,94,95]. DDS can also be engineered to specifically reroute a drug to targets whose impairment will trigger a cell signaling cascade likely to entail apoptotic cell death [96]. The design of adaptive systems sensitive to microenvironmental changes – namely, environment- (pH [97], enzyme, and reductive environment [98]) responsive DDSs – further allows specific targeting and triggered drug release. Controlled release of the drug over time and the subsequent modulation of its pharmacokinetic profile may improve its therapeutic benefit [45,88,99–104].

Although DDSs are of great interest in extending the lifetime of the drug in the general circulation and protecting it from deactivation until it reaches its target, alternative routes have been investigated to circumvent physiological barriers. In animal models, the local infusion of liposomes [105], nanoparticles [70,106], or polymeric micelles [56] by CED in the brain parenchyma was reported to substantially enhance the distribution volume of the system compared with the free drug, as well as to reduce toxicity and prolong half-life [45,105,107].

Endocytosis has been extensively described as the key mechanism of DDS cellular uptake [89,95,97,102,108–110]. Protected from deactivation by the plasma membrane vesicle, quanta of active molecules are conveyed from early endosomes to late endosomes and lysosomes, like a 'Trojan Horse',

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favoring drug release in the close vicinity of the nucleus and subsequently promoting interactions with DNA [91,94,95,110]. Lipoplatin™, a liposomal formulation of cisplatin, was reported to bypass membrane transporters and subsequent intracellular trafficking by direct fusion with the cell membrane [94,111].

Thanks to the reduced systemic toxicity that accompanies nanovectorization, new effective drug combinations may be considered. Furthermore, the resort to agents modulating drug-resistance mechanisms is of particular interest to enhance cell sensitivity to chemotherapy. **Poloxamers** have been reported to accumulate in resistant cancer cells and intracellular organelles from where they alter metabolic processes involved in drug efflux and detoxification [94,112]. Similarly, micelles loaded with a Pt(IV) prodrug based on an ethacrynic acid backbone achieved substantial reversal of cisplatin resistance owing to effective GST inhibition, leading to tumor necrosis in vivo [103]. Codelivery of platinum derivatives and miRNA whose involvement in tumorigenesis was specifically identified could also help for impeding tumor cell proliferation and invasiveness [113].

Alternatives that combine nanomedicine and other key therapeutic strategies may also have great potential in the clinic. One example relies on the radiosensitizing effect of gold nanoparticles due to high X-ray absorption [114]. The incorporation of high-Z platinum compounds into various DDSs also potentiates drug efficacy in synergy with RT [31]. Surface functionalization of DDSs with radiopharmaceutics could further allow targeted molecular nuclear medicine, providing nanosystems with an additional imaging modality. This way towards 'theranostics' may be a promising application of DDSs in the near future.

From the perspective of personalized medicine, multifunctional nanoplatforms may enable the gathering of large amounts of information relevant to patient care [115]. Therefore, combinatorial systems have been developed to allow real-time monitoring of treatment efficacy. Some of these systems require a specific stimulus, either physical (light or heat) or chemical (hypoxic conditions or oxidative stress), to release their pharmaceutically active payload [99,116,117]. The therapeutic benefit of such tunable nanosystems is improved by real-time monitoring of their biodistribution within the organism together with the evaluation of patients' early response to the treatment [118]. Thus, the rise of various DDSs with integrated smart functions has already pushed the frontiers of science by making it possible to develop hybrid systems that are able not only to drive the drug to its target but also to monitor its impact, or even intensify it.

Outstanding Questions

Will strategies bypassing resistance mechanisms faced by alkylating agents and platinum derivatives ultimately provide favorable outcomes in cancer treatment?

Should we focus on alternative molecular pathways or subcellular organelles, as yet neglected in current clinical practice, to improve the therapeutic index of alkylating agents and platinum derivatives?

Which treatment schedule will be the most accurate, combined with efficient topographic delivery of alkylating agents and platinum complexes in cancer cells?

Will the therapeutic arsenal of alkylating agents and platinum derivatives benefit from nanomedicines in the management of solid tumors?

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Concluding Remarks

Owing to their broad anticancer spectrum, alkylating agents and platinum derivatives are key in the management of solid tumors. Still, they suffer from acute systemic toxicity, suboptimal treatment schedules, intrinsic or acquired resistance, and inadequate routing at both the tissue and the cellular level. In this context, this review envisions promising alternatives to the conventional use of alkylating agents and platinum derivatives in clinical practice, including their administration by appropriate routes depending on the tumor location, optimized subcellular rerouting, synergistic strategies, and the development of an arsenal of smart nanocarriers. Driven by the necessity to rethink their use through rather simple potentiating therapeutic strategies relevant to daily needs and clinical practice – instead of developing new drugs that would quickly face the same issues in terms of limited therapeutic index (see Outstanding Questions) – we do believe that these old molecules have great promise for future applications in the management of solid tumors.

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References

1. Hanahan, D. and Weinberg, R.A. (2011) Hallmarks of cancer: the next generation. *Cell* 144, 646–674
2. Ye, W. (2016) The complexity of translating anti-angiogenesis therapy from basic science to the clinic. *Dev. Cell* 37, 114–125
3. Yang, W.-H. et al. (2017) Revision of the concept of anti-angiogenesis and its applications in tumor treatment. *Chronic Dis. Transl. Med.* 3, 33–40
4. Mezencev, R. (2015) Interactions of cisplatin with non-DNA targets and their influence on anticancer activity and drug toxicity: the complex world of the platinum complex. *Curr. Cancer Drug Targets* 14, 794–816
5. Gibson, D. (2019) Multi-action Pt(IV) anticancer agents; do we understand how they work? *J. Inorg. Biochem.* 191, 77–84
6. Kelland, L. (2007) The resurgence of platinum-based cancer chemotherapy. *Nat. Rev. Cancer* 7, 573–584
7. Johnstone, T.C. et al. (2015) Third row transition metals for the treatment of cancer. *Philos. Trans. R. Soc. Lond. A Math. Phys. Eng. Sci.* 373, 20140185
8. Wishart, D.S. et al. (2018) DrugBank 5.0: a major update to the DrugBank database for 2018. *Nucleic Acids Res.* 46, D1074–D1082
9. Yang, F. et al. (2014) Doxorubicin, DNA torsion, and chromatin dynamics. *Biochim. Biophys. Acta* 1845, 84–89
10. Babu, N.J. et al. (2013) Temozolomide hydrochloride dihydrate. *CrystEngComm* 15, 666–671

11. Siddik, Z.H. (2002) Mechanisms of action of cancer chemotherapeutic agents: DNA-interactive alkylating agents and antitumour platinum-based drugs. In *The Cancer Handbook* (1st edn) (Alison, M.R., ed.), pp. 1295–1313, John Wiley & Sons
12. Corte-Rodríguez, M. et al. (2015) Quantitative evaluation of cellular uptake, DNA incorporation and adduct formation in cisplatin sensitive and resistant cell lines: comparison of different Pt-containing drugs. *Biochem. Pharmacol.* 98, 69–77
13. Shen, D.-W. et al. (2012) Cisplatin resistance: a cellular self-defense mechanism resulting from multiple epigenetic and genetic changes. *Pharmacol. Rev.* 64, 706–721
14. Dasari, S. and Tchounwou, P.B. (2014) Cisplatin in cancer therapy: molecular mechanisms of action. *Eur. J. Pharmacol.* 740, 364–378
15. Stewart, D.J. (2007) Mechanisms of resistance to cisplatin and carboplatin. *Crit. Rev. Oncol. Hematol.* 63, 12–31
16. Garrido, N. et al. (2008) Cisplatin-mediated impairment of mitochondrial DNA metabolism inversely correlates with glutathione levels. *Biochem. J.* 414, 93–102
17. Galluzzi, L. et al. (2014) Systems biology of cisplatin resistance: past, present and future. *Cell Death Dis.* 5, e1257-18
18. Cheung-Ong, K. et al. (2013) DNA-damaging agents in cancer chemotherapy: serendipity and chemical biology. *Chem. Biol.* 20, 648–659
19. Amable, L. (2016) Cisplatin resistance and opportunities for precision medicine. *Pharmacol. Res.* 106, 27–36
20. Fennell, D.A. et al. (2016) Cisplatin in the modern era: the backbone of first-line chemotherapy for non-small cell lung cancer. *Cancer Treat. Rev.* 44, 42–50
21. Ostrom, Q. et al. (2013) Gene markers in brain tumors: what the epileptologist should know. *Epilepsia* 54, 25–29
22. Van Thuijl, H.F. et al. (2015) Evolution of DNA repair defects during malignant progression of low-grade gliomas after temozolomide treatment. *Acta Neuropathol.* 129, 597–607
23. Hegi, M.E. et al. (2005) MGMT gene silencing and benefit from temozolomide in glioblastoma. *N. Engl. J. Med.* 352, 997–1003
24. O'Grady, S. et al. (2014) The role of DNA repair pathways in cisplatin resistant lung cancer. *Cancer Treat. Rev.* 40, 1161– 1170
25. Hoebers, F.J.P. et al. (2008) Cisplatin–DNA adduct formation in patients treated with cisplatin-based chemoradiation: lack of correlation between normal tissues and primary tumor. Cancer Chemother. *Pharmacol*. 61, 1075–1081
26. Chalmers, A.J. et al. (2009) Cytotoxic effects of temozolomide and radiation are additive- and schedule-dependent. *Int. J. Radiat. Oncol. Biol. Phys.* 75, 1511–1519
27. Eckert, F. et al. (2010) Definitive radiotherapy and single-agent radiosensitizing ifosfamide in patients with localized, irresectable soft tissue sarcoma: a retrospective analysis. *Radiat. Oncol.* 5, 55
28. Wilson, G.D. et al. (2006) Biologic basis for combining drugs with radiation. *Semin. Radiat. Oncol.* 16, 2–9
29. Reboul, F.L. (2004) Radiotherapy and chemotherapy in locally advanced non-small cell lung cancer: preclinical and early clinical data. *Hematol. Oncol. Clin. North Am.* 18, 41–53
30. Tippayamontri, T. et al. (2012) Synergism in concomitant chemoradiotherapy of cisplatin and oxaliplatin and their liposomal formulation in the human colorectal cancer HCT116 model. *Anticancer Res.* 32, 4395–4404
31. Miladi, I. et al. (2015) Combining ultrasmall gadolinium-based nanoparticles with photon irradiation overcomes radioresistance of head and neck squamous cell carcinoma. Nanomed. Nanotechnol. *Biol. Med.* 11, 247–257
32. Tomoda, K. et al. (2015) Examination of gossypol-pluronic micelles as potential radiosensitizers. *AAPS J.* 17, 1369–1375
33. De Felice, F. et al. (2015) Head and neck cancer: metronomic chemotherapy. *BMC Cancer* 15, 677
34. Goldberg, R.M. et al. (2004) A randomized controlled trial of fluorouracil plus leucovorin, irinotecan, and oxaliplatin combinations in patients with previously untreated metastatic colorectal cancer. *J. Clin. Oncol.* 22, 23–30

35. Carethers, J.M. (2008) Systemic treatment of advanced colorectal cancer: tailoring therapy to the tumor. *Ther. Adv. Gastroenterol.* 1, 33–42
36. Oldenburg, J. et al. (2013) Testicular seminoma and non-seminoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann. Oncol.* 24, vi125–vi132
37. Bellmunt, J. et al. (2014) Bladder cancer: ESMO Practice Guidelines for diagnosis, treatment and follow-up. *Ann. Oncol.* 25, iii40–iii48
38. Lordick, F. et al. (2016) Oesophageal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann. Oncol.* 27, v50–v57
39. Novello, S. et al. (2016) Metastatic non-small-cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann. Oncol.* 27, v1–v27
40. Marth, C. et al. (2017) Cervical cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann. Oncol.* 28, iv72–iv83
41. Postmus, P.E. et al. (2017) Early and locally advanced nonsmall-cell lung cancer (NSCLC): ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann. Oncol.* 28, iv1–iv21
42. Wilson, T. et al. (2014) Glioblastoma multiforme: state of the art and future therapeutics. *Surg. Neurol. Int.* 5, 132138
43. Lombardi, M.Y. and Assem, M. (2017) Glioblastoma genomics: a very complicated story. In *Glioblastoma* (De Vleeschouwer, S., ed.), pp. 3–25, Codon
44. Laquintana, V. et al. (2009) New strategies to deliver anticancer drugs to brain tumors. *Expert Opin. Drug Deliv.* 6, 1017–1032
45. Zhou, J. et al. (2012) Novel delivery strategies for glioblastoma. *Cancer J.* 18, 89–99
46. Fernandes, C. et al. (2017) Current standards of care in glioblastoma therapy. In *Glioblastoma* (De Vleeschouwer, S., ed.), pp. 197–241, Codon
47. Hanif, F. et al. (2017) Glioblastoma multiforme: a review of its epidemiology and pathogenesis through clinical presentation and treatment. *Asian Pac. J. Cancer Prev.* 18, 3–9
48. Arshad, A. et al. (2015) Convection-enhanced delivery of carboplatin PLGA nanoparticles for the treatment of glioblastoma. *PLoS One* 10, e0132266
49. Garg, T. et al. (2015) Current strategies for targeted delivery of bio-active drug molecules in the treatment of brain tumor. *J. Drug Target.* 23, 865–887
50. Vogelbaum, M.A. and Aghi, M.K. (2015) Convection-enhanced delivery for the treatment of glioblastoma. *Neuro Oncol.* 17, ii3–ii8
51. Cheff, D.M. and Hall, M.D. (2017) A drug of such damned nature. 1. Challenges and opportunities in translational platinum drug research. *J. Med. Chem.* 60, 4517–4532
52. Shi, M. et al. (2015) Convection-enhancement delivery of platinum-based drugs and Lipoplatin™ to optimize the concomitant effect with radiotherapy in F98 glioma rat model. *Invest. New Drugs* 33, 555–563
53. Vogelbaum, M.A. (2007) Convection enhanced delivery for treating brain tumors and selected neurological disorders: symposium review. *J. Neurooncol.* 83, 97–109
54. White, E. et al. (2012) A Phase I trial of carboplatin administered by convection-enhanced delivery to patients with recurrent/progressive glioblastoma multiforme. *Contemp. Clin. Trials* 33, 320–331
55. Siegal, T. (2013) Change clinical practice for brain tumor. *Neuro Oncol.* 15, 656–669
56. Zhang, R. et al. (2016) Convection-enhanced delivery of SN-38loaded polymeric micelles (NK012) enables consistent distribution of SN-38 and is effective against rodent intracranial brain tumor models. *Drug Deliv.* 23, 2780–2786
57. Yang, W. et al. (2011) Convection-enhanced delivery of carboplatin in combination with radiotherapy for the treatment of brain tumors. *J. Neurooncol.* 101, 379–390
58. Jaaback, K. et al. (2016) Intraperitoneal chemotherapy for the initial management of primary epithelial ovarian cancer (review). *Cochrane Database Syst. Rev.* 11, CD005340

59. Jandial, D.A. et al. (2017) A Phase I pharmacokinetic study of intraperitoneal bortezomib and carboplatin in patients with persistent or recurrent ovarian cancer: an NRG Oncology/Gynecologic Oncology Group study. *Gynecol. Oncol.* 145, 236–242
60. Scharovsky, O.G. et al. (2009) Metronomic chemotherapy: changing the paradigm that more is better. *Curr. Oncol.* 16, 7–15
61. Zhidkov, N. et al. (2013) Continuous intraperitoneal carboplatin delivery for the treatment of late-stage ovarian cancer. *Mol. Pharm.* 10, 3315–3322
62. Su, W.H. et al. (2012) Metronomic therapy for gynecologic cancers. Taiwan. *J. Obstet. Gynecol.* 51, 167–178
63. Ye, H. et al. (2015) Sustained, low-dose intraperitoneal cisplatin improves treatment outcome in ovarian cancer mouse models. *J. Control. Release* 220, 358–367
64. Pasquier, E. et al. (2010) Metronomic chemotherapy: new rationale for new directions. *Nat. Rev. Clin. Oncol.* 7, 455–465
65. Kubota, H. et al. (2017) Feasibility of metronomic chemotherapy with tegafur-uracil, cisplatin, and dexamethasone for docetaxelrefractory prostate cancer. *J. Rural Med.* 12, 112–119
66. Patil, V.M. et al. (2015) A prospective randomized Phase II study comparing metronomic chemotherapy with chemotherapy (single agent cisplatin), in patients with metastatic, relapsed or inoperable squamous cell carcinoma of head and neck. *Oral Oncol.* 51, 279–286
67. Specenier, P. and Vermorken, J.B. (2018) Optimizing treatments for recurrent or metastatic head and neck squamous cell carcinoma. *Expert Rev. Anticancer Ther.* 18, 901–915
68. Correale, P. et al. (2011) Phase II trial of bevacizumab and dose/ dense chemotherapy with cisplatin and metronomic daily oral etoposide in advanced non-small-cell-lung cancer patients. *Cancer Biol. Ther.* 12, 112–118
69. Tippayamontri, T. et al. (2011) Cellular uptake and cytoplasm/ DNA distribution of cisplatin and oxaliplatin and their liposomal formulation in human colorectal cancer cell HCT116. *Invest. New Drugs* 29, 1321–1327
70. Lajous, H. et al. (2018) Hybrid Gd3+/cisplatin cross-linked polymer nanoparticles enhance platinum accumulation and formation of DNA adducts in glioblastoma cell lines. *Biomater*. Sci. 6, 2386–2409
71. Cullen, K.J. et al. (2007) Mitochondria as a critical target of the chemotherapeutic agent cisplatin in head and neck cancer. *J. Bioenerg. Biomembr.* 39, 43–50
72. Van Gisbergen, M.W. et al. (2015) How do changes in the mtDNA and mitochondrial dysfunction influence cancer and cancer therapy? Challenges, opportunities and models. *Mutat. Res.* 764, 16–30
73. Fonseca, S.B. et al. (2011) Rerouting chlorambucil to mitochondria combats drug deactivation and resistance in cancer cells. *Chem. Biol.* 18, 445–453
74. Wisnovsky, S.P. et al. (2013) Targeting mitochondrial DNA with a platinum-based anticancer agent. *Chem. Biol.* 20, 1323–1328
75. Gibson, D. (2016) Platinum(IV) anticancer prodrugs – hypotheses and facts. *Dalton Trans.* 45, 12983–12991
76. Rocha, C.R.R. et al. (2014) Glutathione depletion sensitizes cisplatin- and temozolomide-resistant glioma cells in vitro and in vivo. *Cell Death Dis.* 5, e1505
77. Villablanca, J.G. et al. (2016) A Phase I. new approaches to neuroblastoma therapy study of buthionine sulfoximine and melphalan with autologous stem cells for recurrent/refractory high-risk neuroblastoma. *Pediatr. Blood Cancer* 63, 1349–1356
78. Vergote, I. et al. (2009) Phase 3 randomised study of canfosfamide (Telcyta[1], TLK286) versus pegylated liposomal doxorubicin or topotecan as third-line therapy in patients with platinum-refractory or -resistant ovarian cancer. *Eur. J. Cancer* 45, 2324–2332
79. Kaina, B. et al. (2010) Targeting O_6-methylguanine-DNA methyltransferase with specific inhibitors as a strategy in cancer therapy. *Cell. Mol. Life Sci.* 67, 3663–3681

80. Warren, K.E. et al. (2012) A Phase II study of O_6-benzylguanine and temozolomide in pediatric patients with recurrent or progressive high-grade gliomas and brainstem gliomas: a Pediatric Brain Tumor Consortium study. *J. Neurooncol.* 106, 643–649
81. Matei, D. et al. (2012) Epigenetic resensitization to platinum in ovarian cancer. *Cancer Res.* 72, 2197–2205
82. Garzon, R. et al. (2009) MicroRNAs in cancer. *Annu. Rev. Med.* 60, 167–179
83. Visone, R. and Croce, C.M. (2009) miRNAs and cancer. *Am. J. Pathol.* 174, 1131–1138
84. Chen, W. et al. (2014) miR-136 targets E2F1 to reverse cisplatin chemosensitivity in glioma cells. *J. Neurooncol.* 120, 43–53
85. Chen, X. et al. (2015) miR-873 acts as a novel sensitizer of glioma cells to cisplatin by targeting Bcl-2. *Int. J. Oncol.* 47, 1603–1611
86. Ventola, C.L. (2017) Progress in nanomedicine: approved and investigational nanodrugs. *Pharm. Ther.* 42, 742–755
87. Peer, D. et al. (2007) Nanocarriers as an emerging platform for cancer therapy. *Nat. Nanotechnol.* 2, 751–760
88. Harper, B.W. et al. (2010) Advances in platinum chemotherapeutics. *Chemistry* 16, 7064–7077
89. Cabral, H. and Kataoka, K. (2014) Progress of drug-loaded polymeric micelles into clinical studies. *J. Control. Release* 190, 465–476
90. Li, J. et al. (2014) Complex of cisplatin with biocompatible poly (ethylene glycol) with pendant carboxyl groups for the effective treatment of liver cancer. *J. Appl. Polym. Sci.* 131, 9246–9254
91. Duan, X. et al. (2015) Polymeric micelle-mediated delivery of DNA-targeting organometallic complexes for resistant ovarian cancer treatment. *Small* 11, 3962–3972
92. Johnstone, T.C. et al. (2016) The next generation of platinum drugs: targeted Pt(II) agents, nanoparticle delivery, and Pt(IV) prodrugs. *Chem. Rev.* 116, 3436–3486
93. Parhizkar, M. et al. (2016) Electrohydrodynamic encapsulation of cisplatin in poly (lactic-co-glycolic acid) nanoparticles for controlled drug delivery. Nanomed. Nanotechnol. *Biol. Med.* 12, 1919–1929
94. Xue, X. et al. (2013) Nanoscale drug delivery platforms overcome platinum-based resistance in cancer cells due to abnormal membrane protein trafficking. *ACS Nano* 7, 10452–10464
95. Lehner, R. et al. (2013) Intelligent nanomaterials for medicine: carrier platforms and targeting strategies in the context of clinical application. *Nanomed. Nanotechnol. Biol. Med.* 9, 742–757
96. Marrache, S. et al. (2014) Detouring of cisplatin to access mitochondrial genome for overcoming resistance. *Proc. Natl. Acad. Sci. U. S. A.* 111, 10444–10449
97. Sun, X. et al. (2017) A pH-responsive yolk-like nanoplatform for tumor targeted dual-mode magnetic resonance imaging and chemotherapy. *ACS Nano* 11, 7049–7059
98. Cajot, S. et al. (2011) Design of reversibly core cross-linked micelles sensitive to reductive environment. *J. Control. Release* 152, 30–36
99. Nishiyama, N. and Kataoka, K. (2006) Current state, achievements, and future prospects of polymeric micelles as nanocarriers for drug and gene delivery. *Pharmacol. Ther.* 112, 630–648
100. Oerlemans, C. et al. (2010) Polymeric micelles in anticancer therapy: targeting, imaging and triggered release. *Pharm. Res.* 27, 2569–2589
101. Stathopoulos, G.P. and Boulikas, T. (2012) Lipoplatin formulation review article. *J. Drug Deliv.* 2012, 581363
102. Patra, H.K. and Turner, A.P.F. (2014) The potential legacy of cancer nanotechnology: cellular selection. *Trends Biotechnol.* 32, 21–31
103. Li, S. et al. (2017) Overcoming resistance to cisplatin by inhibition of glutathione S-transferases (GSTs) with ethacraplatin micelles *in vitro* and *in vivo*. *Biomaterials* 144, 119–129
104. Xu, S. et al. (2017) Supramolecular cisplatin–vorinostat nanodrug for overcoming drug resistance in cancer synergistic therapy. *J. Control. Release* 266, 36–46

105. Krauze, M.T. et al. (2005) Real-time visualization and characterization of liposomal delivery into the monkey brain by magnetic resonance imaging. *Brain Res. Protoc.* 16, 20–26
106. Zhang, C. et al. (2017) Convection enhanced delivery of cisplatin-loaded brain penetrating nanoparticles cures malignant glioma in rats. *J. Control. Release* 263, 112–119
107. Saito, R. et al. (2005) Gadolinium-loaded liposomes allow for real-time magnetic resonance imaging of convection-enhanced delivery in the primate brain. *Exp. Neurol.* 196, 381–389
108. Hillaireau, H. and Couvreur, P. (2009) Nanocarriers' entry into the cell: relevance to drug delivery. *Cell. Mol. Life Sci.* 66, 2873–2896
109. Bregoli, L. et al. (2016) Nanomedicine applied to translational oncology: a future perspective on cancer treatment. *Nanomed. Nanotechnol. Biol. Med.* 12, 81–103
110. Cai, Y. et al. (2017) Supramolecular "Trojan Horse" for nuclear delivery of dual anticancer drugs. *J. Am. Chem. Soc.* 139, 2876– 2879
111. Boulikas, T. (2009) Clinical overview on Lipoplatin™: a successful liposomal formulation of cisplatin. *Expert Opin. Invest. Drugs* 18, 1197–1218
112. Exner, A.A. et al. (2005) Enhancement of carboplatin toxicity by pluronic block copolymers. *J. Control. Release* 106, 188–197
113. Yang, T. et al. (2016) Anti-tumor efficiency of lipid-coated cisplatin nanoparticles co-loaded with microRNA-375. *Theranostics* 6, 142–154
114. Hainfeld, J.F. et al. (2013) Gold nanoparticle imaging and radiotherapy of brain tumors in mice. *Nanomedicine* 8, 1601–1609
115. McCarthy, J.R. and Weissleder, R. (2008) Multifunctional magnetic nanoparticles for targeted imaging and therapy. *Adv. Drug Deliv. Rev.* 60, 1241–1251
116. Zhu, H. et al. (2012) Responsive fluorescent Bi2O3@PVA hybrid nanogels for temperature-sensing, dual-modal imaging, and drug delivery. *Biomaterials* 33, 3058–3069
117. Cao, Y. et al. (2015) Photo-triggered fluorescent theranostic prodrugs as DNA alkylating agents for mechlorethamine release and spatiotemporal monitoring. *Org. Biomol. Chem.* 13, 6742– 6748
118. Yuan, Y. et al. (2014) Targeted theranostic platinum(IV) prodrug with a built-in aggregation-induced emission light-up apoptosis sensor for noninvasive early evaluation of its therapeutic responses *in situ. J. Am. Chem. Soc.* 136, 2546–2554
119. Choi, Y.M. et al. (2015) Mechanism of cisplatin-induced cytotoxicity is correlated to impaired metabolism due to mitochondrial ROS generation. *PLoS One* 10, e0135083
120. Mandal, R. et al. (2006) Mass spectrometry study of hemoglobin–oxaliplatin complexes in colorectal cancer patients and potential association with chemotherapeutic responses.
Rapid Commun. Mass Spectrom. 20, 2533–2538