Rethinking alkylating(-like) agents for solid tumor management

Hélène Lajous^{a,b}, Bénédicte Lelièvre^c, Elodie Vauléon^{d,e}, Philippe Lecomte^{b,*} and Emmanuel Garcion^{a,*}

^a CRCINA, INSERM, Université de Nantes, Université d'Angers, Angers, France

^b Center for Education and Research on Macromolecules (CERM), CESAM Research Unit, University of Liege, B6a Sart-Tilman, B-4000 Liege, Belgium

^c Centre régional de pharmacovigilance, Laboratoire de pharmacologie-toxicologie, CHU Angers, 4 rue Larrey, F-49100 Angers, France

^d Centre Eugène Marquis, Rennes, France

^e INSERM U1242, Université de Rennes 1, Rennes, France

* Equivalent contribution

Correspondence: emmanuel.garcion@univ-angers.fr (E. Garcion)

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 encapsulation of bioactive molecules within smart multifunctional drug delivery systems, have shown promising results to potentiate anticancer activity while circumventing such hurdles. Furthermore, questioning the old paradigm according to which nuclear DNA is the critical target of their anticancer activity has shed light upon subcellular alternative and neglected targets that obviously participate in mediating cytotoxicity or resistance. Thus, rethinking the use of these pivotal antineoplastic agents appears critical to improve clinical outcomes in the management of solid tumors.

Key words: alkylating agents, cisplatin, local treatment, synergies, nanomedicine, solid tumors

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, still consist in the mainstay of the treatment of solid tumors by addressing specific mechanisms involved in tumorigenesis. More **targeted therapies** (see Glossary), such as anti-angiogenesis 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 for developing 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 for exerting its anticancer activity while limiting adverse side effects. In this context, alkylating agents are old molecules still widely used in the front-line 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 patients experience platinum-based drug therapy [4,5]. As such, the clinical relevance of platinum compounds is key in the 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 ways, 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.

Rationale and conventional use of alkylating agents and platinum derivatives in clinical practice

After the attack of Bari Harbor in 1943 revealed the effects of mustard gas on bone marrow depletion and first therapeutic outcomes on lymphoma, alkylating agents gradually became a gold-standard as first-line treatment in 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 that 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, 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 1-(a) strictly differs from that of alkylating agents [9]. Alkylators allow for the transfer of an **alkyl group** from one to another molecule 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 1-(b) in the case of **temozolomide** [10]. Since exposure to alkylating agents leads to chromosomal aberrations in dividing cells, DNA stands for the key target site of alkylation within 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 RNA and proteins. Alkylation mainly occurs during S phase, while DNA is

replicating: both 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 N₇, O₆, N₂ and N₃), adenine (N₃ and N₇) and cytosine bases (N₃). 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 even though they do not interact with biological macromolecules through an alkyl group but rather by complexation. Cisplatin, whose mechanism of action is illustrated on Figure 1-(c), enabled to dramatically improve the prognosis of germinal cancer cells and is still currently used as a gold-standard in the treatment of various solid tumors [6,7]. Contrary to alterations caused by mono-functional alkylating agents such as nitrosoureas, inter- or intracatenary bridges induced by platinum derivatives between both DNA strands are extremely difficult to repair.

Resistance

Intrinsic or acquired resistance to alkylating agents and platinum derivatives is considered as 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] or 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 (miRNA)**, and could be identified as relevant predictive biomarkers of patient response to a treatment with the perspective of providing more accurate and personalized chemotherapeutic regimen [24]. Figure 2-(a), 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 within the tumor margins that are insensitive to radio- or chemotherapy, is of particular interest to circumvent drug resistance [6].

Radiosensitization

Radiotherapy (RT) constitutes a key strategy in the treatment of several solid tumors, including glioma, lung, breast, head and neck, uterine cervix, rectum, vulvar and prostate cancers. Radiation beam causes direct DNA damages but also indirectly impact 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 further evidenced the superior efficacy of concomitant chemoradiotherapy in various solid tumors compared to RT alone [25,26,30]. This synergistic effect depicted in Figure 2-(b) can be explained by a more accurate locoregional control of the pathology with a reduced or at least contained tumor cell proliferation that would otherwise quickly entails radioresistance, resulting in a better prognosis. Paradoxically, radioresistance can also occur from disruption of blood supply to the altered tissue after surgery 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 effect [31,32]. The time schedule between chemotherapy and RT is a key point for 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 reach 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 metabolism 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 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 consists of the combination of 5-fluorouracil, leucovorin and oxaliplatin, demonstrated a 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 to reach up to 80 % of cure rates [6]. Additional adjunctive drugs can also be considered to modulate platinum activity or toxicity [36–41].

Optimization of the use of alkylating agents and platinum derivatives

Although dramatically limited by resistance mechanisms and a 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 an optimization of their use in anticancer therapy and suggests that time may have come to bring these old molecules back on the stage again.

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. 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**[®]) within the resection cavity at the end of the surgery [42,43]. In such aqueous environment, the anhydride bonds of the biodegradable polymeric matrix get hydrolyzed, allowing for a controlled and sustained release of the drug that can diffuse within the surrounding parenchyma during several weeks. After degradation, the active metabolite can alkylate DNA, cross-link with RNA and entail proteins carbamylation, ultimately leading to cell apoptosis [43]. Although Gliadel[®] demonstrated a 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 strong enough to allow carmustine for penetrating deep into the brain tissue and for distributing 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 of the tumor burden and maximize their anticancer activity.

In this context, the locoregional administration of chemotherapy directly within the brain enables both to bypass the blood brain barrier that prevents most macromolecules and therapeutic drugs from reaching the central nervous system and to locally increase drug concentration. Convection-enhanced delivery (CED) consists of infusing the drug at high concentration directly within 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 to 10 $\mu\text{l min}^{-1}$. As such, CED achieves homogeneous elliptical to spherical distribution of molecules of various molar masses over large distances compared to suboptimal therapeutic doses reached by passive diffusion from 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 within the brain tumor in regard with traditional administration routes while reducing systemic toxicity [52]. Although safety and feasibility have been demonstrated in phase I clinical trials, translation to the clinics failed so far because of surgical complications [53,54]. In addition, increased interstitial fluid pressure within brain tumors and leakage into the cerebrospinal fluid drastically reduce 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 diseases, 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 for recovering 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 a stabilization of the disease over time for maintenance of quality of life. As hyperfractionated radiotherapy suggests that a continuous low-dose schedule may be more efficient in killing highly-proliferative cells than standard radiotherapy by avoiding tumor cells reparation, metronomic chemotherapy consists in the chronic and equally-spaced administration of drugs at low dose ($1/10^{\text{th}}$ to $1/3^{\text{rd}}$ 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 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 the dividing vascular endothelial cells, thus demonstrating additional anti-angiogenic potential, while the stimulation of the anticancer immune response may further contribute to force tumor dormancy [33,60,62,64]. Besides, metronomic chemotherapy results in more convenient treatment administration and promotes maintenance of patients quality of life [65]. Economic reasons can also favor oral metronomic chemotherapy as a minimal cost but still compelling alternative to current standard-of-care, particularly in developing countries [66,67]. Metronomic regimens based on alkylating agents or platinum derivatives have demonstrated a therapeutic benefit in patients with solid tumors [60,65,68]. However, large-scale studies and controlled randomized trials that compare conventional MTD to 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 neither intracellular drug accumulation nor 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 suggest the role of 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 chlorambucil through engineered mitochondria-penetrating peptides (MPPs) that are able to cross the dense and highly hydrophobic membranes of mitochondria demonstrated a dramatic potentiation of its anticancer activity in various cancer cell lines by promoting apoptosis and evading deactivation processes that commonly occur within 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 implementing innovative and efficient anticancer strategies. Furthermore, since platinum complexes demonstrate 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 as many potential targets of their 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 whole amount of cisplatin covalently bound to biomolecules within cells [4,13,17]. Therefore, the proper significance of the multifactorial mechanisms that mediate cytotoxicity in a highly concerted way both at the cellular and molecular levels 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 2-(b) with the example of cisplatin. Inhibition of 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, a significant inhibition of the tumor growth was achieved in animal groups treated with BSO in combination with either temozolomide or cisplatin compared to 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 from 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]. Bio-mimicking molecules can also be synthesized to supersede their bio-analogs within the organism. Methylation of the gene's promoter of **6-O-methylguanine-DNA methyltransferase (MGMT)**, a common feature in glioblastoma diagnosed patients, is of good prognosis since it improves cell sensitivity to temozolomide and results in an increase in median survival [21–23]. O6-benzylguanine, a structural analog of O6-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 play a role in circumventing drug resistance too [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 of 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]. 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 reach a synergistic effect through miRNA that play a key role 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 2-(c). Various **drug delivery systems (DDS)** have been engineered to locally deliver their bioactive cargo, thus concentrating drug efficacy at the tumor site while preventing from systemic toxicity [86]. Indeed, DDS 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 DDS, 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]. Designing adaptive systems sensitive to micro-environmental changes, namely environment- (pH [97], enzyme and reductive environment [98]) responsive DDS, further allows for specific targeting and triggered drug release. A 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 DDS are of high interest to extend the drug lifetime in the general circulation and protect 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 within the brain parenchyma was reported to substantially enhance the distribution volume of the system in comparison 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”, favoring drug release in close vicinity of the nuclear and subsequently promoting interactions with DNA [91,94,95,110]. As such, LipoplatinTM, 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 goes along with 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 within 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 owed to effective GST inhibition, leading to tumor necrosis *in vivo* [103]. Co-delivery of platinum derivatives and miRNA whose involvement in tumorigenesis was specifically identified could also enable to impede tumor cell proliferation and invasiveness [113].

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

From the perspective of personalized medicine, multifunctional nanoplatforms may enable to gather large amount of information relevant to patient care [115]. Therefore, combinatorial systems have been developed to allow for real-time monitoring of the 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 patient early response to the treatment [118]. As such, the rise of various DDS 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.

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, sub-optimal treatment schedule, intrinsic or acquired resistance and inadequate routing both at the tissue and cellular levels. 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, an 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 the daily needs and clinical practice – instead of developing plenty of 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.

Acknowledgements

This work was supported by the “Institut National de la Santé et de la Recherche Médicale” (INSERM), by the University of Angers (Angers, France) and by the University of Liege (Liege, Belgium). It is also related to the LabEx IRON “Innovative Radiopharmaceuticals in Oncology and Neurology” as part of the French government “Investissements d’Avenir” program, to the INCa (Institut National du Cancer) MARENGO consortium “MicroRNA agonist and antagonist Nanomedicines for GliOblastoma treatment: from molecular programming to preclinical validation through the PL-BIO 2014-2020 grant and to the MuMoFRaT project “Multi-scale Modeling & simulation of the response to hypo-Fractionated Radiotherapy or repeated molecular radiation Therapies” supported by “La Région Pays-de-la-Loire” and by the Cancéropôle Grand-Ouest (tumor targeting and radiotherapy network). Hélène Lajous was a Ph.D. student involved in the Erasmus Mundus Joint Doctorate program for Nanomedicine and pharmaceutical innovation (EMJD NanoFar) and received a fellowship from “La Région Pays-de-la-Loire”.

Disclosures

There are no conflicts of interest to declare.

References

- 1 Hanahan, D. and Weinberg, R.A. (2011) Hallmarks of cancer: The next generation. *Cell* 144, 646–74
- 2 Ye, W. (2016) The complexity of translating anti-angiogenesis therapy from basic science to the clinic. *Dev. Cell* 37, 114–25
- 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

- 405 5 Gibson, D. (2019) Multi-action Pt(IV) anticancer agents; do we understand how they
406 work? *J. Inorg. Biochem.* 191, 77–84
- 407 6 Kelland, L. (2007) The resurgence of platinum-based cancer chemotherapy. *Nat. Rev.*
408 *Cancer* 7, 573–84
- 409 7 Johnstone, T.C. *et al.* (2015) Third row transition metals for the treatment of cancer.
410 *Philos. Trans. R. Soc. A Math. Phys. Eng. Sci.* 373, 20140185
- 411 8 Wishart, D.S. *et al.* (2018) DrugBank 5.0: A major update to the DrugBank database
412 for 2018. *Nucleic Acids Res.* 46, D1074–D1082
- 413 9 Yang, F. *et al.* (2014) Doxorubicin, DNA torsion, and chromatin dynamics. *Biochim.*
414 *Biophys. Acta* 1845, 84–9
- 415 10 Babu, N.J. *et al.* (2013) Temozolomide hydrochloride dihydrate. *CrystEngComm* 15,
416 666–71
- 417 11 Siddik, Z.H. (2002) Mechanisms of action of cancer chemotherapeutic agents: DNA-
418 interactive alkylating agents and antitumour platinum-based drugs. In *The Cancer*
419 *Handbook 1st Edition* (Alison, M. R., ed), John Wiley & Sons, Ltd
- 420 12 Corte-Rodríguez, M. *et al.* (2015) Quantitative evaluation of cellular uptake, DNA
421 incorporation and adduct formation in cisplatin sensitive and resistant cell lines:
422 Comparison of different Pt-containing drugs. *Biochem. Pharmacol.* 98, 69–77
- 423 13 Shen, D.-W. *et al.* (2012) Cisplatin resistance: A cellular self-defense mechanism
424 resulting from multiple epigenetic and genetic changes. *Pharmacol. Rev.* 64, 706–21
- 425 14 Dasari, S. and Tchounwou, P.B. (2014) Cisplatin in cancer therapy: Molecular
426 mechanisms of action. *Eur. J. Pharmacol.* 740, 364–78
- 427 15 Stewart, D.J. (2007) Mechanisms of resistance to cisplatin and carboplatin. *Crit. Rev.*
428 *Oncol. Hematol.* 63, 12–31
- 429 16 Garrido, N. *et al.* (2008) Cisplatin-mediated impairment of mitochondrial DNA
430 metabolism inversely correlates with glutathione levels. *Biochem. J.* 414, 93–102
- 431 17 Galluzzi, L. *et al.* (2014) Systems biology of cisplatin resistance: Past, present and
432 future. *Cell Death Dis.* 5, e1257-18
- 433 18 Cheung-Ong, K. *et al.* (2013) DNA-damaging agents in cancer chemotherapy:
434 Serendipity and chemical biology. *Chem. Biol.* 20, 648–59
- 435 19 Amable, L. (2016) Cisplatin resistance and opportunities for precision medicine.
436 *Pharmacol. Res.* 106, 27–36
- 437 20 Fennell, D.A. *et al.* (2016) Cisplatin in the modern era: The backbone of first-line
438 chemotherapy for non-small cell lung cancer. *Cancer Treat. Rev.* 44, 42–50

- 439 21 Ostrom, Q. *et al.* (2013) Gene markers in brain tumors: What the epileptologist should
440 know. *Epilepsia* 54, 25–9
- 441 22 Van Thuijl, H.F. *et al.* (2015) Evolution of DNA repair defects during malignant
442 progression of low-grade gliomas after temozolomide treatment. *Acta Neuropathol.*
443 129, 597–607
- 444 23 Hegi, M.E. *et al.* (2005) MGMT gene silencing and benefit from temozolomide in
445 glioblastoma. *N. Engl. J. Med.* 352, 997–1003
- 446 24 O’Grady, S. *et al.* (2014) The role of DNA repair pathways in cisplatin resistant lung
447 cancer. *Cancer Treat. Rev.* 40, 1161–70
- 448 25 Hoebers, F.J.P. *et al.* (2008) Cisplatin-DNA adduct formation in patients treated with
449 cisplatin-based chemoradiation: Lack of correlation between normal tissues and
450 primary tumor. *Cancer Chemother. Pharmacol.* 61, 1075–81
- 451 26 Chalmers, A.J. *et al.* (2009) Cytotoxic effects of temozolomide and radiation are
452 additive- and schedule-dependent. *Int. J. Radiat. Oncol. Biol. Phys.* 75, 1511–9
- 453 27 Eckert, F. *et al.* (2010) Definitive radiotherapy and single-agent radiosensitizing
454 ifosfamide in patients with localized, irresectable soft tissue sarcoma: A retrospective
455 analysis. *Radiat. Oncol.* 5, 55
- 456 28 Wilson, G.D. *et al.* (2006) Biologic basis for combining drugs with radiation. *Semin.*
457 *Radiat. Oncol.* 16, 2–9
- 458 29 Reboul, F.L. (2004) Radiotherapy and chemotherapy in locally advanced non-small
459 cell lung cancer: Preclinical and early clinical data. *Hematol. Oncol. Clin. North Am.*
460 18, 41–53
- 461 30 Tippayamontri, T. *et al.* (2012) Synergism in concomitant chemoradiotherapy of
462 cisplatin and oxaliplatin and their liposomal formulation in the human colorectal cancer
463 HCT116 model. *Anticancer Res.* 32, 4395–404
- 464 31 Miladi, I. *et al.* (2015) Combining ultrasmall gadolinium-based nanoparticles with
465 photon irradiation overcomes radioresistance of head and neck squamous cell
466 carcinoma. *Nanomedicine Nanotechnology, Biol. Med.* 11, 247–57
- 467 32 Tomoda, K. *et al.* (2015) Examination of gossypol-pluronic micelles as potential
468 radiosensitizers. *AAPS J.* 17, 1369–75
- 469 33 De Felice, F. *et al.* (2015) Head and neck cancer: Metronomic chemotherapy. *BMC*
470 *Cancer* 15, 677
- 471 34 Goldberg, R.M. *et al.* (2004) A randomized controlled trial of fluorouracil plus
472 leucovorin, irinotecan, and oxaliplatin combinations in patients with previously

473 untreated metastatic colorectal cancer. *J. Clin. Oncol.* 22, 23–30

474 35 Carethers, J.M. (2008) Systemic treatment of advanced colorectal cancer: Tailoring
475 therapy to the tumor. *Therap. Adv. Gastroenterol.* 1, 33–42

476 36 Oldenburg, J. *et al.* (2013) Testicular seminoma and non-seminoma: ESMO Clinical
477 Practice Guidelines for diagnosis, treatment and follow-up. *Ann. Oncol.* 24, vi125-
478 vi132

479 37 Bellmunt, J. *et al.* (2014) Bladder cancer: ESMO Practice Guidelines for diagnosis,
480 treatment and follow-up. *Ann. Oncol.* 25, iii40-iii48

481 38 Lordick, F. *et al.* (2016) Oesophageal cancer: ESMO Clinical Practice Guidelines for
482 diagnosis, treatment and follow-up. *Ann. Oncol.* 27, v50–v57

483 39 Novello, S. *et al.* (2016) Metastatic non-small-cell lung cancer: ESMO Clinical
484 Practice Guidelines for diagnosis, treatment and follow-up. *Ann. Oncol.* 27, v1–v27

485 40 Marth, C. *et al.* (2017) Cervical cancer: ESMO Clinical Practice Guidelines for
486 diagnosis, treatment and follow-up. *Ann. Oncol.* 28, iv72-iv83

487 41 Postmus, P.E. *et al.* (2017) Early and locally advanced non-small-cell lung cancer
488 (NSCLC): ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up.
489 *Ann. Oncol.* 28, iv1-iv21

490 42 Wilson, T. *et al.* (2014) Glioblastoma multiforme: State of the art and future
491 therapeutics. *Surg. Neurol. Int.* 5, 132138

492 43 Lombardi, M.Y. and Assem, M. (2017) Glioblastoma genomics: A very complicated
493 story. In *Glioblastoma* pp. 3–25, Codon Publications

494 44 Laquintana, V. *et al.* (2009) New strategies to deliver anticancer drugs to brain tumors.
495 *Expert Opin. Drug Deliv.* 6, 1017–32

496 45 Zhou, J. *et al.* (2012) Novel delivery strategies for glioblastoma. *Cancer J.* 18, 89–9

497 46 Fernandes, C. *et al.* (2017) Current standards of care in glioblastoma therapy. In
498 *Glioblastoma* pp. 197–241, Codon Publications

499 47 Hanif, F. *et al.* (2017) Glioblastoma multiforme: A review of its epidemiology and
500 pathogenesis through clinical presentation and treatment. *Asian Pacific J. Cancer Prev.*
501 18, 3–9

502 48 Arshad, A. *et al.* (2015) Convection-enhanced delivery of carboplatin PLGA
503 nanoparticles for the treatment of glioblastoma. *PLoS One* 10, e0132266

504 49 Garg, T. *et al.* (2015) Current strategies for targeted delivery of bio-active drug
505 molecules in the treatment of brain tumor. *J. Drug Target.* 23, 865–87

506 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, 4517–32

52 Shi, M. *et al.* (2015) Convection-enhancement delivery of platinum-based drugs and LipoplatinTM to optimize the concomitant effect with radiotherapy in F98 glioma rat model. *Invest. New Drugs* 33, 555–63

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, 320–31

55 Siegal, T. (2013) Change clinical practice for brain tumor. *Neuro. Oncol.* 15, 656–69

56 Zhang, R. *et al.* (2016) Convection-enhanced delivery of SN-38-loaded polymeric micelles (NK012) enables consistent distribution of SN-38 and is effective against rodent intracranial brain tumor models. *Drug Deliv.* 23, 2780–6

57 Yang, W. *et al.* (2011) Convection-enhanced delivery of carboplatin in combination with radiotherapy for the treatment of brain tumors. *J Neurooncol* 101, 379–90

58 Jaaback, K. *et al.* (2016) Intraperitoneal chemotherapy for the initial management of primary epithelial ovarian cancer (Review). In *Cochrane Database of Systematic Reviews* pp. CD005340, John Wiley & Sons, Ltd

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, 236–42

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, 3315–22

62 Su, W.H. *et al.* (2012) Metronomic therapy for gynecologic cancers. *Taiwan. J. Obstet. Gynecol.* 51, 167–78

63 Ye, H. *et al.* (2015) Sustained, low-dose intraperitoneal cisplatin improves treatment outcome in ovarian cancer mouse models. *J. Control. Release* 220, 358–67

64 Pasquier, E. *et al.* (2010) Metronomic chemotherapy: New rationale for new directions. *Nat. Rev. Clin. Oncol.* 7, 455–65

65 Kubota, H. *et al.* (2017) Feasibility of metronomic chemotherapy with tegafur-uracil,

cisplatin, and dexamethasone for docetaxel-refractory prostate cancer. *J Rural Med* 12, 112–9

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, 279–86

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, 901–15

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, 112–8

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, 1321–7

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, 2386–409

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, 445–53

74 Wisnovsky, S.P. *et al.* (2013) Targeting mitochondrial DNA with a platinum-based anticancer agent. *Chem. Biol.* 20, 1323–8

75 Gibson, D. (2016) Platinum(IV) anticancer prodrugs - hypotheses and facts. *Dalton Trans.* 45, 12983–91

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, 1349–56

575 78 Vergote, I. *et al.* (2009) Phase 3 randomised study of canfosfamide (Telcyta®,
576 TLK286) versus pegylated liposomal doxorubicin or topotecan as third-line therapy in
577 patients with platinum-refractory or -resistant ovarian cancer. *Eur. J. Cancer* 45, 2324–
578 32

579 79 Kaina, B. *et al.* (2010) Targeting O6-methylguanine-DNA methyltransferase with
580 specific inhibitors as a strategy in cancer therapy. *Cell. Mol. Life Sci.* 67, 3663–81

581 80 Warren, K.E. *et al.* (2012) A phase II study of O6-benzylguanine and temozolomide in
582 pediatric patients with recurrent or progressive high-grade gliomas and brainstem
583 gliomas: A Pediatric Brain Tumor Consortium study. *J. Neurooncol.* 106, 643–9

584 81 Matei, D. *et al.* (2012) Epigenetic resensitization to platinum in ovarian cancer. *Cancer*
585 *Res.* 72, 2197–205

586 82 Garzon, R. *et al.* (2009) MicroRNAs in cancer. *Annu. Rev. Med.* 60, 167–79

587 83 Visone, R. and Croce, C.M. (2009) MiRNAs and cancer. *Am. J. Pathol.* 174, 1131–8

588 84 Chen, W. *et al.* (2014) MiR-136 targets E2F1 to reverse cisplatin chemosensitivity in
589 glioma cells. *J. Neurooncol.* 120, 43–53

590 85 Chen, X. *et al.* (2015) MiR-873 acts as a novel sensitizer of glioma cells to cisplatin by
591 targeting Bcl-2. *Int. J. Oncol.* 47, 1603–11

592 86 Ventola, C.L. (2017) Progress in nanomedicine: Approved and investigational
593 nanodrugs. *Pharm. Ther.* 42, 742–55

594 87 Peer, D. *et al.* (2007) Nanocarriers as an emerging platform for cancer therapy. *Nat.*
595 *Nanotechnol.* 2, 751–60

596 88 Harper, B.W. *et al.* (2010) Advances in platinum chemotherapeutics. *Chem. - A Eur. J.*
597 16, 7064–77

598 89 Cabral, H. and Kataoka, K. (2014) Progress of drug-loaded polymeric micelles into
599 clinical studies. *J. Control. Release* 190, 465–76

600 90 Li, J. *et al.* (2014) Complex of cisplatin with biocompatible poly(ethylene glycol) with
601 pendant carboxyl groups for the effective treatment of liver cancer. *J. Appl. Polym. Sci.*
602 131, 9246–54

603 91 Duan, X. *et al.* (2015) Polymeric micelle-mediated delivery of DNA-targeting
604 organometallic complexes for resistant ovarian cancer treatment. *Small* 11, 3962–72

605 92 Johnstone, T.C. *et al.* (2016) The next generation of platinum drugs: Targeted Pt(II)
606 agents, nanoparticle delivery, and Pt(IV) prodrugs. *Chem. Rev.* 116, 3436–86

607 93 Parhizkar, M. *et al.* (2016) Electrohydrodynamic encapsulation of cisplatin in poly
608 (lactic-co-glycolic acid) nanoparticles for controlled drug delivery. *Nanomedicine*

609 *Nanotechnology, Biol. Med.* 12, 1919–29

610 94 Xue, X. *et al.* (2013) Nanoscale drug delivery platforms overcome platinum-based
611 resistance in cancer cells due to abnormal membrane protein trafficking. *ACS Nano* 7,
612 10452–64

613 95 Lehner, R. *et al.* (2013) Intelligent nanomaterials for medicine: Carrier platforms and
614 targeting strategies in the context of clinical application. *Nanomedicine*
615 *Nanotechnology, Biol. Med.* 9, 742–57

616 96 Marrache, S. *et al.* (2014) Detouring of cisplatin to access mitochondrial genome for
617 overcoming resistance. *Proc. Natl. Acad. Sci.* 111, 10444–9

618 97 Sun, X. *et al.* (2017) A pH-responsive yolk-like nanoplatform for tumor targeted dual-
619 mode magnetic resonance imaging and chemotherapy. *ACS Nano* 11, 7049–59

620 98 Cajot, S. *et al.* (2011) Design of reversibly core cross-linked micelles sensitive to
621 reductive environment. *J. Control. Release* 152, 30–6

622 99 Nishiyama, N. and Kataoka, K. (2006) Current state, achievements, and future
623 prospects of polymeric micelles as nanocarriers for drug and gene delivery. *Pharmacol.*
624 *Ther.* 112, 630–48

625 100 Oerlemans, C. *et al.* (2010) Polymeric micelles in anticancer therapy: Targeting,
626 imaging and triggered release. *Pharm. Res.* 27, 2569–89

627 101 Stathopoulos, G.P. and Boulikas, T. (2012) Lipoplatin formulation review article. *J.*
628 *Drug Deliv.* 2012, 581363

629 102 Patra, H.K. and Turner, A.P.F. (2014) The potential legacy of cancer nanotechnology:
630 Cellular selection. *Trends Biotechnol.* 32, 21–31

631 103 Li, S. *et al.* (2017) Overcoming resistance to cisplatin by inhibition of glutathione S-
632 transferases (GSTs) with ethacraplatin micelles in vitro and in vivo. *Biomaterials* 144,
633 119–29

634 104 Xu, S. *et al.* (2017) Supramolecular cisplatin-vorinostat nanodrug for overcoming drug
635 resistance in cancer synergistic therapy. *J. Control. Release* 266, 36–46

636 105 Krauze, M.T. *et al.* (2005) Real-time visualization and characterization of liposomal
637 delivery into the monkey brain by magnetic resonance imaging. *Brain Res. Protoc.* 16,
638 20–6

639 106 Zhang, C. *et al.* (2017) Convection enhanced delivery of cisplatin-loaded brain
640 penetrating nanoparticles cures malignant glioma in rats. *J. Control. Release* 263, 112–
641 9

642 107 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, 381–9
- 108 Hillaireau, H. and Couvreur, P. (2009) Nanocarriers' entry into the cell: Relevance to drug delivery. *Cell. Mol. Life Sci.* 66, 2873–96
- 109 Bregoli, L. *et al.* (2016) Nanomedicine applied to translational oncology: A future perspective on cancer treatment. *Nanomedicine Nanotechnology, 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, 2876–9
- 111 Boulikas, T. (2009) Clinical overview on LipoplatinTM: A successful liposomal formulation of cisplatin. *Expert Opin. Investig. Drugs* 18, 1197–218
- 112 Exner, A.A. *et al.* (2005) Enhancement of carboplatin toxicity by Pluronic block copolymers. *J. Control. Release* 106, 188–97
- 113 Yang, T. *et al.* (2016) Anti-tumor efficiency of lipid-coated cisplatin nanoparticles co-loaded with microRNA-375. *Theranostics* 6, 142–54
- 114 Hainfeld, J.F. *et al.* (2013) Gold nanoparticle imaging and radiotherapy of brain tumors in mice. *Nanomedicine* 8, 1601–9
- 115 McCarthy, J.R. and Weissleder, R. (2008) Multifunctional magnetic nanoparticles for targeted imaging and therapy. *Adv. Drug Deliv. Rev.* 60, 1241–51
- 116 Zhu, H. *et al.* (2012) Responsive fluorescent Bi₂O₃@PVA hybrid nanogels for temperature-sensing, dual-modal imaging, and drug delivery. *Biomaterials* 33, 3058–69
- 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, 6742–8
- 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, 2546–54
- 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, 2533–8

677 **Figure legends**

(B)

(C)

678

679

680 **Figure 1. Various mechanisms of action of DNA targeting agents. (a) Intercalation of**
681 **doxorubicin between DNA strands.** Doxorubicin forms with guanine a covalent bond
682 (formaldehyde equivalent) on one DNA strand and hydrogen bonds on the opposite strand to
683 stabilize the structure. Consequently, DNA stays unwound and replication becomes
684 impossible. Interactions with DNA preferentially occur with neighboring GC base pairs [9].
685 **(b) DNA methylation by temozolomide.** Temozolomide acts as a prodrug spontaneously
686 hydrolyzed at physiological pH in its active metabolite MTIC, subsequently converted to AIC
687 and methyldiazonium [10]. This highly reactive cation methylates purine bases, preferentially
688 O₆ and N₇ guanines and to a lesser extent A₃ adenine, thus inhibiting DNA replication.
689 Excision of O₆-methylguanine adducts by MMR enzymes may induce either mutations
690 continuously recovered along replications or DNA single- or double-strand breaks responsible
691 for cell apoptosis [43]. **(c) DNA complexation with cisplatin.** Cisplatin (**1**) requires the
692 substitution of at least one chloride group by water for its activation, a process called
693 aquation. This hydrolysis automatically occurs once cisplatin is internalized because of the
694 small intracellular chloride concentration. Reactivity of Pt(II) complexes, **(4)** > **(2),(5)** >
695 **(1),(3)** >> **(6)**, is determined by the ability of every ligand to be substituted by a nucleophile
696 [4]. Active Pt(II) species complex with nucleophilic intracellular ligands: N₇-sites of purine
697 DNA or RNA bases, mainly guanine and to a lesser extent adenine, and nucleophilic sites on
698 several proteins [7,12]. Guanine intrastrand cross-linking with cisplatin impedes DNA
699 replication and transcription [6,11].

Figure 2, Key Figure. Strategies to overcome cellular resistance and enhance the therapeutic index of a drug: The example of cisplatin. (a) **Cellular resistance mechanisms to cisplatin.** (1) Impaired influx through altered transported-mediated uptake (CTR1) [13,14], or conversely (2) active efflux outside the cell (ATP7A/B, MRP2) [6] are responsible for (3) a 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 within 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]. Besides, glutathione may quench Pt-DNA monoadducts before their conversion into cytotoxic DNA cross-links and (5) reduce cisplatin-induced oxidative stress within cells [15,16]. To overcome drug cytotoxicity, tumor cells trigger an overall abnormal phenotype by silencing or activating multiple genes notably

involved in (6) the modulation of the expression of miRNA, (7) of GTPases, ribosomal and **heat shock proteins (HSP)**, in (8) the overexpression of markers of the epithelial to mesenchymal transition (EMT) pathway, in (9) histone acetylation, (10) aberrant DNA methylation, (11) DNA-damage repair and (14) apoptotic signaling pathways [13]. (11-a) DNA cross-linking recognition and reparation mechanisms allow for (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 recurrence of aggressive and resistant tumor cells [16]. Among other indications, cisplatin provided a breakthrough in the management of testis cancer attributed to an intrinsic cellular hypersensitivity together with a 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 regimen [13,15,17,24]. (13) An enhanced tolerance to DNA damage and (14) the 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 MGMT activity whose expression counters temozolomide efficacy in glioma treatment [76]. (3) An alternative to reverse resistance induced detoxification processes consists in taking advantage of the elevated levels of GSH in resistant cancer cells to specifically damage them [6,78] or to inhibit the glutathione S-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 an optimized drug delivery to tumor cells for (1) locoregional confinement in specific environments and/or for selective targeting of receptors in relation with administration routes and modalities [87,94,95]. They can also be engineered by 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 transporters

mediation, endocytosis of nanosized DDS enables the 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]. Besides, 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 an increased formation of DNA adducts and a markedly enhanced antitumor activity whatever the resistance status of the cells [69,91,97,103,104]. The versatility of structure of smart DDS also allows for (6) a 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 radiation therapy or modulation of the expression of resistance signals through miRNA agonist or antagonist strategies may reinforce the cytotoxicity of nanovectorized cisplatin.

Figure 3. Cisplatin alternative and neglected targets. Cisplatin binds to various intracellular non DNA components that constitute potential targets and factors of efficacy or resistance. (1) Cisplatin interactions with proteins account for most adducts within cells due to high reactivity of thiol and thioether protein constitutive residues and their abundance within

the cytosol [4]. (2) Nucleotides and nucleosides are characterized by a lower steric hindrance compared to their analogs involved in DNA that may result in easier interactions with Pt(II) complexes. (3) Kinetics considerations showed faster and higher complexation rates of cisplatin with RNA than DNA *in vitro*, even though resulting in less stable adducts. Cross-linking with mRNA was reported to inhibit translation *in vitro*. Interactions with non-coding RNA may impair downstream cellular and molecular processes [4]. (4) Positively charged Pt(II) activated species were reported to accumulate within negatively charged mitochondria due to electrostatic interactions. There, cisplatin produces a significantly higher amount of adducts with mitochondrial DNA than with nuclear DNA, subsequently impairing response and clinical outcome of cancer patients [16,72,119]. Besides, since resistance to cisplatin is partly linked to an extensive repair of Pt-DNA adducts by the 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 persist following an oxaliplatin-based treatment was correlated with an increased risk of disease progression in patients with colorectal cancer, the impact of cisplatin interactions with extracellular components has not been reported yet [120].

Tables

Table 1. FDA-approved alkylating agents and affiliated compounds for anticancer therapy ^a.

Marketing authorization and clinical practice guidelines are likely to evolve over time and depending on the country.

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

Drug	Approval year	Indication	Delivery type	Route
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
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 Abbreviations: IV, intravenous

Glossary

Alkyl group: a univalent group derived from alkanes by 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} .

Cisplatin, *cis*-diamminedichloroplatinum(II): 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 *E. 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, absorption of X-rays of a given frequency increases with higher Z atomic number of the penetrated material, which explains the radiosensitizing properties of platinum derivatives.

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

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

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

Heat shock proteins (HSP): produced by living organisms in response to a stress such as temperature or exposition to heavy metals, overexpressed in cisplatin resistant cells, HSP prevent proteins from 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 constituted of high amounts of sulfur-rich amino acids, namely cysteine. Exhibiting high affinity to metals, they play a key role in drug detoxification.

MGMT, 6-O-methylguanine-DNA methyltransferase: enzyme involved in the repair of methylated DNA adducts.

817 **microRNA (miRNA):** regulatory endogenous non-coding RNAs produced by the genome.

818 **p53:** tumor suppressor notably involved in cell cycle regulation and apoptotic cell death, its
819 mutation is a common feature in human cancer cells.

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

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

825 **Temozolomide:** small orally available lipophilic molecule of high interest in the treatment of
826 malignant gliomas due to its ability to cross the blood-brain barrier.

827 **Theranostics:** merger between the words “therapy” and “diagnosis”.