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# 1 Rethinking alkylating(-like) agents for solid tumor management

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13

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14 **Abstract**

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

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

## 28 **Strategic paths towards anticancer therapy**

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

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

57 In the following, we provide an up-to-date review of the rationale and conventional  
58 use of alkylating agents and platinum derivatives in clinical practice. Then, we focus on  
59 optimization ways, synergies and innovative alternatives that pave the way for rethinking how

60 to potentiate their anticancer efficacy, laying down future challenges for these old molecules  
61 in the treatment of solid tumors, with the ultimate view of personalized medicine.

## 62 **Rationale and conventional use of alkylating agents and platinum** 63 **derivatives in clinical practice**

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

### 76 *Mechanism*

77 Anticancer agents are traditionally classified in chemical families according to their  
78 mode of action. Intercalating and alkylating agents are reported to directly interact with DNA  
79 by inter- or intrastrand crosslinking. However, the mechanism of action of intercalating agents  
80 that form formaldehyde-based covalent bonds with DNA bases as shown through the example  
81 of anthracycline antibiotics on Figure 1-(a) strictly differs from that of alkylating agents [9].  
82 Alkylators allow for the transfer of an **alkyl group** from one to another molecule under  
83 physiological conditions. Such nucleophilic substitutions occur by an  $S_N1$  or  $S_N2$  mechanism  
84 depending on the kinetics of the reaction and result in covalent binding to an organic  
85 macromolecule as depicted in Figure 1-(b) in the case of **temozolomide** [10]. Since exposure  
86 to alkylating agents leads to chromosomal aberrations in dividing cells, DNA stands for the  
87 key target site of alkylation within cells. This hypothesis is further supported by its high molar  
88 mass, which makes DNA the major nucleophilic substrate for alkylation within the organism,  
89 far ahead RNA and proteins. Alkylation mainly occurs during S phase, while DNA is

90 replicating: both strands are separated making nucleophilic substrates easily reachable. A  
91 blockage in G2-phase was also reported [11]. Alkylating agents are more likely to bind to  
92 exposed nucleophilic sites in the grooves of the DNA double helix: guanine (positions N<sub>7</sub>, O<sub>6</sub>,  
93 N<sub>2</sub> and N<sub>3</sub>), adenine (N<sub>3</sub> and N<sub>7</sub>) and cytosine bases (N<sub>3</sub>). The resulting adducts prevent  
94 strands from uncoiling and separating, making DNA replication and downstream RNA  
95 transcription impossible where the alkylation occurred. Platinum complexes are stabilized by  
96 various ligands that can be substituted by nucleophilic substrates to form a strong  
97 coordination bound with the central platinum atom. In this respect, platinum compounds were  
98 historically considered as alkylating agents even though they do not interact with biological  
99 macromolecules through an alkyl group but rather by complexation. Cisplatin, whose  
100 mechanism of action is illustrated on Figure 1-(c), enabled to dramatically improve the  
101 prognosis of germinal cancer cells and is still currently used as a gold-standard in the  
102 treatment of various solid tumors [6,7]. Contrary to alterations caused by mono-functional  
103 alkylating agents such as nitrosoureas, inter- or intracatenary bridges induced by platinum  
104 derivatives between both DNA strands are extremely difficult to repair.

#### 105 *Resistance*

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

## 120 *Radiosensitization*

121 Radiotherapy (RT) constitutes a key strategy in the treatment of several solid tumors,  
122 including glioma, lung, breast, head and neck, uterine cervix, rectum, vulvar and prostate  
123 cancers. Radiation beam causes direct DNA damages but also indirectly impact cell-death  
124 through the formation of highly reactive oxygen species (ROS). Modulation of the tumor  
125 response to RT can be achieved by resorting to various antineoplastic agents and has been  
126 extensively investigated in alkylating agents and platinum-based strategies with the aim of  
127 amplifying the differential effects between tumor and normal cells [25–28]. Due to their  
128 ability to form DNA adducts leading to double-strand breaks and the heavy platinum atom  
129 that locally enhances the effect of external beam radiation respectively, alkylating agents and  
130 platinum compounds are particularly used in combination with RT as effective  
131 radiosensitizing chemotherapy [25,27–29]. Clinical studies have further evidenced the  
132 superior efficacy of concomitant chemoradiotherapy in various solid tumors compared to RT  
133 alone [25,26,30]. This synergistic effect depicted in Figure 2-(b) can be explained by a more  
134 accurate locoregional control of the pathology with a reduced or at least contained tumor cell  
135 proliferation that would otherwise quickly entails radioresistance, resulting in a better  
136 prognosis. Paradoxically, radioresistance can also occur from disruption of blood supply to  
137 the altered tissue after surgery and chemotherapy, leading to hypoxic foci. Tumor  
138 radiosensitivity can then be modulated by chemical radiosensitizers that simultaneously  
139 enhance the therapeutic benefit of RT locally and exert their own cytotoxic effect [31,32]. The  
140 time schedule between chemotherapy and RT is a key point for effective combination owing  
141 to dose- and time-dependent cytotoxicity of the drug, leading to synergism or at least to an  
142 additive effect on tumor cells [30]. Polychemotherapy, i.e. the combination of several drugs,  
143 offers another way to reach synergism in anticancer treatment.

## 144 *Polychemotherapy*

145 Alkylating agents as well as platinum compounds are commonly used concurrently  
146 with other antineoplastic agents, including targeted drugs and antibodies, in the management  
147 of solid tumors. The combination of drugs that exert their anticancer activity through various  
148 mechanisms of action induces cell damage and metabolism dysfunction by altering several  
149 molecular targets and signaling pathways involved in tumorigenesis [14,33]. This option is  
150 therefore commonly considered in clinical practice to potentiate drug efficacy and reverse  
151 acquired drug resistance like in ovarian, biliary tract, lung, breast and prostate cancers that

152 primary respond to a platinum-based treatment but ultimately relapse. For instance, the  
153 standard treatment for patients with advanced colorectal cancer that consists of the  
154 combination of 5-fluorouracil, leucovorin and oxaliplatin, demonstrated a potentiation of the  
155 anticancer activity of oxaliplatin with fluoropyrimidines resulting in a significant  
156 improvement in overall survival. The design of complementary targeted drugs since the 2000s  
157 has further reinforced this trend [34,35]. In parallel, in the mid-1970s, a breakthrough in the  
158 treatment of men with metastatic testicular cancer arose from a combinatory regimen based on  
159 cisplatin supplemented with bleomycin and vinblastine, leading to an increase in complete  
160 response rates from 5 % to 60 %. Substitution of vinblastine with etoposide further enabled to  
161 reach up to 80 % of cure rates [6]. Additional adjunctive drugs can also be considered to  
162 modulate platinum activity or toxicity [36–41].

### 163 **Optimization of the use of alkylating agents and platinum derivatives**

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

#### 170 *Drug administration and dosage*

171 Chemotherapy is often limited by systemic injection which causes drug dilution within  
172 the organism and is responsible for severe side effects, especially on highly proliferative cells.  
173 High systemic toxicity of conventional anticancer agents can be overcome by using a more  
174 suited route of administration depending on the tumor type. In the case of operable patients  
175 with glioblastoma, an alternative to temozolomide relies on the implantation of carmustine-  
176 loaded wafers (**Gliadel**<sup>®</sup>) within the resection cavity at the end of the surgery [42,43]. In such  
177 aqueous environment, the anhydride bonds of the biodegradable polymeric matrix get  
178 hydrolyzed, allowing for a controlled and sustained release of the drug that can diffuse within  
179 the surrounding parenchyma during several weeks. After degradation, the active metabolite  
180 can alkylate DNA, cross-link with RNA and entail proteins carbamylation, ultimately leading  
181 to cell apoptosis [43]. Although Gliadel<sup>®</sup> demonstrated a high therapeutic efficacy in animal  
182 models, clinical translation is limited by side effects and poor diffusion within the damaged



183 parenchyma since the concentration gradient may not be strong enough to allow carmustine  
184 for penetrating deep into the brain tissue and for distributing through the tumor margins [44–  
185 47]. Although alkylating agents have provided therapeutic efficacy and improved patient  
186 outcomes in the management of brain cancer, alternative strategies are required to reach  
187 therapeutic doses in close vicinity of the tumor burden and maximize their anticancer activity.

188 In this context, the locoregional administration of chemotherapy directly within the  
189 brain enables both to bypass the blood brain barrier that prevents most macromolecules and  
190 therapeutic drugs from reaching the central nervous system and to locally increase drug  
191 concentration. Convection-enhanced delivery (CED) consists of infusing the drug at high  
192 concentration directly within the brain or the tumor *via* intraparenchymal microcatheters [44].  
193 A constant hydrostatic positive pressure gradient is established by an infusion pump that  
194 forces convection of the therapeutic solution at a rate of 0.1 to 10  $\mu\text{l min}^{-1}$ . As such, CED  
195 achieves homogeneous elliptical to spherical distribution of molecules of various molar  
196 masses over large distances compared to suboptimal therapeutic doses reached by passive  
197 diffusion from concentration gradient [45,48–50]. Because they cannot easily cross the blood  
198 brain barrier, platinum derivatives do not reach brain tumors in optimal therapeutic  
199 concentrations when administered intravenously [51]. In animal models, CED was shown to  
200 dramatically increase the concentration of cisplatin and carboplatin within the brain tumor in  
201 regard with traditional administration routes while reducing systemic toxicity [52]. Although  
202 safety and feasibility have been demonstrated in phase I clinical trials, translation to the  
203 clinics failed so far because of surgical complications [53,54]. In addition, increased  
204 interstitial fluid pressure within brain tumors and leakage into the cerebrospinal fluid  
205 drastically reduce drug concentration at the targeted site and can even induce neurotoxicity  
206 [55,56]. Thus, technical advances are expected to fill the gap between the view of CED as a  
207 promising strategy to deliver therapeutic agents *in situ* to large and clinically-relevant brain  
208 volumes and the current state of an invasive technique in which continuous or repeated  
209 administration is at risk due to infection, hemorrhage or neurologic disorders related to  
210 catheter positioning inside the brain parenchyma [45,55,57]. In case of localized diseases,  
211 other clinically-relevant routes of administration have been investigated such as  
212 intraperitoneal chemotherapy for primary or recurrent ovarian cancer [58,59].

213 The use of drugs at their **maximum tolerated dose (MTD)** requires intermittent drug-  
214 free periods between two cycles of chemotherapy that should allow the patient for recovering  
215 from acute toxicities. However, tumor cells can regenerate during that resting time, and

216 selected clones may develop resistance to the treatment [60,61]. As a result, the traditional  
217 rationale according to which higher doses are necessary for tumor eradication is slowly  
218 shifting to the concept that “less is more”, which favors a stabilization of the disease over time  
219 for maintenance of quality of life. As hyperfractionated radiotherapy suggests that a  
220 continuous low-dose schedule may be more efficient in killing highly-proliferative cells than  
221 standard radiotherapy by avoiding tumor cells reparation, metronomic chemotherapy consists  
222 in the chronic and equally-spaced administration of drugs at low dose ( $1/10^{\text{th}}$  to  $1/3^{\text{rd}}$  of the  
223 MTD) without extended rest periods [33,62]. Whereas drug administration by intermittent  
224 bolus generally results in high peak plasma concentrations that are further responsible for  
225 severe toxicity, “dose-dense” strategies have shown encouraging results with evidence of  
226 disease stabilization and improved outcomes associated with a low toxicity profile in patients  
227 with solid tumors [61,63–65]. Interestingly, the frequent low-dose administration of  
228 traditional drugs makes them able to target the dividing vascular endothelial cells, thus  
229 demonstrating additional anti-angiogenic potential, while the stimulation of the anticancer  
230 immune response may further contribute to force tumor dormancy [33,60,62,64]. Besides,  
231 metronomic chemotherapy results in more convenient treatment administration and promotes  
232 maintenance of patients quality of life [65]. Economic reasons can also favor oral metronomic  
233 chemotherapy as a minimal cost but still compelling alternative to current standard-of-care,  
234 particularly in developing countries [66,67]. Metronomic regimens based on alkylating agents  
235 or platinum derivatives have demonstrated a therapeutic benefit in patients with solid tumors  
236 [60,65,68]. However, large-scale studies and controlled randomized trials that compare  
237 conventional MTD to the same metronomic administration regimen are required to define the  
238 optimal drug dosage and schedule.

### 239 *Alternative and neglected targets*

240 Since chromosomal aberrations in dividing cells were an outstanding feature of  
241 mustard gas intoxication, most hypotheses postulated that nuclear DNA was the most critical  
242 pharmacological target of alkylating agents and platinum derivatives [13,14]. However, in the  
243 case of platinum-based treatments, the level of Pt-DNA adducts does not necessarily correlate  
244 with neither intracellular drug accumulation nor cytotoxicity, suggesting that other cellular or  
245 molecular components must be involved with various degrees of specificity and severity in  
246 anticancer activity [4,16,25,69,70]. Growing evidence notably suggest the role of  
247 mitochondria in cisplatin anticancer activity [13,17,71,72]. Mitochondria are involved in the  
248 apoptotic pathway through the release of cytochrome c into the cytosol and subsequent

249 activation of caspases 8 and 9, thus constituting a critical target for cytotoxic drugs. Rerouting  
250 chlorambucil through engineered mitochondria-penetrating peptides (MPPs) that are able to  
251 cross the dense and highly hydrophobic membranes of mitochondria demonstrated a dramatic  
252 potentiation of its anticancer activity in various cancer cell lines by promoting apoptosis and  
253 evading deactivation processes that commonly occur within the cytosol [73]. Interestingly, the  
254 development of a cisplatin analog from MPPs showed that mtDNA damage was sufficient to  
255 induce cytotoxicity and promote apoptotic cell death without impairing nuclear DNA or  
256 entailing cell cycle arrest [74]. Therefore, mitochondria-specific targeting should be  
257 reconsidered for implementing innovative and efficient anticancer strategies. Furthermore,  
258 since platinum complexes demonstrate high affinity for nucleophilic sites, various studies  
259 have investigated their ability to trigger interactions at the molecular level by binding to  
260 various intracellular non DNA components that constitute as many potential targets of their  
261 cytotoxicity or resistance. This rationale is schematized in Figure 3 with the example of  
262 cisplatin whose participation in DNA adducts accounts for only about 10 % of the whole  
263 amount of cisplatin covalently bound to biomolecules within cells [4,13,17]. Therefore, the  
264 proper significance of the multifactorial mechanisms that mediate cytotoxicity in a highly  
265 concerted way both at the cellular and molecular levels should be reconsidered with the  
266 perspective of giving traditional drugs a new impetus [5,75].

### 267 *Innovative synergies*

268 The combination of alkylating agents or platinum derivatives with relevant therapeutic  
269 strategies capable of promoting a synergistic effect and therefore potentiating anticancer  
270 activity is of paramount interest in the treatment of solid tumors, as illustrated in Figure 2-(b)  
271 with the example of cisplatin. Inhibition of abundant thiol- and thioether-containing amino  
272 acids and proteins for which platinum complexes exhibit high affinity can hamper drug  
273 detoxification processes [4]. Based on *in vitro* assays that demonstrated enhanced cell  
274 sensitivity to DNA damage and apoptosis in glioblastoma cell lines exposed to buthionine  
275 sulfoximine (BSO) beforehand, a significant inhibition of the tumor growth was achieved in  
276 animal groups treated with BSO in combination with either temozolomide or cisplatin  
277 compared to animal groups treated with each of these drugs independently. Thanks to a  
278 putative synergistic effect, even low doses of anticancer agents were sufficient to achieve  
279 substantial outcomes while preventing from severe side effects. According to these promising  
280 results, the authors suggest that the combination of **glutathione (GSH)** inhibitors with  
281 alkylating agents or platinum complexes may improve the clinical outcome in brain cancer

282 patients [76,77]. Conversely, advantage can be taken of the elevated levels of GSH in resistant  
283 cancer cells to specifically damage them [6,78]. Bio-mimicking molecules can also be  
284 synthesized to supersede their bio-analogs within the organism. Methylation of the gene's  
285 promoter of **6-O-methylguanine-DNA methyltransferase (MGMT)**, a common feature in  
286 glioblastoma diagnosed patients, is of good prognosis since it improves cell sensitivity to  
287 temozolomide and results in an increase in median survival [21–23]. O6-benzylguanine, a  
288 structural analog of O6-methylguanine, is able to divert and irreversibly inactivate the MGMT  
289 enzyme, preventing it from repairing DNA adducts induced by temozolomide. Such  
290 synergistic combination is expected to lead to the restoration of tumor sensitivity and to  
291 maximize drug cytotoxicity. Despite promising preliminary results, the efficacy of this  
292 strategy was limited in clinical practice by severe side effects attributed to the inactivation of  
293 the MGMT enzyme also in normal tissues [79,80]. Epigenetic modulations that may alter the  
294 DNA repair machinery can play a role in circumventing drug resistance too [6,13,24]. DNA  
295 demethylating agents are able to reverse hypermethylation of genes involved in the DNA  
296 mismatch repair (MMR) pathway whose alteration participates in cell resistance to platinum  
297 compounds and is of bad prognosis for patients with ovarian carcinoma. A phase II clinical  
298 trial in patients with platinum-resistant ovarian carcinoma supported impairment of gene  
299 methylation by low-dose decitabine administration and subsequent alteration of the MMR  
300 pathway to restore sensitivity to carboplatin, resulting in high response rates and extended  
301 progression-free survival [81]. The expression of a panel of genes involved in cell sensitivity  
302 or resistance mechanisms and the molecular pathways below may also be modulated to  
303 reverse drug resistance and reach a synergistic effect through miRNA that play a key role in  
304 cellular development but also in oncogenesis, cancer progression and drug resistance [82–85].

### 305 *Development of smart nanocarriers*

306 Nanotechnologies may offer tremendous opportunities in the field of medicine due to  
307 their size and versatility of structure, as described with the example of cisplatin in Figure 2-  
308 (c). Various **drug delivery systems (DDS)** have been engineered to locally deliver their  
309 bioactive cargo, thus concentrating drug efficacy at the tumor site while preventing from  
310 systemic toxicity [86]. Indeed, DDS have been described to passively target tumor cells  
311 through the enhanced permeability and retention effect (EPR) [87,88]. Although  
312 controversial, this paradigm has given rise to the development of various DDS, including for  
313 vectorization of platinum derivatives [70,88–93]. Interestingly, active targeting can be  
314 achieved by functionalizing nanocarriers with various ligands that specifically bind to

315 receptors overexpressed on the surface of cancer cells such as folate or epidermal growth  
316 factor [87,94,95]. DDS can also be engineered to specifically reroute a drug to targets whose  
317 impairment will trigger a cell signaling cascade likely to entail apoptotic cell death [96].  
318 Designing adaptive systems sensitive to micro-environmental changes, namely environment-  
319 (pH [97], enzyme and reductive environment [98]) responsive DDS, further allows for  
320 specific targeting and triggered drug release. A controlled release of the drug over time and  
321 the subsequent modulation of its pharmacokinetic profile may improve its therapeutic benefit  
322 [45,88,99–104].

323 Although DDS are of high interest to extend the drug lifetime in the general  
324 circulation and protect it from deactivation until it reaches its target, alternative routes have  
325 been investigated to circumvent physiological barriers. In animal models, the local infusion of  
326 liposomes [105], nanoparticles [70,106] or polymeric micelles [56] by CED within the brain  
327 parenchyma was reported to substantially enhance the distribution volume of the system in  
328 comparison with the free drug, as well as to reduce toxicity and prolong half-life  
329 [45,105,107].

330 Endocytosis has been extensively described as the key mechanism of DDS cellular  
331 uptake [89,95,97,102,108–110]. Protected from deactivation by the plasma membrane vesicle,  
332 quanta of active molecules are conveyed from early endosomes to late endosomes and  
333 lysosomes, like a “Trojan horse”, favoring drug release in close vicinity of the nuclear and  
334 subsequently promoting interactions with DNA [91,94,95,110]. As such, Lipoplatin<sup>TM</sup>, a  
335 liposomal formulation of cisplatin, was reported to bypass membrane transporters and  
336 subsequent intracellular trafficking by direct fusion with the cell membrane [94,111].

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

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

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

## 366 **Concluding remarks**

367 Owing to their broad anticancer spectrum, alkylating agents and platinum derivatives  
368 are key in the management of solid tumors. Still, they suffer from acute systemic toxicity,  
369 sub-optimal treatment schedule, intrinsic or acquired resistance and inadequate routing both at  
370 the tissue and cellular levels. In this context, this review envisions promising alternatives to  
371 the conventional use of alkylating agents and platinum derivatives in clinical practice,  
372 including their administration by appropriate routes depending on the tumor location, an  
373 optimized subcellular rerouting, synergistic strategies, and the development of an arsenal of  
374 smart nanocarriers. Driven by the necessity to rethink their use through rather simple  
375 potentiating therapeutic strategies relevant to the daily needs and clinical practice – instead of  
376 developing plenty of new drugs that would quickly face the same issues in terms of limited

377 therapeutic index (see Outstanding Questions), we do believe that these old molecules have  
378 great promise for future applications in the management of solid tumors.

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## 393 **Disclosures**

394 There are no conflicts of interest to declare.

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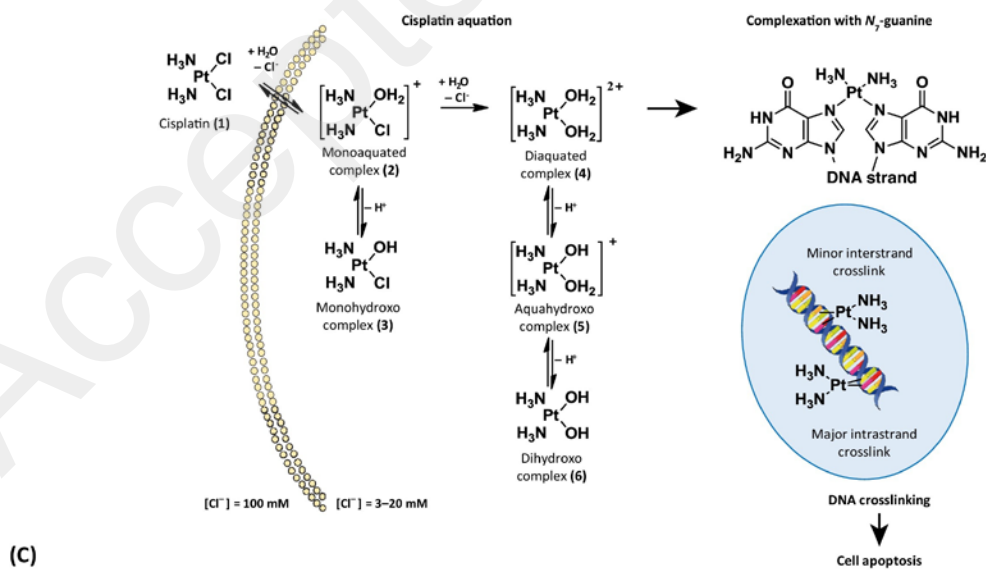
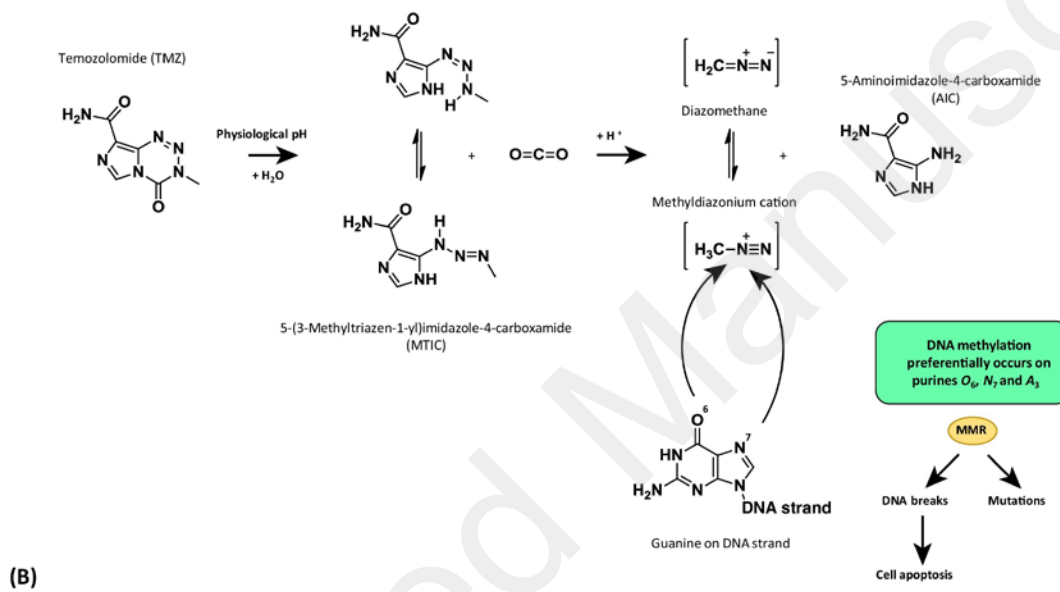
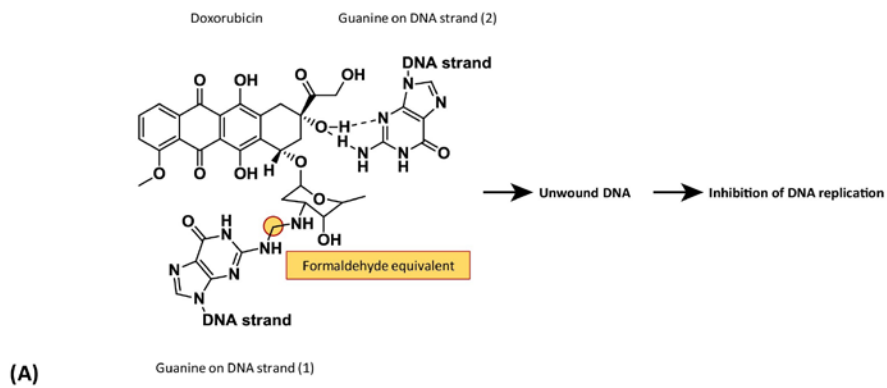
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677 **Figure legends**

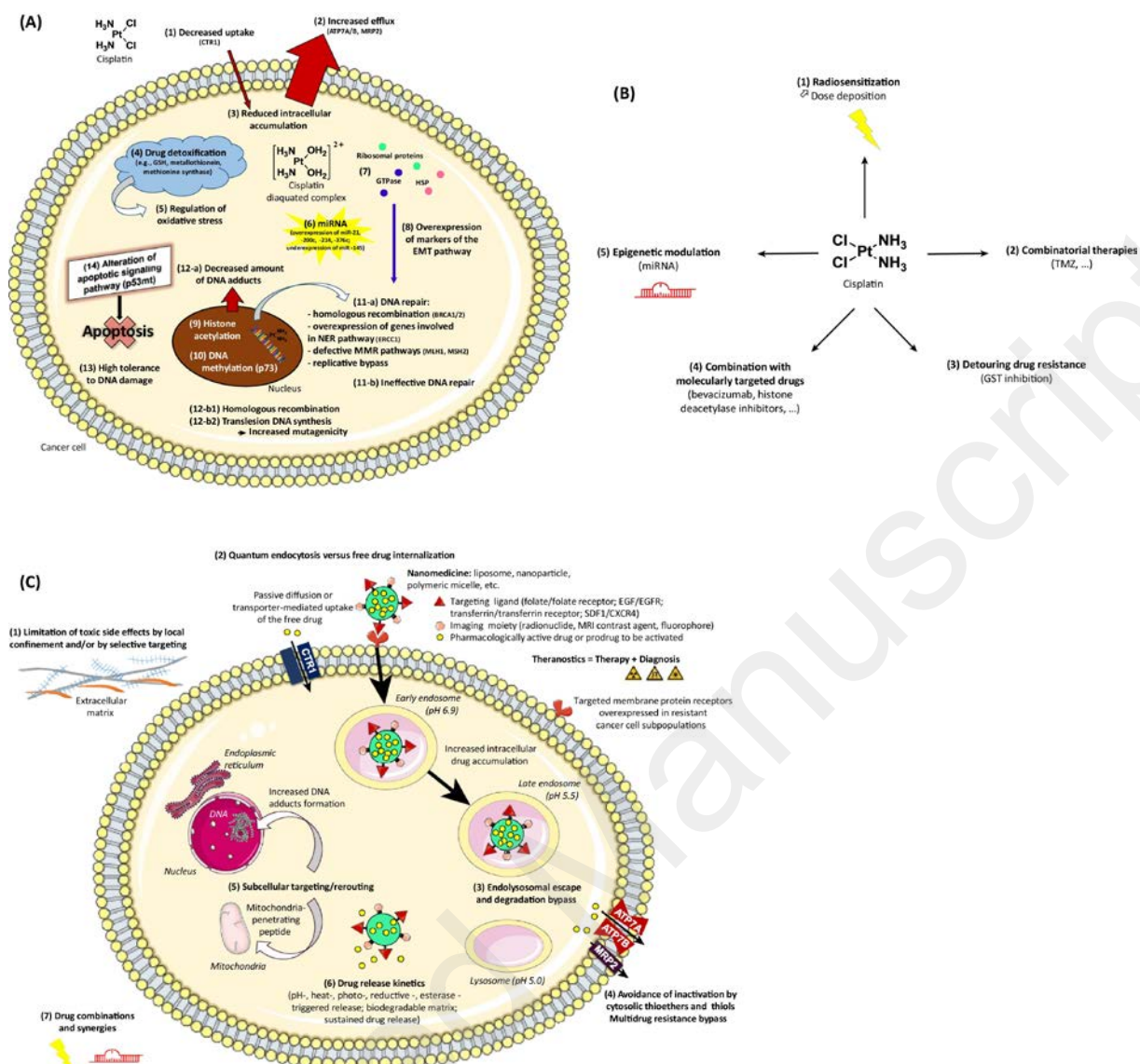


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680 **Figure 1. Various mechanisms of action of DNA targeting agents. (a) Intercalation of**  
681 **doxorubicin between DNA strands.** Doxorubicin forms with guanine a covalent bond  
682 (formaldehyde equivalent) on one DNA strand and hydrogen bonds 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 methyl diazonium [10]. This highly reactive cation methylates purine bases, preferentially  
688 O<sub>6</sub> and N<sub>7</sub> guanines and to a lesser extent A<sub>3</sub> adenine, thus inhibiting DNA replication.  
689 Excision of O<sub>6</sub>-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: N<sub>7</sub>-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].



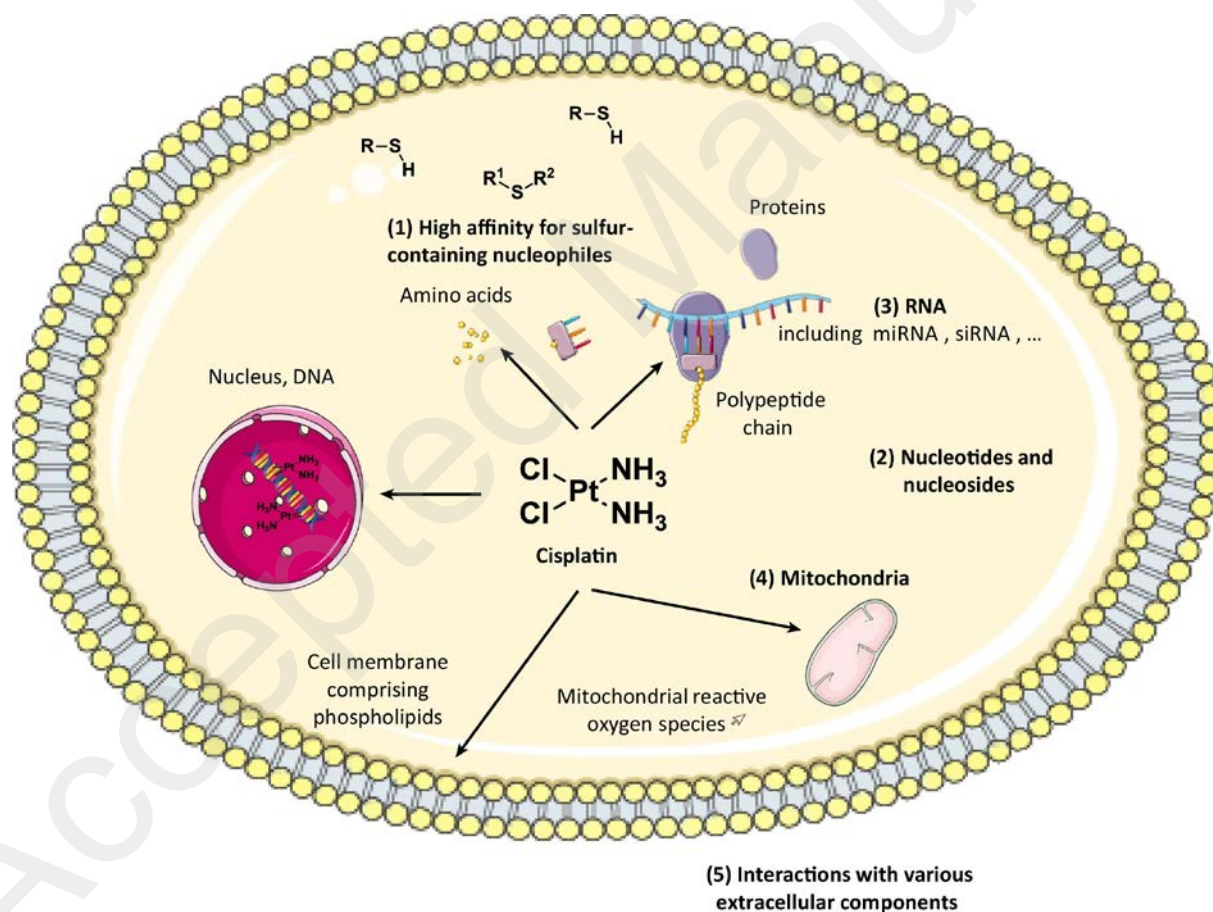
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Trends in Pharmacological Sciences

701 **Figure 2, Key Figure. Strategies to overcome cellular resistance and enhance the**  
 702 **therapeutic index of a drug: The example of cisplatin. (a) Cellular resistance**  
 703 **mechanisms to cisplatin. (1)** Impaired influx through altered transported-mediated uptake  
 704 (CTR1) [13,14], or conversely **(2)** active efflux outside the cell (ATP7A/B, MRP2) [6] are  
 705 responsible for **(3)** a reduced total intracellular accumulation of the drug [12]. Cisplatin efflux  
 706 from the nucleus back to the cytoplasm also reduces drug distribution to nuclear DNA [15].  
 707 **(4)** The abundance within the cytosol of thiol- and thioether-containing amino acids and  
 708 proteins for which cisplatin exhibits high affinity is responsible for detoxification processes  
 709 that lead to drug sequestration and inactivation [4,16,17,73]. Besides, glutathione may quench  
 710 Pt-DNA monoadducts before their conversion into cytotoxic DNA cross-links and **(5)** reduce  
 711 cisplatin-induced oxidative stress within cells [15,16]. To overcome drug cytotoxicity, tumor  
 712 cells trigger an overall abnormal phenotype by silencing or activating multiple genes notably

713 involved in **(6)** the modulation of the expression of miRNA, **(7)** of GTPases, ribosomal and  
714 **heat shock proteins (HSP)**, in **(8)** the overexpression of markers of the epithelial to  
715 mesenchymal transition (EMT) pathway, in **(9)** histone acetylation, **(10)** aberrant DNA  
716 methylation, **(11)** DNA-damage repair and **(14)** apoptotic signaling pathways [13]. **(11-a)**  
717 DNA cross-linking recognition and reparation mechanisms allow for **(12-a)** a decreased  
718 amount of DNA adducts, whereas **(11-b)** ineffective DNA repair leads to **(12-b1)** homologous  
719 recombination or **(12-b2)** translesion DNA synthesis that further results in genome instability  
720 and recurrence of aggressive and resistant tumor cells [16]. Among other indications, cisplatin  
721 provided a breakthrough in the management of testis cancer attributed to an intrinsic cellular  
722 hypersensitivity together with a reduced ability to repair DNA adducts through the nucleotide  
723 excision repair (NER) pathway [6]. Other molecular pathways are also involved in the  
724 efficacy/toxicity of platinum-based regimen [13,15,17,24]. **(13)** An enhanced tolerance to  
725 DNA damage and **(14)** the alteration of the apoptotic signaling pathway, especially **p53**  
726 mutation (p53mt), result in cell escape from apoptosis and acquired resistance [6,15]. **(b)**  
727 **Innovative synergies capable of detouring drug resistance mechanisms.** **(1)** Cisplatin is  
728 conventionally used in combination with radiotherapy (RT) in the treatment of various solid  
729 tumors as it enhances **dose deposition** [25,28,29]. RT can increase the cellular uptake of  
730 cisplatin and promote the activation of toxic Pt(II) complexes. Conversely, cisplatin may stop  
731 the cell cycle and inhibit the molecular repair machinery that tackles radiation-induced DNA  
732 damage [28]. **(2)** Interestingly, cisplatin was reported to decrease the MGMT activity whose  
733 expression counters temozolomide efficacy in glioma treatment [76]. **(3)** An alternative to  
734 reverse resistance induced detoxification processes consists in taking advantage of the  
735 elevated levels of GSH in resistant cancer cells to specifically damage them [6,78] or to  
736 inhibit the glutathione S-transferase (GST) [103]. **(4)** Combination with histone deacetylase  
737 inhibitors prevents histones from binding to DNA, leaving it more accessible to  
738 alkylation/complexation [104]. **(5)** Sensitivity can also be restored through epigenetic  
739 modulations involving miRNAs for permissive or synergistic effects [82–85]. **(c) Advantages**  
740 **of cisplatin nanovectorization over traditional regimens.** Multifunctional nanocarriers are  
741 developed and evaluated towards an optimized drug delivery to tumor cells for **(1)**  
742 locoregional confinement in specific environments and/or for selective targeting of receptors  
743 in relation with administration routes and modalities [87,94,95]. They can also be engineered  
744 by using radionuclides, MRI contrast agents or fluorophores to assess the patient response in  
745 real-time and adjust the treatment [116,118]. **(2)** Whereas free molecules individually enter  
746 the intracellular space by passive diffusion through the membrane or by transporters

747 mediation, endocytosis of nanosized DDS enables the internalization of the drug in a quantum  
 748 form [109]. Nanocarriers have been synthesized to bypass (3) endolysosomal degradation, (4)  
 749 detoxification processes and drug elimination by multidrug resistance efflux [94]. Besides,  
 750 tailored nanosystems can mediate (5) the rerouting or subcellular trafficking of the drug to  
 751 target specific organelles [96]. The high intracellular platinum accumulation favored by the  
 752 endocytic process is associated with an increased formation of DNA adducts and a markedly  
 753 enhanced antitumor activity whatever the resistance status of the cells [69,91,97,103,104].  
 754 The versatility of structure of smart DDS also allows for (6) a sustained drug release mediated  
 755 by environmental triggers in the intracellular compartment or in the extracellular space  
 756 [98,116]. (7) Drug combinations and synergies with alternative approaches such as adjuvant  
 757 radiation therapy or modulation of the expression of resistance signals through miRNA  
 758 agonist or antagonist strategies may reinforce the cytotoxicity of nanovectorized cisplatin.



759 Trends in Pharmacological Sciences

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

764 the cytosol [4]. (2) Nucleotides and nucleosides are characterized by a lower steric hindrance  
765 compared to their analogs involved in DNA that may result in easier interactions with Pt(II)  
766 complexes. (3) Kinetics considerations showed faster and higher complexation rates of  
767 cisplatin with RNA than DNA *in vitro*, even though resulting in less stable adducts. Cross-  
768 linking with mRNA was reported to inhibit translation *in vitro*. Interactions with non-coding  
769 RNA may impair downstream cellular and molecular processes [4]. (4) Positively charged  
770 Pt(II) activated species were reported to accumulate within negatively charged mitochondria  
771 due to electrostatic interactions. There, cisplatin produces a significantly higher amount of  
772 adducts with mitochondrial DNA than with nuclear DNA, subsequently impairing response  
773 and clinical outcome of cancer patients [16,72,119]. Besides, since resistance to cisplatin is  
774 partly linked to an extensive repair of Pt-DNA adducts by the NER machinery, rerouting the  
775 drug towards mitochondria whose DNA lacks such repair mechanisms may overcome  
776 resistance and enhance therapeutic efficacy [96]. (5) Although the amount of Pt(II) complexes  
777 with hemoglobin that persist following an oxaliplatin-based treatment was correlated with an  
778 increased risk of disease progression in patients with colorectal cancer, the impact of cisplatin  
779 interactions with extracellular components has not been reported yet [120].

780

781 **Tables**782 **Table 1.** FDA-approved alkylating agents and affiliated compounds for anticancer therapy <sup>a</sup>.783 *Marketing authorization and clinical practice guidelines are likely to evolve over time and*  
784 *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 <sup>®</sup> )	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

785 <sup>a</sup> Abbreviations: IV, intravenous

786 **Glossary**

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

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

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

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

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

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

808 **Heat shock proteins (HSP):** produced by living organisms in response to a stress such as  
809 temperature or exposition to heavy metals, overexpressed in cisplatin resistant cells, HSP  
810 prevent proteins from impairment.

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

813 **Metallothioneins:** proteins constituted of high amounts of sulfur-rich amino acids, namely  
814 cysteine. Exhibiting high affinity to metals, they play a key role in drug detoxification.

815 **MGMT, 6-O-methylguanine-DNA methyltransferase:** enzyme involved in the repair of  
816 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”.