Antitumour metal compounds: more than theme and variations

Michael A. Jakupec,* Markus Galanski, Vladimir B. Arion, Christian G. Hartinger and Bernhard K. Keppler*

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Triggered by the resounding success of cisplatin, the past decades have seen tremendous efforts to produce clinically beneficial analogues. The recent achievement of oxaliplatin for the treatment of colon cancer should, however, not belie the imbalance between a plethora of investigated complexes and a very small number of clinically approved platinum drugs. Strategies opening up new avenues are increasingly being sought using complexes of metals other than platinum such as ruthenium or gallium. Based on the chemical differences between these metals, the spectrum of molecular mechanisms of action and potential indications can be broadened substantially. Other approaches focus on complexes with tumour-targeting properties, thereby maximizing the impact on cancer cells and minimizing the problem of adverse side effects, and complexes with biologically active ligands.

Introduction

For decades, research articles on the subject of tumour-inhibiting metal compounds have almost stereotypically begun with a reference to Barnett Rosenberg's serendipitous discovery of the antineoplastic properties of cisplatin,[1] which, apart from its activity in various tumour diseases, has remained the only antineoplastic agent with highly curative effects in a solid malignancy, *viz.* testicular cancer, since its first approval in 1978. However, this legitimate appreciation of a ground-breaking finding by an exemplary exploratory spirit, which indeed formed the historical point of origin for this field of research, has unfortunately raised the misconception now commonly encountered in the non-specialist public that antitumoral metal complexes can in some way invariably be regarded as analogues of cisplatin. We begin to realize that this notion has involuntarily become an impediment to the development of novel agents with unique modes of action having in common with cisplatin the mere presence of a metal centre, necessitating great efforts to convince those bearing responsibility for the clinical drug development process of the contrary. Ironically, we continue this critically observed practice even by arguing against the misconception provoked by it.

The increasing number of platinum complexes having failed the test of clinical evaluation,[2,3] most of which are indeed close cisplatin analogues, in the best case disparagingly classified as "me too" drugs, has contributed to this situation. Moreover, the plethora of compounds showing a certain cytotoxicity not paralleled by therapeutic efficacy *in vivo* prompted the National Cancer Institute (Bethesda, USA), the world's largest institution exploring anticancer activities of synthetic compounds and extracts from natural sources, to refuse to test new metal-containing compounds without a clear-cut rationale some years ago (in one breath with anthracyclins, camptothecins or taxanes, each class, unlike metal compounds, indeed consisting of derivatives of a single lead structure). This is all the more remarkable as the procedures applied have actually been designed for indiscriminate

Institute of Inorganic Chemistry, University of Vienna, Waehringer Strasse 42, 1090 Vienna, Austria. E-mail: michael.jakupec@univie.ac.at, bernhard.keppler@univie.ac.at

Michael A. Jakupec

Michael A. Jakupec graduated as a Master of Science in biology from the University of Vienna in 1998 and as a Doctor of Science in bioinorganic chemistry from the same university in 2004. He joined the group of Bernhard Keppler as a research assistant in 1998 and has led the cell biological laboratory at the Institute of Inorganic Chemistry since 2000.

Bernhard K. Keppler

Bernhard K. Keppler received his diploma and Ph.D. in chemistry from the University of Heidelberg in 1979 and 1981, respectively, and a Ph.D. in medicine from the German Cancer Research Center of Heidelberg in 1986. He habilitated and gained the qualification of a university lecturer in inorganic chemistry at the University of Heidelberg in 1990. In 1995, he joined the Institute of Inorganic Chemistry at the University of Vienna as a full professor. He is Head of the Institute of Inorganic Chemistry and Vicedean of the Faculty of Chemistry at the University of Vienna.

bulk screening. As a matter of fact (not restricted to platinum compounds), the yield of approved anticancer drugs as compared to the number of compounds studied in high-throughput cell line screens is not impressive. Not detracting from the merits to lead structure identification in general, it should be thought-provoking that carboplatin, despite being listed in the standard agent database and being, in fact, one of the platinum drugs firmly established in clinical cancer therapy today, has a cytotoxic potency which is too low to meet the requirements for classification as an active compound in the cell line screen of this institution. (The beneficial therapeutic effects of carboplatin, which is tremendously less potent than cisplatin *in vitro* but which is much more tolerable *in vivo*, have been recognised prior to the onset of routine cytotoxicity screens.) The consequences of this dilemma are double-edged: On the one hand, it calls for a more rational drug design, and on the other hand it suggests reconsidering the prevalent drug evaluation strategies.

Cisplatin, carboplatin and oxaliplatin (Fig. 1) are at present the only metal-based anticancer agents in worldwide clinical use. The metal complexes are used in about 50% of all tumour therapies and display a remarkable therapeutic activity in a series of solid tumours. Nevertheless, severe dose-limiting side effects and intrinsic or acquired resistance are the main drawbacks associated with this kind of therapy. Nearly 40 platinum complexes have been investigated in clinical trials up to now. Of these, satraplatin, an octahedral platinum(IV) complex, is at present the most interesting candidate in an advanced clinical stage. Contrary to the clinically established square-planar platinum(II) complexes, which are administered intravenously, satraplatin can be applied orally due to its kinetic inertness.

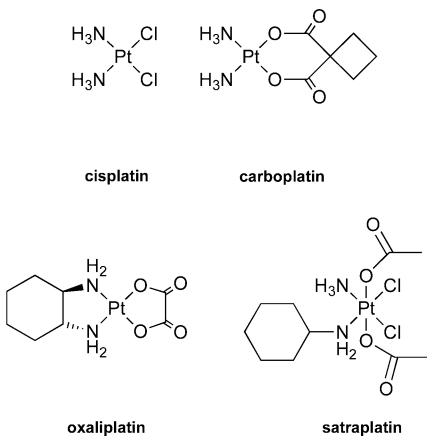


Fig. 1 Clinically established anticancer platinum(II) complexes (cisplatin, carboplatin and oxaliplatin) and satraplatin, at present the most promising candidate in phase III clinical trials.

During the last four decades, immense efforts have been undertaken in order to synthesise novel and innovative platinum anticancer drugs and to shed more light onto their mechanism of action. A series of reviews,[3,4,5,6] comprehensive articles in books[7,8,9] and a journal issue[10] have recently been published, giving an in-depth overview of the current developments in this very prolific field of research. Hitherto, thousands of platinum complexes have been synthesised and investigated in preclinical settings *in vitro* and *in vivo*. The chance to find active platinum complexes with a simple set of ligands and better therapeutic properties in comparison to cisplatin, carboplatin and oxaliplatin has nearly vanished. Consequently, two main routes build the basis for the recent platinum-based drug discovery process: (i) synthesis of platinum complexes containing ligands with the specific function of improving tumour selectivity; and (ii) development of non-classic platinum complexes clearly violating the classic structure–activity relationships.

While non-classic platinum complexes are increasingly being developed because they do not mimic cisplatin in their modes of action, metals other than platinum inherently have more or less proper preconditions for this purpose. Differences in coordination geometry, binding preferences according to the HSAB (hard and soft acids and bases) principle, electrovalency and redox activity, kinetics of ligand exchange reactions, or even the simple capacity of replacement of essential metals form the chemical basis for a diversity of pharmacologically relevant interactions with biomolecules. The antineoplastic potential of compounds of several other metals such as gallium, ruthenium and titanium was recognised more than two decades ago, but the stimulus for the development of non-platinum metal compounds was not nearly as huge as the impact cisplatin had on the platinum field of research. Although the mechanisms by which these compounds exert their effects are still not completely understood, present knowledge of their transport into tumour cells, their molecular targets and downstream effects as well as early clinical experience (in particular with the ruthenium complex KP1019 and the gallium complex KP46) now strongly argue for discrete pharmaceutical potentials distinct from those of platinum complexes. The state of knowledge summarized in the following sections should encourage more intense developmental activities, in order to fully exploit these obviously great potentials for tumour therapy.

The growing field of metal complexes with biologically active ligands deserves a separate section because of the different rationales involved. In these cases, the activity of the metal centre is usually subordinate to the activity of the ligands, and the metal ions rather serve to modulate this activity or make potent organic ligands applicable that are not particularly suited for drug development in an uncoordinated form for various reasons. Although evidence for clear-cut advantages *in vivo* is still missing and the products of this approach are still far from entering the clinical stage of development, the potential of this strategy should not be underestimated.

Ruthenium complexes

The advantages of utilising ruthenium in the development of metal-based antitumour drugs have been considered in a number of excellent reviews.[11,12,13] Briefly, the benefits of exploiting ruthenium include: (i) a well developed preparative coordination chemistry of this transition metal, providing reliable routes to novel compounds; (ii) a rate of ligand exchange often comparable to that of platinum or which can be tuned by coordination of appropriate ancillary ligands; (iii) octahedral coordination geometry in contrast to the square-planar geometry of platinum(II) complexes, implying a reactivity and mode of function different from cisplatin; (iv) accessibility of oxidation states 2+, 3+ and 4+ under physiological conditions and the ability to tune the electron transfer rates and redox potentials; (v) the ability of ruthenium to mimic iron in binding to biomolecules such as human serum

transferrin and other proteins, which makes ruthenium-based agents markedly less toxic than platinum drugs; and (vi) increasing knowledge about the biological effects of ruthenium complexes.

Ruthenium-based drugs are much less toxic than the worldwide approved platinum-based drugs. This can at least in part be explained by the ability of ruthenium to mimic iron in binding to biological molecules, such as human serum albumin and transferrin.[14] These are present in human serum at concentrations of 35–50 mg ml^{-1} and 2.5–3.5 mg ml^{-1}, correspondingly.[15,16] Platinum(II)-based antitumour agents are also capable of binding to these proteins; their coordination geometry (square-planar) is, however, distinct from that of ruthenium(III) or iron(III) (octahedral). This difference between platinum(II) and ruthenium(III) makes the delivery of platinum(II)-based drugs into cells *via* transferrin receptor-mediated endocytosis at least less likely. The "activation-by-reduction" mechanism could also be responsible for the lower general toxicity of some ruthenium-containing agents.[17,18] This mechanism, proposed about three decades ago,[19] is supposed to be operative in solid tumours with low oxygen level as compared to the normoxic tissue, enabling the reduction of ruthenium(III) to the kinetically more reactive ruthenium(II) species. The reductive microenvironment arises in rapidly growing tumours because of insufficient formation of new blood vessels and poor blood supply. Tumour hypoxia is a main factor contributing to failure of radiotherapy or chemotherapy.[20,21] The low oxygen content, together with the lower extracellular pH and the presence of appreciable amounts of cellular reducing agents such as glutathione provide favourable conditions for a selective reduction of drugs with the physiologically accessible Ru^{III}/Ru^{II} redox potential. The reducing capability of ruthenium(III)-based drugs depends on their ligand environment. The knowledge of the net electron donation from ligands to metal enables the prediction of metal-centred redox potentials and creation of drugs with desired redox parameters.[13,22,23] However, the ruthenium(III) prodrug can undergo hydrolysis or protein binding prior to reduction, changing the redox properties of the resulting metabolite significantly. In particular, $[Ru^{III}Cl_3(H_2O)(1H\text{-indazole})_2]$ ($E_{1/2} = -0.16$ V *vs.* NHE) is significantly easier to reduce than $[Ru^{III}Cl_4(1H\text{-indazole})_2]^-$ ($E_{p/2} = -0.43$ V *vs.* NHE).[13]

Since all major genes involved in iron metabolism respond to oxygen depletion, it is tempting to assume that hypoxia generally sensitises tumour cells to compounds interfering with iron-dependent processes. In particular, hypoxia has been shown to induce elevated transferrin receptor expression in tumour cells,[24] suggesting that hypoxia-activated prodrugs capable of transferrin-mediated cellular uptake attain their tumour selectivity not only by a dual, but a cooperative mechanism. The "activation-by-reduction" hypothesis implies that the compound should be much more readily reducible under the hypoxic conditions of solid tumours than under the normoxic conditions of normal tissues, in order to obtain a tumour-selective activity and high therapeutic index. For ruthenium(III) compounds, an appropriate redox potential is therefore probably a more important parameter than cytotoxic potency under normal conditions. Paradox as it might seem, a high cytotoxicity must be considered disadvantageous in this case, because it reflects an easy reducibility under normoxic conditions.[17]

All the clinically established platinum drugs as well as the ruthenium-based drug candidate KP1019, indazolium [*trans*-tetrachlorobis(1H-indazole)ruthenate(III)] (Fig. 2), are administered intravenously and, therefore, proteins are among the first available binding partners in the blood stream. The role of protein binding of metallodrugs has not been clarified unambiguously. The binding of platinum complexes to serum proteins is thought to contribute to the side effects, while the binding of KP1019 to transferrin seems to be an important step in the mode of action.[25] Considering the enhanced permeability and retention (EPR) effect and the higher expression of transferrin receptors on tumour cells, binding of drugs to serum proteins appears promising as a targeting concept.

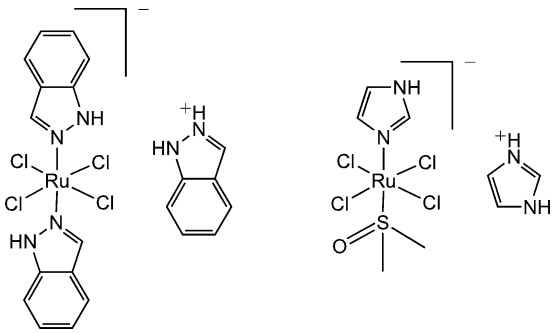


Fig. 2 Structures of the investigational ruthenium drugs KP1019 (left) and NAMI-A (right).

In the last few years, many analytical methods, *e.g.*, capillary electrophoresis and high performance liquid chromatography, both offline and online coupled to molecular and elemental mass spectrometric detectors, nuclear magnetic resonance, X-ray diffraction, infrared, circular dichroism and UV/Vis spectroscopy, *etc.*, have been applied to the characterisation of drug–protein systems in terms of binding and rate constants, metallation stoichiometry and coordination sites. Human serum albumin and transferrin as the most important transport proteins in the blood were extensively exploited for studies as potential target molecules for a variety of metal complexes.[25] In general, the metal complexes were found to bind with lower binding constants to these proteins than, for example, organic drugs do. For the drug candidate KP1019, the attachment to transferrin and its effect on the mode of action is well characterised (Fig. 3).[26] The protein was shown to preferentially bind two ruthenium moieties to the iron binding sites which most probably influence the protein's structure.[14,27] Notably, the reaction of KP1019 with transferrin is slightly faster than with human serum albumin. The structural change imposed by attaching two ruthenium moieties to transferrin probably prevents the protein from binding to its receptor, reflected in a lower accumulation of ruthenium in the cell. However, loading transferrin with physiologically normal amounts of iron prior to binding of one ruthenium unit led to a markedly increased cellular uptake.[14] The release from the protein is thought to take place in the endosomes at a lower pH in the presence of biological chelators.[28]

In an initial, simplified assumption of analogy with platinum drugs, DNA has been considered the critical target of KP1019, and binding to nucleotides and DNA has therefore been investigated in various studies.[18,26,29,30,31] The sensitisation of cells to the sodium salt analogue KP1339 by inhibitors of DNA repair seems to argue for this assumption, but since the pattern of sensitisation differs

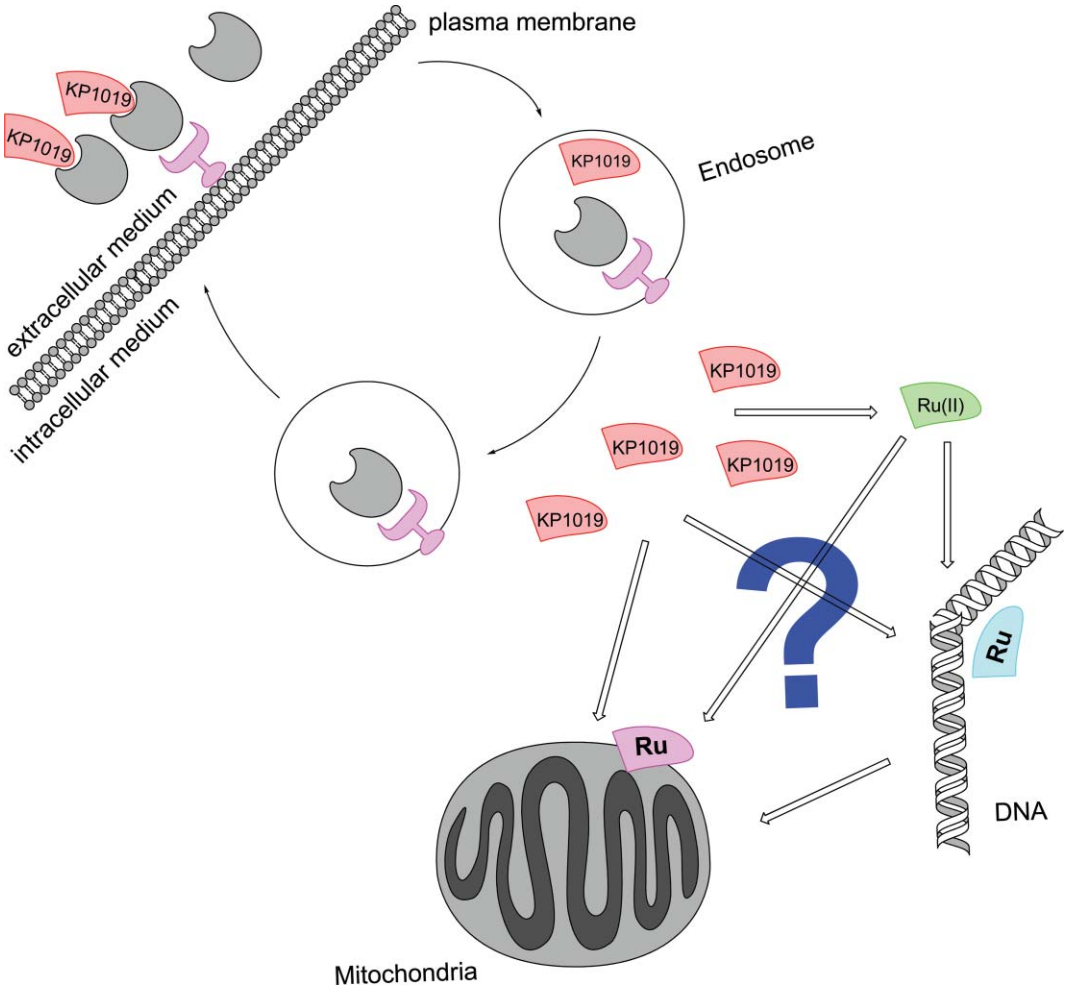


Fig. 3 Schematic representation of the mode of action of KP1019.

from that of cisplatin, cytotoxic DNA lesions are probably processed differently by the cell.[26] The induction of apoptosis in cancer cells by the intrinsic mitochondrial pathway[32] does not exclude the possibility that DNA binding triggers the apoptotic process, but the comparatively rapid onset of membrane depolarisation in mitochondria suggests that a direct interaction with mitochondria might be involved in the mechanism of action of KP1019. Taking into account the similarities between ruthenium and iron, it is tempting to assume an interference with iron-dependent metabolic processes. In this context, the capacity to bind to cytochrome c is noteworthy. Although a direct interaction with the heme group has not been reported, binding to this protein results in marked conformational changes in the heme environment which might affect biological functionality.[33]

Despite its moderate cytotoxicity, KP1019 exerts impressive effects in non-toxic doses in a variety of tumour models, probably reflecting a high degree of tumour selectivity mediated by the mechanisms mentioned above. The predictive power of the chosen models, in particular chemically induced autochthonous colorectal carcinoma in the rat, which responded with a complete remission of one third of tumours,[34] and primary cultures of human tumour cells, a high proportion of which (including clinically chemoresistant specimens and cells from metastatic lesions) proved sensitive to this compound,[35] excites confidence that a therapeutic benefit will be confirmed by further clinical studies. The phase I dose escalation study conducted already in patients with advanced solid tumours yielded encouraging results with disease stabilisations in five of six evaluable patients despite the fact that the majority of patients are not treated with the therapeutically optimal dose in this type of study.[36]

Another ruthenium complex under clinical investigation, NAMI-A, imidazolium [*trans*-tetrachloro(dimethylsulfoxide)-imidazoleruthenate(III)] (Fig. 2),[37] exhibits, despite its structural relationship to KP1019, a quite different biological activity. This compound reduces the formation of metastases and appears to inhibit their growth as a result of a delayed process of metastasis, but has little impact on primary tumours in animal models.[38] NAMI-A interferes with the interactions of tumour cells with the extracellular matrix, including an increase of actin-dependent cell adhesion,[39,40] inhibition of matrix degradation by matrix metalloproteinases,[41] and reduction of cell invasiveness and migration,[38,41] resulting in a less malignant cell phenotype. A contribution of antiangiogenic effects to the antimetastatic properties has also been suggested,[42] whereas the low capacity of DNA binding[43] is unlikely to account for the antimetastatic activity.

The unique properties of NAMI-A imply that its effects are mainly directed against the process of metastasis, while its inhibitory effects on established tumour lesions are much less pronounced, corresponding to its negligible cytotoxicity. Whether these properties are sufficient for a significant clinical benefit remains to be seen. Clinical experience suggests that established metastases can only be treated effectively with compounds that have the capacity to exert their effects on the primary tumour as well, comprehensible from the common tissue characteristics shared by primary and secondary tumours.

Aside from Ru(III) compounds in clinical trials, organometallic Ru(II) complexes have attracted interest in recent years, some of which show similar antimetastatic activity to NAMI-A, *i.e.*, RAPTA compounds,[44] and others were proven to exert their activity by a DNA binding mechanism.[45] Furthermore, the disadvantage of the small therapeutic window of multinuclear platinum complexes (see section "Non-classic platinum complexes") might be overcome by using multinuclear ruthenium (or other non-platinum) complexes, since ruthenium complexes are usually less toxic than platinum compounds.

Gallium complexes

Gallium(III) displays coordination characteristics similar to other group IIIa metal ions, *e.g.* Al^{3+} and In^{3+}, but also, in particular, to the group VIII metal ion iron(III). Mutually shared physicochemical characteristics with iron(III) include ionic radius, electronegativity, electron affinity, ligand affinity and coordination geometry.[46] The gallium(III) octahedral ionic radius of 0.62 Å is comparable with that of high-spin octahedral iron(III) (0.645 Å). Both Ga^{3+} and Fe^{3+}, as hard Lewis acids, show strong affinity toward hard and border-line Lewis bases and, in particular, to oxygen and nitrogen donors.[47] Therefore, gallium(III) is believed to follow biochemical pathways similar to those found in iron metabolism. However, it is the difference between these two metal ions, which enables the utilisation of gallium(III) as a therapeutic agent. Gallium(III) possesses a stable outer electronic configuration (d^{10}) resulting from a loss of two 4s electrons and one 4p electron. The oxidation state 2+ is energetically unfavourable, compared to 1+, which is found in a number of gallium compounds. However, the oxidation state 1+ can not be reached easily. Hence, gallium(III) is considered to be redox-inactive under physiological conditions. This property prevents the insertion of this metal ion into proteins involved in oxygen transport and the participation in other redox processes of biological relevance.[48] However, gallium is able to bind to proteins that require the trivalent form of iron, therefore perturbing the normal cellular homeostasis. In this context, it is worth mentioning that the availability of Ga^{3+} ions at pH 7.4 is higher than that of Fe^{3+}, considering that the solubility of Ga^{3+} is 1 μM, compared to $\sim 10^{-18}$ M of Fe^{3+} which tends to form insoluble polymers of the composition FeO(OH).

Remarkably, gallium exerts antineoplastic effects in the form of simple salts such as gallium nitrate, and the interference with cellular iron metabolism seems to be crucial for this activity.[46,49,50,51] In particular, gallium affects cellular acquisition of iron by a competitive binding to transferrin, which mediates a large proportion of cellular gallium uptake,[52] and by inhibitory effects on acidification of endosomes which is essential for the intracellular release of iron from transferrin.[53] Tumour hypoxia has been shown to conduce to cellular gallium accumulation by its stimulating effect on transferrin receptor expression.[54] Apart from transferrin-mediated uptake, gallium does not strictly follow the routes of cellular iron trafficking. Since transport of iron from endosomes to the cytosol and its deposition in the iron storage protein ferritin involves reduction and subsequent re-oxidation, the mechanisms of intracellular gallium transport probably differ. In fact, gallium is incorporated into ferritin to a much lesser extent than iron and mainly present in the form of a labile pool (*i.e.* chelatable by desferrioxamine).[55]

It is generally agreed that the critical cellular target of gallium is the enzyme ribonucleotide reductase, which catalyses the reduction of ribonucleotides to deoxyribonucleotides required for DNA synthesis and has long been recognised as a suitable target for cancer chemotherapy. The activity of this enzyme is inhibited by binding of gallium to the iron site of the R2 subunit and the resulting destabilisation of the tyrosyl radical essential for enzymatic activity.[56] This results in the depletion of dNTP pools, impaired DNA synthesis, cell cycle perturbations[48] and apoptosis through the mitochondrial pathway involving activation of the proapoptotic factor Bax and caspase-3 (Fig. 4).[57]

Furthermore, gene expression analyses of gallium nitrate-treated cells suggest that gallium interacts with cellular pathways involved in zinc metabolism, while zinc-induced metallothionein expression protects cells from the cytotoxicity of gallium.[58] Microtubule-disrupting effects suggest an antimitotic component of activity[59] and may explain the varying results of cell cycle analyses reported in the literature. On the other hand, direct interactions with nucleotides and DNA have been described only at high gallium-to-nucleotide ratios, making their relevance for antitumour activity doubtful.[50]

In addition, gallium nitrate is effective against hypercalcaemia of malignancy, a life-threatening complication frequently observed in several forms of advanced cancer, probably by inhibition of vacuolar-type proton-translocating ATPases (V-ATPases) which are responsible for the acidic secretion involved in the osteolytic activity of osteoclasts.[46] Life-prolonging effects were observed in patients with advanced multiple myeloma having received gallium nitrate for the attenuation of bone resorption.[60]

Gallium nitrate showed notable anticancer activity in phase II trials in lymphoma and bladder cancer.[61] However, the applicability of gallium nitrate as an anticancer drug was questioned by nephrotoxicity (in the case of short infusions) and occasional severe optical neuropathy (in the case of continuous infusions). An improved therapeutic index is expected to result from prolonged exposure to low steady-state plasma gallium concentrations, but attempts to accomplish this with oral administration of gallium chloride yielded unsatisfactory results because of insufficient bioavailability.[62] Therefore, the only approved application remains low-dose gallium nitrate for the treatment of cancer-related hypercalcaemia.

Alternatively, the complexation of gallium with suitable chelators has been pursued to stabilise gallium against hydrolysis, which is the major impediment for intestinal absorption, and to facilitate membrane permeation. Two of these complexes, namely tris(8-quinolinolato)gallium(III) (KP46) and tris(3-hydroxy-2-methyl-4H-pyran-4-onato)gallium(III) (gallium maltolate), are currently being evaluated in the clinical setting (Fig. 5). Both

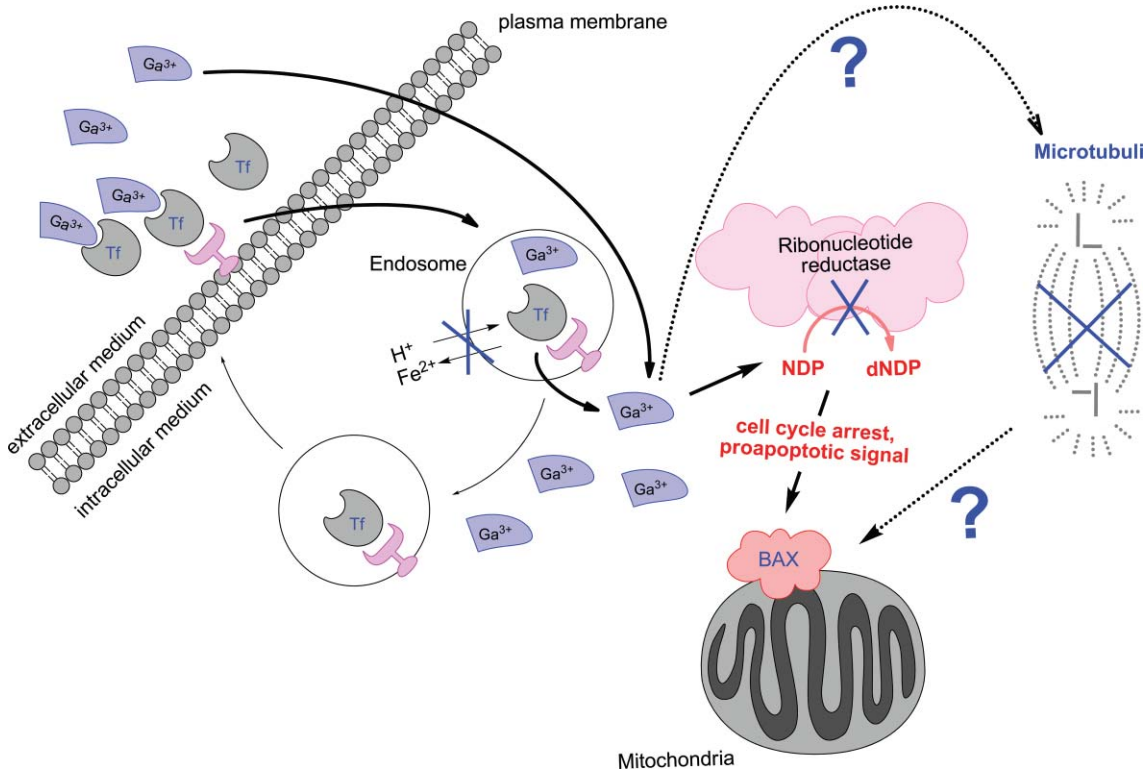


Fig. 4 Schematic representation of the mode of action of gallium compounds.

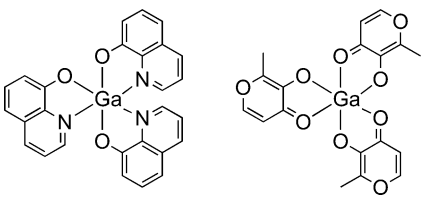


Fig. 5 Structures of the investigational gallium drugs KP46 (left) and gallium maltolate (right).

complexes show a high bioavailability in animal models when administered *via* the oral route[63,64] and are more potent inhibitors of tumour cell proliferation *in vitro* than gallium salts.[65,66] Given the differences in complex stability, it is unlikely that their pharmacological properties are equal. Moreover, the question whether the mode of action outlined in Fig. 4 applies to these compounds in the same manner has not been completely clarified yet.

No dose-limiting toxicities were encountered in phase I studies, neither with gallium maltolate nor with KP46,[67,68] reflecting the higher than expected tolerability of these compounds. Upon administration of oral gallium maltolate to patients, gallium is present in serum mainly in a transferrin-bound form.[69] For KP46, there is preliminary evidence for activity in renal cell carcinoma, with one partial response and two disease stabilisations for up to 11 months.[68] Clinical activity in this malignancy, which is otherwise largely chemoresistant probably due to effective detoxification mechanisms, is unprecedented in the development of anticancer metal compounds.

Platinum compounds with improved tumour selectivity

Selectivity for the tumour tissue and tumour cells can be reached by various strategies consequently focusing on the differences to normal tissues and cells. But which are the differences to be targeted? Growth of most solid tumours is comparatively fast. In parallel, angiogenesis, the formation of new blood vessels, proceeds in an uncontrolled manner. As a consequence, the vascular endothelium is defective with large gaps in endothelial cell–cell junctions. Besides, permeability mediators are over-expressed, whereas the lymphatic drainage is impaired. These unique pathophysiological characteristics lead to an enhanced permeability and retention of macromolecules in tumour tissue (EPR effect).[70] In order to exploit this effect, macromolecular constructs have been synthesised in the hope for an increased accumulation of cytotoxic platinum moieties at the tumour site. In this context, diammineplatinum(II) and (*trans*-cyclohexane-1,2-diamine)platinum(II) fragments were coordinated to *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymers, resulting in AP5280 and AP5346, respectively, which were already investigated in patients.[71,72] Also, liposomal formulations of cisplatin (lipoplatin)[73,74] and oxaliplatin (lipoxal)[75] recently found their way into clinical evaluation. Additionally, micelles built up of poly(ethylene glycol)–poly(aspartatic acid), or poly(ethylene glycol)–poly(glutamic acid) block copolymers with incorporated diammineplatinum(II) or (*trans*-cyclohexane-1,2-diamine)platinum(II) moieties have been developed,[76,77] showing a significantly higher accumulation in solid tumours in comparison to cisplatin. The prototypical polymeric drug carrier poly(ethylene glycol) also shows an enhanced accumulation in tumours due to the EPR effect. Logically, pegylated (*trans*-cyclohexane-1,2-diamine)platinum(II) complexes were synthesised and investigated

with respect to their cytotoxic potential.[78] A further strategy trusts in the use of low-molecular-weight platinum complexes with the capability to bind very selectively to proteins.[79] For that purpose, a maleimido residue was coordinated to the platinum(II) centre *via* a linker which reacts very efficiently with thiol groups (*e.g.*, cysteine-34 in albumin).

The classic approach to target antineoplastic drugs *via* an organ- or receptor-specific carrier-mediated transport is also extensively being applied in the field of platinum-based anticancer complexes. In this context, the liver is an attractive target, since on the one hand liver parenchymal cells exclusively express high levels of galactose receptors and on the other hand bile acids synthesised in the liver are excreted to a small extent because of a very efficient re-uptake by ileal cells and hepatocytes. Therefore, platinum complexes with branched and unbranched galactose units[80,81] as well as platinum compounds containing bile acids[82,83,84] as part of the coordination sphere have been synthesised and tested with respect to their cytotoxic properties *in vitro* and *in vivo*. Furthermore, phosphonatoplatinum(II) complexes displaying a high affinity to bone tissue are of high interest for treatment of bone tumours and bone metastases (about 50% of all tumours have metastases located in the bone tissue) and were subject of intensive investigations.[85,86,87] Remarkably, lethal ossifying lung metastases derived from a bone tumour could also be treated efficiently in animal experiments.[88] In line with this concept, platinum(II) complexes with coordinated diethyl [(methylsulfinyl)methyl]phosphonate were developed.[89,90] Remarkably, as known for classic bis(phosphonates), inhibition of matrix metalloproteinases was reported.[91] Active targeting of receptors over-expressed in some kinds of tumours is a promising strategy in the fight against cancer. Breast, endometrial and prostate tumours have a high steroid hormone receptor status, whereas ovarian and endometrial cancers have a high affinity for folic acid, whereas overexpression of the peripheral benzodiazepine receptor is known in many tumour types. Consequently, a series of complexes with non-steroidal estrogens, antioestrogens, steroid derivatives,[92,93,94,95] platinum prodrugs exhibiting folate[96] or the peripheral benzodiazepine receptor ligand TZ6[97] were prepared and tested for their biological activity.

Despite an extensive angiogenesis, the blood supply in solid tumours is insufficient. As one consequence, the oxygen concentration is decreasing with increasing tumour size, resulting in a hypoxic milieu. Compounds, which can be reduced under such conditions, will show selectivity for the tumour tissue (activation by reduction). In platinum-based chemistry, octahedrally coordinated platinum(IV) complexes can be reduced to the corresponding square-planar platinum(II) complexes under release of the axial ligands, therefore acting as prodrugs.[98] The most interesting candidate at present, being in phase III clinical trials, is satraplatin (Fig. 1),[99] a platinum(IV) complex active in hormone-refractory prostate cancer. A second consequence of an insufficient supply of rapidly growing tumours with oxygen is their anaerobic metabolism resulting in an increased intra- and especially extracellular proton concentration. Extracellular pH values as low as 5.5 have been reported, offering a further possibility to activate platinum complexes. Two classes of platinum agents showing an enhanced cytotoxicity under slightly acidic conditions are known: bis(*O*-alkyldithiocarbonato)platinum(II)[100] and bis(2-aminoalcoholato-$\kappa^2 N,O$)platinum(II) complexes.[101,102]

Non-classic platinum complexes

Cleare and Hoeschele investigated a series of platinum complexes and published, two years after the first patient had been treated with cisplatin in a clinical trial, structure–activity relationships for platinum-based agents:[103] (i) the platinum complexes should contain two (or one bidentate) labile leaving ligands; (ii) two more kinetically (or one bidentate) inert am(m)ine ligands should be coordinated to the platinum(II) centre; and (iii) the complexes should be neutral and should have a *cis* configuration. In other words, *e.g.*, *trans* configured platinum(II) complexes should be equipped with a low anticancer potential or be inactive at all.

The main target of platinum complexes is nuclear DNA. Consequently, one strategy in anticancer chemotherapy trusts in the design of complexes forming unprecedented DNA adducts, which are processed differently by the cellular machinery in comparison to those derived from cisplatin, carboplatin and oxaliplatin. Non-classic platinum complexes *per se* inherently fulfil these requirements. Three main classes of non-classic platinum-based agents are known: (i) complexes with *trans* geometry; (ii) multinuclear platinum complexes; and (iii) complexes with intercalating properties, but exhibiting a non-classic coordination sphere at the platinum centre (Fig. 6).

The prototype of a non-classic platinum complex is transplatin (Fig. 6, **I**), being devoid of anticancer activity. Therefore, it is even more remarkable that transplatin attains cytotoxic properties comparable to cisplatin when treated cells are irradiated with UVA light. This photo-activation results in interstrand and DNA-protein cross-links and was published very recently by Sadler *et al.*[104] Transplatin analogues with iminoether ligands (Fig. 6, **II**) have been investigated in detail *in vitro* and *in vivo*[105] and were further developed to complexes with cyclic 3,4-dihydro-5-methoxypyrrole or 4,5-dihydro-2-methyloxazole ligands mimicking the iminoethers but avoiding *Z*/*E* isomerisation.[106] The formation of stable monofunctional adducts on DNA and the high propensity for forming DNA-protein cross-links clearly distinguishes these compounds from classic platinum drugs.[107] Additionally, *trans* configured complexes with mixed aliphatic amine ligands (*e.g.*, **III**, Fig. 6),[108] acetimines[109] and cycloaliphatic amines[110] are currently under investigation. While the active *trans* complexes recognised so far are not more or only marginally more cytotoxic than their *cis* congeners, we have recently identified a pair of complexes with two acetoxime ligands as the first example for a marked reversion of structure–activity relationships. The hydroxyl functions of the acetoxime ligands renders these compounds stronger H-bonding donors, which might be relevant for stabilising monofunctional DNA adducts.[111]

Cross-linking of DNA nucleobases over a longer distance can be accomplished by multinuclear platinum complexes with two binding sites separated by a linker of variable length. Complex **V** (BBR3464) (Fig. 6) is the most thoroughly investigated compound of this type.[112] Interestingly, the central tetramineplatinum(II) unit plays a crucial role, although it is unable to bind to DNA in a coordinative manner. It is positively charged and interacts with the negatively charged DNA *via* preassociation.[113] Very recently, a cytotoxic trinuclear complex with three tetramineplatinum(II) moieties, TriplatinNC, which is not at all capable of binding to DNA in a coordinative manner, was also reported.[114] In contrast, the dinuclear platinum complex **IV** (Fig. 6) cross-links

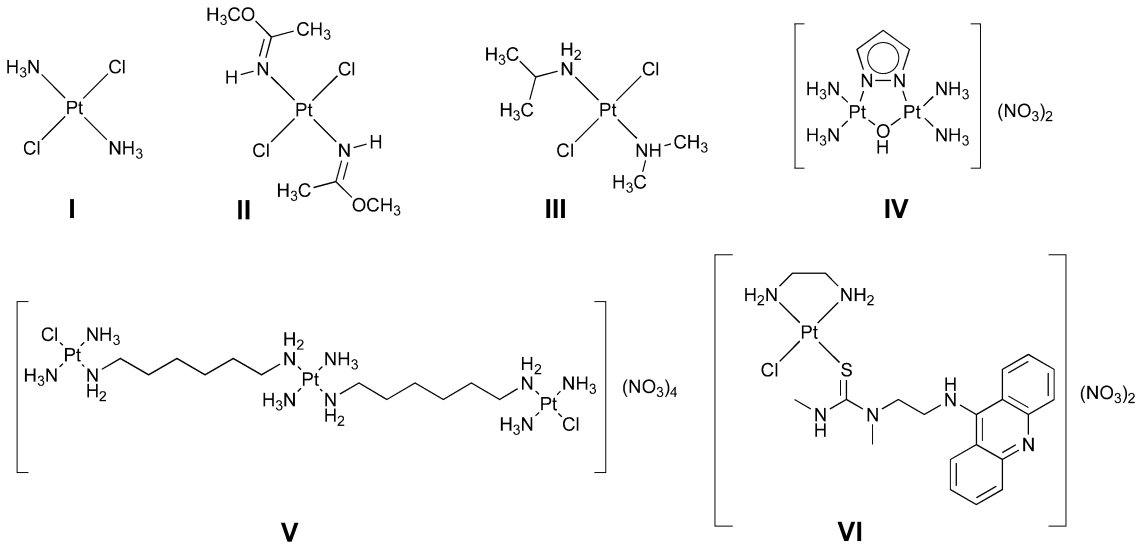


Fig. 6 Representatives of non-classic platinum complexes with *trans* geometry (**I–III**), multinuclear platinum complexes (**IV** and **V**) and complexes with intercalating properties (**VI**) exhibiting a non-classic coordination sphere at the platinum centre.

two adjacent guanines like cisplatin but without changing the directionality of the helix axis.[115] A new series of platinum-acridine complexes (*e.g.*, **VI**, Fig. 6) displaying unprecedented platinum–DNA interactions has recently been developed.[116] In contrast to the majority of platinum complexes, they bind to N3 of adenine in the minor groove based on an intercalator-mediated minor-groove association at adenine-containing base pair steps.

BBR3464 (**V**) has been claimed as the first platinum compound with a DNA binding mode fundamentally different from cisplatin to be studied in clinical trials. However, phase II studies yielded only sporadic responses in patients with non-small and small cell lung cancer,[117,118] ovarian cancer[119] and gastric or gastro-oesophageal cancer.[120] Notably, the maximum tolerated dose of BBR3464 (0.9 mg m^{-2} every three weeks or 1.1 mg m^{-2}) is manifold lower than that of cisplatin. Severe, dose-limiting gastro-intestinal and haematological side effects render the confirmation of the activity profile, expected according to preclinical experience to extend to cisplatin-resistant tumours, impossible. Thus, the high cytotoxic potency of BBR3464 (one to two orders of magnitude higher than that of cisplatin) is accompanied by a narrow therapeutic range, which was not predicted by animal studies. On the contrary, all ten investigated xenograft models responded at least to some degree to the maximum tolerated dose of this compound.[121] Irrespective of pharmacokinetic differences in mice and humans, one must be aware that the definitions of both tolerability and efficacy commonly applied in therapeutic experiments in animals deviate from the clinical setting. Since a thorough monitoring of side effects is hardly practicable, changes in body weight are usually taken as the principal indicator for toxicity. In fact, higher toxicities are accepted, as reflected by the definition of the maximum tolerated dose, allowing $\leq$15% body weight loss and $\leq$10% lethality, which may imply a burden of side effects not tolerated in patients. On the other hand, a statistically significant inhibition/deceleration of tumour growth, which is usually taken as sufficient for efficacy in animals, must not be mistaken for response in the clinical sense of the term, which requires a reduction of the size of measurable tumour lesions (the extent depending on the criteria applied)—a distinction justifiable by the fast tumour growth in animal models.

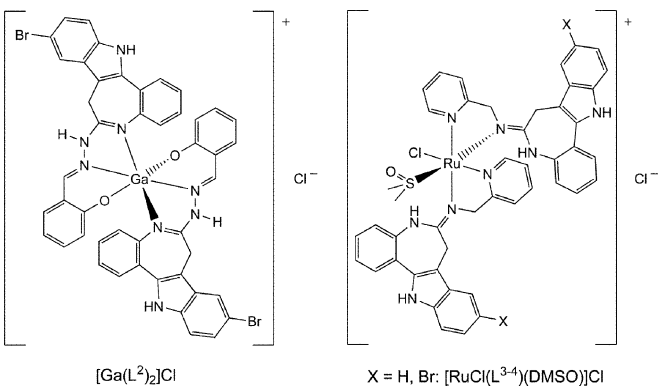
Metal complexes with biologically active ligands

The search for tumour-inhibiting compounds through the coordination of biologically active ligands capable of exerting their own antineoplastic effects to metal scaffolds is an interesting and promising field of research.[11,122,123] Favourable effects upon complexation include: (i) the stabilisation of certain, sometimes unusual ligand geometries; (ii) acquired redox-activity; (iii) increased solubility; (iv) enhanced cellular uptake; (v) different modes of action; and (vi) synergistic effects from metal and ligand(s).[124–132] We focus our attention on different classes of organic ligands which exhibit high antiproliferative activity *in vitro*, and in particular on thiosemicarbazones, which are the strongest known inhibitors of ribonucleotide reductase (RR), both in cell-free assays and in intact tumour cells,[133,134] and on paullone derivatives, some of which were found to show an *in vitro* activity similar to flavopiridol, a well-known inhibitor of cyclin-dependent kinases (CDKs).[135,136]

The enzyme RR which catalyses the conversion of ribonucleotides to deoxyribonucleotides is produced at the transition from the G_1 to the S phase of the cell cycle as a prerequisite for DNA replication and is highly expressed in tumour cells, making it a suitable and well-established target for cancer therapy.[44] The combination of ^{4}N-substituted α-N-heterocyclic thiosemicarbazones with a gallium(III) ion, which is also a well-known inhibitor of the enzyme RR, resulted in a series of highly potent antiproliferative complexes.[137,138] Although the pharmacological properties of these complexes must be primarily attributed to the thiosemicarbazone ligands, gallium(III) unequivocally and specifically increases their cytotoxic potency. In contrast, coordination to iron(III) impairs the biological activity of these thiosemicarbazones. From an *in vitro* point of view, coordination to metals only modulates the activity to a certain degree, but the impact on pharmacokinetic behaviour and biodistribution might still be appreciable.

Although slow tyrosyl radical (Y*) quenching on the hour timescale has been found in a dithiothreitol-containing solution of the R2 subunit of mouse RR without the thiosemicarbazone ligand, the much faster reaction on the minute timescale in the presence of the ligand clearly showed that the Y* in mammalian R2 protein is a direct and preferred target of α-N-heterocyclic thiosemicarbazones under slightly reducing conditions. The reversed order of cytotoxic activity $[Ga(L^1)_2][PF_6]$ > HL^1 > $[Fe(L^1)_2][PF_6]$ as compared to Y* quenching kinetics $[Fe(L^1)_2][PF_6]$ > HL^1 > $[Ga(L^1)_2][PF_6]$ (HL^1 = 2-acetylpyridine *N*,*N*-dimethylthiosemicarbazone) displayed the difference between a complex whole cell and a purified protein solution. In addition, target(s) other than RR are imaginable for this solely quantitative difference.

Although a large number of kenpaullone derivatives have been documented in the literature,[135,136] the majority of them are characterised by low aqueous solubility and bioavailability, making their application impossible. The effect of metallation is being explored to overcome existing limitations and to improve pharmacokinetic and pharmacodynamic properties. By chemical modifications of the thiolactam moiety in kenpaullone, two types of ligands were synthesised, one containing a tridentate binding site for gallium(III)[139] and the other with an N,N-chelating moiety able to bind ruthenium(II).[140] The complexes $[Ga(L^2)_2]Cl$ and $[RuCl(L^{3-4})(DMSO)]Cl$ (Fig. 7) show remarkable cytotoxicities *in vitro*. The first was found to be 1.5- to 18-fold more cytotoxic than HL^2. However, the limited water solubility along with low general hydrolytic stability of the complex impeded further development of this antiproliferative agent. The second type of complex is significantly more resistant to hydrolysis, with aqueous solubility of the complex with HL^3 higher than of that with HL^4. The IC_{50} values of $[RuCl(L^3)(DMSO)]Cl$ in three different cell lines (A549, CH1 and SW480) ranged from 2.5 to >23 μM. The kinetic inertness of the complex toward hydrolysis of the Ru–Cl bond and binding to GMP, along with fainting of ethidium bromide staining of the plasmid pTZ18u, provide evidence that the species responsible for cytotoxicity is the intact monocation intercalating into DNA.



$[Ga(L^2)_2]Cl$ X = H, Br: $[RuCl(L^{3-4})(DMSO)]Cl$

Fig. 7 The first gallium(III) and ruthenium(II) paullone derivatives.

Concluding remarks

Various aspects of coordination chemistry as well as mechanistic studies point out that tumour-inhibiting metal complexes constitute a heterogeneous class of compounds with diverse mechanisms of action. Ruthenium and gallium compounds, selectively discussed here because of their high relevance as drug candidates, have little in common with the established platinum drugs, making comparisons with cisplatin and its analogues, albeit frequently drawn, misleading. In our opinion, this even applies to the non-classic platinum complexes which probably share with cisplatin DNA damage as the critical mechanism of action, but differ distinctly by the nature of the adducts produced.

A common practice in anticancer drug development is to modify lead structures in a way that maximises cytotoxic potency in cancer cells. This might be an appropriate procedure if chemically and pharmacologically very similar compounds are compared with each other. However, there are prominent examples for established and investigational drugs with low cytotoxicity and high tolerability which do not meet the frequently applied criteria for activity in cell line screens (*e.g.*, carboplatin, KP1019) as well as for highly potent compounds which did not come up to expectations in clinical studies because of a low therapeutic index (*e.g.*, BBR3464). Cytotoxicity in cancer cells should therefore always be viewed in relation to general toxicity and not be mistaken for anticancer activity. It is of utmost importance to critically assess in animal models the therapeutic window, which is actually decisive for the applicability and efficacy of a drug, already in an early stage of preclinical evaluation and not to prematurely discard compounds with a moderate activity *in vitro*. Assessing the preclinical performance and the chances for clinical success of an investigational drug requires a deeper understanding of the predictivity of the preclinical tumour models applied. *In vivo* tumour models closely resembling the clinical situation in terms of histology, orthotopic growth and chemosensitivity profile, such as chemically induced autochthonous tumours, probably enable the most reliable predictions.[141] In xenograft experiments with human tumours, orthotopic rather than subcutaneous transplantation is preferable from this point of view.[142] The Human Tumour Cloning Assay (HTCA) using primary human tumour cell cultures has proved of high predictive value for sensitivity and even more so for resistance, provided that clinically achievable plasma concentrations of the drug are known or can be estimated.[143] However, the laboriousness of these techniques makes them unsuitable for large numbers of test compounds and explains why they are not frequently applied in experimental cancer therapy.

The design of agents that are rather inert under the conditions prevailing in normal tissues but are accumulated and activated in the environment of solid tumours is considered the most promising strategy for the development of new metal-based antineoplastic agents. In this context, we have to be aware that this tumour environment does not reflect properly in cultures of tumour cells and can only be simulated imperfectly by special techniques. But even though the increased knowledge since the time of Rosenberg's discovery certainly forms a sounder basis for rational drug design, this should not go without the willingness to keep an open mind for less calculated or unconventional approaches.

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