Advantageous Reactivity of Unstable Metal Complexes: Potential Applications of Metal-Based Anticancer Drugs for Intratumoral Injections

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Abstract: Injections of highly cytotoxic or immunomodulating drugs directly into the inoperable tumor is a procedure that is increasingly applied in the clinic and uses established Pt-based drugs. It is advantageous for less stable anticancer metal complexes that fail administration by the standard intravenous route. Such hydrophobic metal-containing complexes are rapidly taken up into cancer cells and cause cell death, while the release of their relatively non-toxic decomposition products into the blood has low systemic toxicity and, in some cases, may even be beneficial. This concept was recently proposed for V(V) complexes with hydrophobic organic ligands, but it can potentially be applied to other metal complexes, such as Ti(IV), Ga(III) and Ru(III) complexes, some of which were previously unsuccessful in human clinical trials when administered via intravenous injections. The potential beneficial effects include antidiabetic, neuroprotective and tissue-regenerating activities for V(V/IV); antimicrobial activities for Ga(III); and antimetastatic and potentially immunogenic activities for Ru(III). Utilizing organic ligands with limited stability under biological conditions, such as Schiff bases, further enhances the tuning of the reactivities of the metal complexes under the conditions of intratumoral injections. However, nanocarrier formulations are likely to be required for the delivery of unstable metal complexes into the tumor.

Keywords: cancer; intratumoral injection; platinum; vanadium; iron; titanium; gallium; ruthenium; Schiff base; nanocarrier formulation

1. Introduction

The treatment of inoperable cancers, particularly those of the brain, head and neck, lung or pancreas, by direct injection of cytotoxic and/or immunomodulating drugs into the tumor is currently transitioning from experimental procedures to mainstream clinical practice [1–5]. Detailed clinical guidelines for intratumoral injections (ITI) have been outlined [6], and hundreds of clinical trials are either underway or have been completed [7]. The treatment of unresectable metastatic melanoma by ITI of an oncolytic virus (T-VEC) has been approved by the Food and Drug Administration (FDA) for human clinical use [8]. A related technique, convection enhanced delivery (CED), which is based on intracranial injections of chemotherapeutic drugs to overcome the blood-brain barrier, continues to be extensively trialed for the treatment of malignant gliomas [9–11]. Another related technique, pressurized intraperitoneal aerosolized chemotherapy (PIPAC), is under development for the treatment of metastatic cancers of the digestive system [12,13]. One of the main aims of these techniques is to maximize the concentrations of cytotoxic drugs within the tumor and to minimize their concentrations in the blood, which reduces the systemic toxicity of the treatment [1–5,9–13]. While ITI, CED and PIPAC treatments are generally regarded as palliative rather than curative, they can be applied in combination...
with systemic chemotherapy to reduce the spread of metastases and significantly prolong the life of cancer patients [14]. Classical Pt(II)-based anticancer drugs (cisplatin, carboplatin and oxaliplatin) [15] are increasingly used in ITI, CED and PIPAC formulations both in pre-clinical studies [14,16–31] and in human clinical trials as shown in Table 1 [7,32].

Table 1. Current and recent clinical trials of ITI and related techniques using Pt-based drugs [7].

| Identifier No | Treatment * | Drug | Disease | Phase | No. of Participants | Institution | Dates ** |
|---------------|-------------|------|---------|-------|---------------------|-------------|---------|
| NCT04311762  | ITI         | cisplatin | stage IV lung cancer | I     | 9                   | University of Vermont, Burlington, VT, USA | February 2020–March 2022 |
| NCT04809103  | ITI         | cisplatin | non-small cell lung cancer | I     | 10                  | University of Vermont, Burlington, VT, USA | March 2021–September 2023 |
| NCT05200650  | ITI         | cisplatin-loaded gel | head and neck cancer | I     | 20                  | Hadassah Medical Center, Jerusalem, Israel | March 2022–November 2022 |
| NCT04781725  | ITI         | new cisplatin formulation (INT230-6) | breast cancer | II    | 90                  | The Ottawa Hospital Research Institute and Cancer Center, Ontario, Canada | March 2021–March 2023 |
| NCT01644955  | CED         | carboplatin | recurrent high-grade gliomas | I     | 10                  | Ohio State University Medical center, Columbus, OH, USA | June 2012–December 2017 |
| NCT03294252  | PIPAC       | oxaliplatin and L-folinic acid | nonresectable peritoneal metastases of digestive cancers | II    | 50                  | Centre Hospitalier Lyon Sud, Pierre-Bénite, France | May 2017–June 2021 |
| NCT04541108  | ITI         | carboplatin (various formulations) | development of master protocol for intratumoral microdosing | 0     | 36                  | Presage Biosciences (various locations in USA) | July 2021–December 2031 |

* ITI = intratumoral injection; CED = convection enhanced delivery; PIPAC = pressurized intraperitoneal aerosolized chemotherapy. ** Start and end dates.

Extensive changes in the speciation of most metal-based drugs typically occur after their administration, due to the abundance of potential biomolecular ligands and reducing (or less commonly, oxidizing) agents in biological fluids [33–42]. One possible solution for this problem is the design of substitutionally inert (mostly organometallic) complexes where the metal ion acts either as a scaffold to build a three-dimensional organic structure for selective binding to protein targets [43–45] or as a catalytic center for intracellular redox reactions [46–48]. Another approach is to use kinetically inert Pt(IV) (see Section 7) or Co(III) prodrugs, which can be converted to their more labile Pt(II) or Co(II) counterparts in the reducing environment of solid tumors [42,49–51]. This approach is often proposed for the targeted delivery of biologically active organic molecules that are bound to such metal ions [51–53]. However, their administration by intravenous injection can result in the reduction of the metal ion by Fe(II) in red blood cells with premature release of the active components [54–56].

A novel concept that was recently proposed by our groups [57] involves the use of reactive metal complexes that have some stability but limited lifetimes in biological media.
Such complexes are ideal agents for ITI and related delivery techniques of anticancer drugs. In this case, the binding of hydrophobic organic ligands to a toxic metal ion assists its efficient uptake into tumor cells and results in high cytotoxicity, while the decomposition products that are released into the blood stream consist of relatively non-toxic ligands and metal–protein complexes (Figure 1) [57]. This approach is expected to exhibit low systemic toxicity, similar to photodynamic therapy [58] or boron neutron capture therapy [59], where highly cytotoxic but short-lived agents are generated locally in the tumor tissue. Similar principles are also applied to organic anticancer prodrugs that hydrolyze in biological media with the formation of highly cytotoxic but short-lived active species [60,61]. Importantly, the decomposition products of some metal anticancer drugs are likely to have beneficial biological effects, as suggested previously for a V(V) complex with hydrophobic organic ligands [57]. In this review, we discuss a number of metal complexes with known anticancer properties that have potential for intratumoral applications.

Figure 1. The principle of the use of reactive and unstable metal complexes in intratumoral injections [57]. Designations: M is the metal ion; and L are the ligands.

2. Vanadium(V) Complexes

Anticancer activities have been reported for V(V/IV) complexes with many different structures [62,63]. The concept of using relatively unstable metal complexes for ITI, where the complexes had some stability and exerted high reactivity, was developed for a non-innocent oxidovanadium(V) complex with a tridentate Schiff base and a redox-active di-3,5-tert-butylcatecholato ligand (1 in Figure 2a) [57,64]. Despite the vanadate–phosphate analogy [65,66], the nature of V–O bond in 1 and in other V(V/IV) complexes with organic ligands is closer to a triple than a double bond (2.5 < n ≤ 3, Figure 2a) due to the presence of one σ and two π bonds, and the bond is thus presented as a triple bond [67,68]. Due to the hydrophobic nature of the ligands [57,64] and sufficient stability of the coordination complex in biological media, 1 is efficiently taken up by cancer cell monolayers and causes high cytotoxicity (IC50 ~ 1–4 µM in 72 h treatments). Complex 1 is ~10-fold more toxic than cisplatin under the same conditions [57,64]. This effect is likely to be caused by changes in cell signaling that could originate from direct interactions of the cell membrane with V-complexes [69–71], inhibition of protein phosphatases by V-derivatives [66,72], as well as from V(V) reactions with cellular reductants that generate reactive oxygen species (ROS); see Figure 2a [73–75]. In parallel, rapid decomposition of 1 in cell culture medium occurs (half-life, ~30 s at 37 °C) [57], which involves hydrolysis of the Schiff base ligand, the release of oxidovanadium(V) species and their binding to serum proteins, predominantly transferrin (Tf, Figure 2a) [37,40,76,77]. This decomposition leads to a decrease in cytotoxicity by an order of magnitude, due to the low cellular uptake of V-Tf adducts and low cytotoxicity of
the ligand fragments [37,57]. Furthermore, V-Tf adducts are likely to be involved in the beneficial biological activities of V, such as the well-known antidiabetic [78,79] and the recently demonstrated neuroprotective and neurostimulatory [80–82] effects. The latter activities, together with the favorable cytotoxicity ratio of fresh and decomposed 1 in human glioma multiforme (T98g) cells, led to the suggestion [57] that 1 can be used in the ITI formulations for this aggressive form of brain cancer. This suggestion is supported by the recently demonstrated low acute oral toxicity of 1 in mice [83]. Neuroprotective and neurostimulatory activities of the decomposition products of 1 may help to fight the neurological and cognitive disorders that commonly occur from cancer itself, or from standard chemotherapy [84,85].

**Figure 2.** Proposed mechanisms of cytotoxic activity (red) and deactivation (blue) of V(V) complexes: (a) a complex with hydrolytically unstable Schiff base ligand (1) [57]; and (b) a complex with stable and cytotoxic salan-type ligand (2) [86]. Potential beneficial activities of the decomposition products are shown in green. Designations: Tf is apo-transferrin; ROS are reactive oxygen species and tBu is tert-butyl.

For comparison, the parent analog of 1 without tert-butyl substituents in the catechol ligand (the simple catechol) decomposes completely within a few seconds in the cell culture medium and is not taken by the cells to a significant extent [64]. Further developments in this field will involve tuning the hydrophobicity and aqueous stability of mixed-ligand V(V) complexes. This will enable optimization of their cellular uptake and decomposition rates and cytotoxic activities for the use in ITI and related techniques [87].

Like 1, V(V) complexes with reduced Schiff base (salan-type) [86,88] ligands, such as 2 in Figure 2b, are efficiently taken into cultured human cancer cells and are highly cytotoxic [86,89]. Unlike for 1, the cytotoxicity of 2 is predominantly due to the release of hydrolytically stable ligands, extracellularly and /or intracellularly (Figure 2b) [86]. Similar ligand-based cytotoxicity mechanisms have been proposed for V(V/IV) complexes with
typical hydrophobic and cytotoxic chelating ligands, such as 1,10-phenanthroline or 8-hydroxyquinoline [39,90,91]. The release of stable and highly cytotoxic ligands into the bloodstream is likely to lead to significant systemic toxicity that complicates the use of 2 and other V(V) complexes with stable cytotoxic ligands in ITI (Figure 2b). However, salan-type ligands in V(V) complexes can also be relatively non-toxic [92], which emphasizes the need for comparative biological activity studies of metal complexes and the corresponding free ligands [36].

Schiff bases, particularly those derived from salicylaldehyde and diamines (salen-type ligands), have long been considered a staple of coordination chemistry. Numerous metal complexes of these ligands have undergone biological activity assays, but none seem to have entered advanced preclinical development, as of yet [88,93,94]. Although the hydrolysis of Schiff bases to the original aldehyde and amine components in neutral aqueous solutions has long been known [95,96], its implications for biological activities of metal Schiff base complexes have not been recognized until recently [88]. For instance, the formation of aldehyde and amine precursors of the Schiff base ligand during the dissolution and subsequent decomposition of 1 in water (Figure 2a) has been demonstrated by $^1$H NMR spectroscopy [64]. The reactivity of the complex and ligand cleavage and V(V) release (Figure 2a) is responsible for the short lifetime of 1 under biologically relevant conditions, which forms the basis of the proposed use of 1 in ITI [57].

3. Iron(III) Schiff Base Complexes

Complexes of Fe(III) with salen-type ligands (3 in Figure 3) [97–100] have recently been highlighted because of their ability to induce uncommon modes of cancer cell death, ferroptosis and necroptosis. Such modes of toxicity reduce the chance of the development of drug resistance [101]. These Fe(III) complexes are thought to bypass normal cellular Fe uptake and metabolism pathways by entering the cell through passive diffusion, which leads to the formation of highly reactive low-molecular mass (LMM) Fe(III/II) complexes and ROS (Figure 3) [97–100]. Although hydrolysis of the ligands has not been reported in the original articles, it is likely to contribute to the decomposition of 3 and related complexes in an extracellular medium. This would assist the binding of the released Fe(III) to Tf (Figure 3) [102], which has been observed experimentally [98]. Furthermore, the release of Fe from 3 and its binding to Tf and other biomolecules is likely to be assisted by the reduction of Fe(III) to Fe(II) in the hypoxic environment of solid tumors [1,50,55].

![Figure 3. Proposed mechanism of cytotoxic activity (red) and deactivation (blue) of a Fe(III) complex with Schiff base ligand (3) [97,98]. Various substituents (X and Y) in the ligand were used, including halogens, CH$_3$, OCH$_3$, NO$_2$, or C(O)XR, where X is O or NH, and R is Et, n-Pr or n-Bu [97,98]. The anticipated hydrolysis of the Schiff base ligand was not reported in the original articles and is based on the data reported for V(V/IV) Schiff base complexes [57,64]. Designations: Tf is apo-transferrin; TfR1 is transferrin receptor 1; LMM is low-molecular-mass; and ROS are reactive oxygen species.](image-url)
The resultant Fe(III)-loaded Tf can then enter cells via a canonical pathway through the binding to its cell surface receptor (TfR1 in Figure 3), followed by receptor-mediated endocytosis [102,103]. Apart from delivering essential Fe into the cells, Fe(III)-Tf binding in the blood plays a protective role by ensuring that no adventitious low-molecular-mass Fe species enter cells and cause excessive oxidative stress [37,102]. Therefore, the amount of Fe that enters cells through Tf-mediated uptake is expected to be lower than that delivered by the passive diffusion of a hydrophobic Fe(III) complex (Figure 3) [37,104].

The flexibility of salen-type ligands to diverse chemical modifications [88,93,94] offers possibilities for the design of Fe(III) complexes with suitable ratios of cellular uptake versus extracellular decomposition rates (Figure 3) for ITI. The use of an essential metal ion, such as Fe(III), enables the exploitation of the natural metal-binding capacity of extracellular fluids, including proteins (mainly transferrin and albumin) and low-molecular-mass ligands (such as citrate and phosphate) [36,40,102] to reduce the possibility of unwanted side effects. Schiff base ligand design can also be used to enable pH-dependent prodrug activation in the acidic extracellular environment surrounding solid tumors [105]. In addition to Schiff bases, other common transition metal ligands, such as (thio)semicarbazones, contain potentially hydrolysable imine functionalities [106]. These compounds are generally stable under physiological conditions and biologically active in their own right, or through the coordination to endogenous Fe(III) and Cu(II) [107,108]. Nevertheless, the possibility of metal- or enzyme-catalyzed hydrolysis of (thio)semicarbazone complexes [107,109] in biological media has potential use in ITI.

4. Titanium(IV) Complexes

Titanocene dichloride and budotitane (4 and 5 in Figure 4) were two of the earliest metal complexes after cisplatin to be developed as potential anticancer drugs in the late 1970s. The design was based initially on their structural similarity with cisplatin with two labile chlorido or ethanolato ligands in a cis arrangement [110–112]. Unfortunately, these complexes did not progress beyond phase I clinical trials because of formulation problems and dose-limiting nephrotoxicity [113]. Notably, 4 and 5 showed low systemic toxicity in animal studies, which is consistent with the generally low toxicity of Ti [112]. Nevertheless, the anticancer activities of 4 and 5 were attributed to the Ti(IV) ion, since this is the only structural element shared between the two complexes (Figure 4). A wide range of effects of Ti(IV) complexes was observed at the cellular level, including induction of apoptosis and paraptosis, inhibition of mitochondrial activity and inactivation of topoisomerases, but the origin of these effects remained uncertain [114]. Recently, interference with the Fe metabolism has emerged as the most likely underlying mechanism of Ti(IV) anticancer activity [115–118].
Figure 4. Typical first-generation (4, 5) and second-generation (6–8) anticancer Ti(IV) complexes [112,116]. Likely decomposition products in an extracellular medium are shown in blue (Tf is apo-transferrin), and their potential beneficial activity is shown in green.

The complicated reactivity of Ti(IV) under biologically relevant conditions has been reviewed recently [117,119]. Complexes 4 and 5 are likely to decompose within seconds after intravenous injection with the formation of a mixture of low-molecular-mass hydrolysis products and Ti(IV)-protein adducts [112,117,119]. Extracellularly, Ti(IV) binds strongly and specifically to the Fe(III) binding sites of Tf [102,117]. This binding is mediated by citrate that helps to maintain Ti(IV) in a soluble form in neutral aqueous solutions [115]. Dependent on the nature of ligands, Ti(IV) complexes can also bind non-covalently to serum albumin [117,118]. Although Ti(IV)-Tf adducts can bind to cell surface TfRI and enter cells through receptor-mediated endocytosis, similarly to Fe(III)-Tf (Figure 4), this uptake is less efficient compared with the passive diffusion of hydrophobic Ti(IV) complexes through the cell membrane [115]. Intracellularly, Ti(IV) complexes are likely to lose their ligands and to displace Fe(III) from the active sites of crucial enzymes, such as ribonucleotide reductase [104,116,117].

Many second- and third-generation anticancer Ti(IV) complexes were developed with the aim to slow down the rate of decomposition in the extracellular medium and to increase cellular uptake and cytotoxicity [112,118,120]. Typical examples (Figure 4)
include increasing lipophilicity of cyclopentadiene ligands (6, titanocene Y) [121], using hexadentate ligands to easily exclude hydrolyzable groups (7) [122] and using ligands that mimic Tf binding sites to prevent extracellular Ti(IV) binding to Tf (8) [116]. It should be noted that the salan-type ligand in 7 is likely to be cytotoxic in its own right [86,88], which means that this complex is unlikely to be suitable for ITI. Some of the complexes shown in Figure 4, as well as other anticancer Ti(IV) complexes described in the literature [112], may be suitable for ITI if the ligand is sufficiently nontoxic. A possible additional advantage of the formation of Ti(IV)-Tf adducts during the decomposition of such complexes outside the cells (Figure 4) is the decrease in availability of Fe(III)-Tf to rapidly growing cancer cells since they have a high metabolic demand for Fe [102,123].

5. Gallium(III) Complexes

Unlike for Ti(IV) complexes, the use of Ga(III) complexes as anticancer drugs was originally based on the concept of chemical similarity of Ga(III) to high-spin Fe(III). This was expected to lead to the disruption of Fe metabolism in rapidly growing cancer cells [113,124–126]. Inorganic Ga(III) salts (nitrate or chloride, 9, Figure 5), injected intravenously in citrate-buffered solutions [127] (shown schematically as 9a, Figure 5) [128,129], reached phase II clinical trials for non-Hodgkin’s lymphoma and advanced melanoma [113]. The use of Ga(III) nitrate was later approved for the treatment of cancer-related calcium overload, but it is currently not used in the clinic [113]. Radiolabeled $^{67}$Ga(III)-citrate injections are still used in the diagnostics of cancer and inflammation, although they are increasingly replaced by $^{18}$F-based positron emission tomography (PET) scans [113,125]. Complexes with hydrophobic organic ligands, such as maltol or 8-hydroxyquinoline (10 and 11, respectively , in Figure 5) were designed to increase the bioavailability of Ga(III) for their potential use as oral anticancer drugs [113,125]. While clinical trials of 10 were discontinued after phase I/II, 11 is still in active trials and has shown promising results against renal cell carcinoma [113].

The cellular uptake of Ga(III) is generally thought to occur through Tf binding and interactions of the resultant Ga(III)-Tf adducts with TfR1 (similar to that for Fe(III) in Figure 3) [124], although the ability of Ga(III)-Tf to bind strongly to TfR1 has been dis-
puted [130]. Speciation studies in bovine serum and in cell culture medium by X-ray absorption spectroscopy showed that 9 was bound to serum proteins, particularly albumin and transferrin, within minutes at 37 °C, 10 decomposed over several hours, and 11 reached partial decomposition after 24 h under these conditions [131–133]. These data suggest that 11 was more likely than 10 to enter cells intact through passive diffusion (Figure 5), although both complexes underwent extensive metabolic changes upon entering the cells [131–133]. The two complexes also differ in the biological activity of their ligands: maltol in 10 is considered non-toxic and is approved as a food additive [78], while 8-hydroxyquinoline in 11 is cytotoxic, probably due to the binding of extracellular Cu(II) and its delivery into cells (Cu ionophore) [134].

The moderate stability of 10 in biological media [132,133] and the non-toxic nature of its ligands make this Ga(III) complex a more suitable candidate for potential use in ITI, compared with 9 or 11. The potential beneficial activities of the decomposition products of 10 (Figure 5) include decreased availability of Fe(III) to rapidly growing cancer cells due to the binding of Ga(III) to Fe(III)-binding sites of Tf [124], in the same way as proposed for Ti(IV) (Figure 4) [102,117]. In addition, the ability of Ga(III) to inhibit bone resorption and Ca(II) release has been reported [135], but the link between Ga(III) and Ca(II) remains much less explored than the similarities between Ga(III) and Fe(III) [124,125]. Recently, inorganic Ga(III) salts and Ga(III) complexes with organic ligands have emerged as potent antibacterial and antifungal agents with low toxicity to animals and humans [136–143]. Such beneficial antimicrobial activities are likely to be based on the differences in both Fe and Ca metabolism between microbial and mammalian cells [138,144]. This activity can potentially be used to help fight opportunistic infections that commonly occur as a result of cancer treatment by chemotherapy [145].

6. Ruthenium(III) Complexes

The anticancer activities of Ru(III) tetrachlorido complexes with axial N-heterocyclic ligands (Figure 6) have been extensively studied since the 1980s [33,113,146–149]. Two of the complexes, NAMI-A (12) and KP1019 (13a), reached human clinical trials but did not proceed beyond phase I/II. A more water-soluble analog of 13a, KP1339 (13b, also known as NKP-1339, IT-139 and BOLD-100) is currently in phase I clinical trials in combination with established anticancer drugs [113,150–153]. The postulated mechanism of action of Ru(III) complexes involves the exchange of labile chlorido ligands for donor groups of various biomolecules, which leads to the binding to numerous intra- and extra-cellular targets [33,34,36,113,146,147,154,155]. Complexes with bulkier, more hydrophobic ligands, such as 13a, rapidly enter the cells and cause significant cytotoxicity, while 12 binds predominantly to extracellular targets and is generally not cytotoxic (Figure 6) [33,113,146,147,156–160]. These complexes decompose in typical cell culture media or in blood serum within ~1 h (12) or ~4 h (13a) at 37 °C with the formation of predominantly Ru(III)-albumin adducts [156,160]. The binding of 12 to albumin involves the complete loss of the original ligands and the formation of covalent bonds with the side chains of the protein, and the resultant Ru(III)-albumin adducts are anti-invasive in cell culture assays [156,160]. The complete loss of the original ligands in NAMI-A during protein binding has been confirmed in several protein crystallography studies [161–163]. Fast non-covalent binding of 13a to albumin occurs through hydrophobic interactions, followed by slower covalent binding [157,159,164]. The addition of trifluoromethyl groups to the indazole ligands in 14 enhances hydrophobic interactions with albumin, which results in increased stability in extracellular media and higher cellular uptake and cytotoxicity (Figure 6) [146].
Figure 6. Structures of anticancer Ru(III) complexes that entered human clinical trials (12,13) and an investigational drug, 14 [113]. Their main modes of action in extra- and intracellular spaces (ECM is extracellular matrix, DAMP is damage-associated molecular pattern) are presented [33,113,147]. Intracellular cytotoxic species are shown in red, extracellular decomposition products are shown in blue, and their potential beneficial activities [151,154–156,165,166] are listed in green.

Based on the results of animal experiments, a unique mode of action of 12 was proposed, in which the drug does not decrease the size of primary tumors but prevents the spread of metastases [147,148]. Covalent binding of 12 to cell surface integrins and to the components of extracellular matrix (ECM), such as collagens (Figure 6), can disrupt the cell–cell and cell–ECM communication and prevent the invasion of aggressive cancer cells [33,147,148,156]. On the other hand, extensive binding to extracellular targets was likely to cause problems observed in the clinical trials of 12, such as the binding to skin collagen that result in painful blisters [147,148]. In these trials, 12 was administered by conventional intravenous injections. It is possible that administration of 12 by ITI could result in the predominant binding to the ECM that surrounds the tumor and to slow the spread of metastases, but this is yet to be established experimentally. More hydrophobic members of the Ru(III) series that have already undergone extensive preclinical development, such as 13a,b, also have a potential for ITI, given that a suitable drug delivery formulation is used (see Section 7) [167]. The administration of such drugs directly into the tumor would result in rapid uptake by cancer cells and in cell death, while the formation
of Ru-containing cell debris could lead to Ru–ECM binding and antimitostatic activity (Figure 6) [33,147,148,156].

The ability of certain metal complexes to promote the expression of damage-associated molecular patterns (DAMPs, Figure 6) on the surface of dying cancer cells, which leads to engagement of immune cells to the tumor (immunogenic activity), is crucial for the future of metal-based anticancer drugs [168,169]. Immunogenic properties have been demonstrated for many established anticancer drugs, including oxaliplatin, while cisplatin is generally considered to be non-immunogenic [168,169]. At least one Ru(III) compound (13b) has demonstrated the ability to induce immunogenic cancer cell death in vitro [151]. Such activity can provide an important additional benefit for the use of Ru(III) complexes in ITI [2,4,170]. Additional potential beneficial effects of the decomposition products of Ru(III) complexes used in ITI (Figure 6) include antimicrobial activity [165] and the disruption of the formation of amyloid aggregates, which are postulated to contribute to Alzheimer’s disease [154,155,166].

7. Drug Formulations for ITI

Producing stable, injectable formulations of poorly water soluble and/or water-sensitive metal-based drugs is a significant challenge [49,167]. Many of the proposed ITI formulations of cytotoxic drugs, including Pt(II) complexes, involve polymeric matrices that are designed for the slow release of the drug [25,171–173], but these are less applicable to unstable metal complexes that have to be delivered rapidly. Some of the possible solutions that can be applied to unstable and reactive V(V) complexes, as well as to other metal complexes, include micellar systems (Figure 7a), graphene quantum dots (Figure 7b), human serum albumin (HSA) adducts (Figure 7c), liposomal systems (Figure 7d) and oncolytic virus–metal complex suspensions (Figure 7e).

A simple approach that is compatible with ITI involves the encapsulation of hydrophobic complexes, such as 1, within micelles that are formed by a mixture of polyethylene glycol and fatty acids or triglycerides (Figure 7a) [174]. More recently, the binding of inorganic vanadate to small peptides that are incorporated into cell-permeable graphene quantum dots has been used for the precise delivery of V(V) to its cellular targets, such as a labile protein tyrosine phosphatase 1B (PTP1B) inhibitor, which was stabilized by the graphene framework (Figure 7b) [64,65]. This delivery system led to pronounced antidiabetic activity in mice [175]. Such technology also enabled the targeting of the compound using protein tyrosine phosphatases (protein tyrosine phosphatase 1B and T-cell protein phosphatase) [175]. Since applications of graphene quantum dots for selective anticancer therapy are under active development [176], a similar approach could potentially be designed for the delivery of unstable anticancer metal complexes to tumors via ITI techniques.

Liposomal formulations of immunomodulating drugs are widely applied for use with ITI [181]. Water-soluble complexes, such as ammonium decavanadate 16, or other polyoxometalates, can be encapsulated within unilamellar liposomes (Figure 7d) [182]. The pH value within the liposomes can be regulated to increase the stability of such complexes (Figure 7d) [183]. This approach may open the way for the wider use of unique biological activities of polyoxometalates that are different from those of mononuclear metal
complexes [182,184,185]. Liposomal formulations have also been developed to enhance the stability of hydrophobic V(V) complexes in biological media [92].

A novel and highly promising way to harness the effect of V complexes on cellular signal transduction [66,70,72,186] is their use in enhancing the effects of oncolytic viruses [187]. Co-administration of a virus with inorganic vanadate (17 in Figure 7e) or selected V complexes enhanced their uptake and cytotoxicity in cultured cancer cells and reduced tumor sizes in mice [187]. Viral infection and cytotoxicity in cancer cells was further enhanced by using more lipophilic V(V) complexes with dipicolinate ligands (18 in Figure 7d) [188], although such complexes are known to be short-lived in aqueous solutions [189]. These findings are of immediate interest for the use in ITI of oncolytic viruses, which is the only ITI application currently approved for clinical use [8].

**Figure 7.** Potential pharmaceutical formulations for intratumoral injections of V(V) complexes (1, 15–18): (a) hydrophobic micelles [174]; (b) protein tyrosine phosphatase (PTP)-targeting graphene quantum dots [175]; (c) adducts with human serum albumin (HSA) [177]; (d) pH-controlled liposomes [182]; and (e) co-administration with oncolytic viruses [187,188]. ‘Bu is tert-butyl.

Injections of well-known cytotoxic Pt(II) complexes [15] directly into the tumor have been extensively trialed (Table 1) [7] to reduce their systemic toxicity compared with standard intravenous injections. However, significant side effects can still occur due to the partial escape of Pt(II) species into the bloodstream [18,26,190]. To overcome this problem, a Pt(IV)-based nanocarrier formulation for ITI was developed recently (19 in Figure 8) [190].
The formulation consists of a Pt(IV)-tocopherol derivative that is bound non-covalently (through hydrophobic interactions) to a hyaluronan-tocopherol adduct (Figure 8) [190]. The resulting nanoparticles are efficiently taken up by cancer cells and reduced by cellular reductants, such as glutathione and ascorbate, to form reactive Pt(II) species (marked with red color in Figure 8) [190,191]. These species enter the cell nucleus and form irreparable Pt(II)-DNA adducts, leading to cell death [15,191]. Importantly, the expressions of DAMPs on the surface of dying cancer cells [168,169] leads to the engagement of immune cells to the tumor and enhances the anticancer activity of 19 in an immunocompetent mouse model [190]. This immunogenic activity provides an additional benefit of using 19 for ITI (shown in green color in Figure 8) [31,181,190]. This example further demonstrates the potential of nanocarrier formulations in enhancing the activity and selectivity of metal complexes for ITI applications.

Figure 8. Structure of a Pt(IV)-tocopherol-hyalouronan nanocarrier 19 [190] and its proposed mechanism of action in ITI (based on a general mechanism of anticancer activity of Pt(IV) complexes) [191]. Proposed cytotoxic species are shown in red, and the beneficial immunogenic activity [168,190] is shown in green. Designations: Red are cellular reductants (e.g., ascorbate or glutathione), and DAMP are damage associated molecular patterns.

8. Conclusions and Future Potential Applications

Metal-based anticancer drugs [113,149,192] often have low stability in biological media [36–42], and this is one of the main obstacles to their wider use in clinical practice. A recent suggestion [57] was to take advantage of this instability and consequently reactivity and use these compounds in ITI applications (Figure 1). This novel concept was based on the results of in vitro stability studies and cell culture assays using a mixed-ligand V(V) complex showing significantly enhanced activity over cisplatin, 1 (Figure 2a) [57]. This literature survey highlights other metal-based anticancer drugs that could potentially be
suitable candidates for ITI injections. Particularly, it focuses on considering anticancer Ti(IV), Ga(III) and Ru(III) complexes that were previously tested in human clinical trials but failed, which was attributed, at least in part, to the low stability when injected into the bloodstream [113].

The question posed for the compounds identified in this review (or related systems) is whether they would have the desired reactivity and sufficient stability to be useful for ITI applications. This approach has been used successfully in clinical trials with established Pt(II) drugs, mostly cisplatin (Table 1) [7] and in pre-clinical studies using a Pt(IV) prodrug [190]. In cell culture models [57,87], V(V) complexes with hydrophobic organic ligands were far superior to cisplatin in causing cancer cell death, particularly in short-term treatments that are relevant to ITI. The use of these biologically active but relatively unstable V(V) complexes can be further enhanced by the development of suitable drug formulations that stabilize the compounds further (Section 7). This is particularly relevant for their use in ITI and CED for the treatment of malignant gliomas [29,30,193]. Based on the low acute toxicity of 1 in healthy mice [83], the next logical step is the use of stabilized formulations of 1 and other hydrophobic metal complexes for intratumoral injections in mouse models of human cancers. These would use similar procedures to those used in previous studies with Pt(II) and Pt(IV) complexes [26,190]. The use of immunocompetent animals is particularly important for the assessment of immunogenic activity of 1 and other metal complexes [31,190].

Successful ITI has a cellular uptake of metal drugs that is faster than the extracellular complex decomposition. Since the proposed ITI approach is dependent on the kinetic competition between cellular uptake and extracellular decomposition, and this is characteristic for transition metal complexes, these complexes are ideal for such ITI applications (Figures 2–6) [33,36]. In addition, Pt(IV) and Co(III) prodrugs that are activated by the reduction in the hypoxic environment of solid tumors (Figure 8) [49–51,53,56,190] can benefit from ITI by avoiding reduction in red blood cells before reaching the tumor target [54–56]. Generally, any cytotoxic metal complex can be considered for the use in ITI if it decomposes in an extracellular medium at a comparable rate with its cellular uptake and the decomposition products show lower toxicity compared with the initial complex [57]. The latter consideration is crucial to exclude the possibility that the cytotoxicity of the metal complex is due to the release of stable and biologically active ligands either inside or outside of the cell, such as 2 in Figure 2b or 11 in Figure 5 [86,90,91]. Under the conditions of ITI, the release of such ligands into the blood stream (Figure 1) is likely to lead to high systemic toxicity. Therefore, metal complexes of the ligands that have limited lifetimes in neutral aqueous solutions, such as Schiff bases (Figures 2a and 3), can be particularly suitable for the use in ITI. More research is urgently needed to follow early kinetic studies [95,96] on the decomposition of such ligands and their complexes under biologically relevant conditions as well as methods that will stabilize these systems and facilitate the administration of these complexes.

An important novel consideration in the use of metal complexes as anticancer drugs for ITI is the potential beneficial activity of their decomposition products (shown in green color in Figures 1–6 and 8), which is unlikely to occur for non-metal-based drugs. Some of the most promising examples include the following: (i) immunogenic activities of some Pt(II), Pt(IV) and Ru(III) complexes [151,168,190]; antidiabetic, tissue regeneration and neurostimulatory activities of V(V/IV) complexes [63,78,79,81,82]; antimicrobial activities of Ga(III) [136–138,140–143], V(V/IV) [194,195] and Ru(III) complexes [165]; and antimetastatic and possibly neuroprotective activity of Ru(III) complexes [147,154–156,166]. The multiple modes of biological activity of many metal ions, dependent on their concentration and speciation in biological compartments [33,35,36,42,196] highlight the unique potential for metal complexes in medicinal applications, which is far from being fully realized at this time [49,52,113].
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References

1. Huang, A.; Pressnall, M.M.; Lu, R.; Huayamares, S.G.; Griffin, J.D.; Groer, C.; DeKosky, B.J.; Forrest, M.L.; Berkland, C.J. Human intratumoral therapy: Linking drug properties and tumor transport of drugs in clinical trials. J. Control. Release 2020, 326, 203–221. [CrossRef] [PubMed]
2. Newman, J.H.; Chesson, C.B.; Herzog, N.L.; Bommarabiy, P.K.; Aspromonte, S.M.; Pepe, R.; Estupinan, R.; Aboelatta, M.M.; Buddhadev, S.; Tarabichi, S.; et al. Intratumoral injection of the seasonal flu shot converts immunologically cold tumors to hot and serves as an immunotherapy for cancer. Proc. Natl. Acad. Sci. USA 2020, 117, 1119–1128. [CrossRef] [PubMed]
3. Kepp, O.; Marabelle, A.; Zitvogel, L.; Kroemer, G. Oncolyis without viruses—Inducing systemic anticancer immune responses with local therapies. Nat. Rev. Clin. Oncol. 2020, 17, 49–64. [CrossRef]
4. Melero, I.; Castanon, E.; Alvarez, M.; Champiat, S.; Marabelle, A. Intratumoural administration and tumour tissue targeting of cancer immunotherapies. Nat. Rev. Clin. Oncol. 2021, 18, 558–576. [CrossRef] [PubMed]
5. Momin, N.; Palmeri, J.R.; Lutz, E.A.; Jialkhani, N.; Mak, H.; Tabet, A.; Chinn, M.M.; Kang, B.H.; Spanoudaki, V.; Hynes, R.O.; et al. Maximizing response to intratumoral immunotherapy in mice by tuning local retention. Nat. Commun. 2022, 13, 109. [CrossRef]
6. Marabelle, A.; Andtbacka, R.; Harrington, K.; Melero, I.; Leidner, R.; de Baere, T.; Robert, C.; Ascierto, P.A.; Baurain, J.F.; Imperiale, M.; et al. Starting the fight in the tumor: Expert recommendations for the development of human intratumoral immunotherapy (HT-IT). Ann. Oncol. 2018, 29, 2163–2174. [CrossRef]
7. NIH, U.S. National Library of Medicine. ClinicalTrials.gov. Available online: https://clinicaltrials.gov (accessed on 2 February 2022).
8. Hamid, O.; Ismail, R.; Puzanov, I. Intratumoral immunotherapy-update 2019. Oncologist 2020, 25, e423–e438. [CrossRef]
9. D’Amico, R.S.; Aghi, M.K.; Vogelbaum, M.A.; Bruce, J.N. Convection-enhanced drug delivery for glioblastoma: A review. J. Neuro-Oncol. 2021, 151, 415–427. [CrossRef]
10. Nwagwu, C.D.; Inmadisetti, A.V.; Jiang, M.Y.; Adeagbo, O.; Adamson, D.C.; Carbonell, A.M. Convection enhanced delivery in the setting of high-grade gliomas. Pharmaceutics 2021, 13, 561. [CrossRef]
11. Kang, J.H.; Desjardins, A. Convection-enhanced delivery for high-grade glioma. Neuro-Oncol. Pract. 2022, 9, 24–34. [CrossRef]
12. Alyami, M.; Hübnner, M.; Grass, F.; Bärich, N.; Villeneuve, L.; Laplace, N.; Passot, G.; Glehen, O.; Kepenekian, V. Pressurised intraperitoneal aerosol chemotherapy: Rationale, evidence, and potential indications. Lancet Oncol. 2019, 20, e368–e377. [CrossRef]
13. de Jong, L.A.W.; van Erp, N.P.; Bijelic, I. Pressurized intraperitoneal aerosol chemotherapy: The road from promise to proof. Clin. Cancer Res. 2021, 27, 1830–1832. [CrossRef]
14. Lu, B.; Sun, L.; Yan, X.; Ai, Z.; Xu, J. Intratumoral chemotherapy with paclitaxel liposome combined with systemic chemotherapy: A new method of neoadjuvant chemotherapy for stage III unresectable non-small cell lung cancer. Med. Oncol. 2014, 32, 345. [CrossRef]
15. Kelland, L. The resurgence of platinum-based cancer chemotherapy. Nat. Rev. Cancer 2007, 7, 573–584. [CrossRef]
16. Khuri, F.R.; Nemunaitis, J.; Ganly, I.; Arseneau, J.; Tannock, I.F.; Romel, L.; Gore, M.; Ironside, J.; MacDougall, R.H.; Heise, C.; et al. A controlled trial of intratumoral ONX-015, a selectively-replicating adenovirus, in combination with cisplatin and 5-fluorouracil in patients with recurrent head and neck cancer. Nat. Med. 2000, 6, 879–885. [CrossRef] [PubMed]
17. Mehta, H.J.; Begnud, A.; Penley, A.M.; Wynne, J.; Malhotra, F.; Fernandez-Bussy, S.; Cope, J.; Shuster, J.J.; Jantz, M.A. Restoration of patency to central airways occluded by malignant endobronchial tumors using intratumoral injection of cisplatin. Ann. Am. Thorac. Soc. 2015, 12, 1345–1350. [CrossRef]
18. Cai, S.; Zhang, T.; Forrest, W.C.; Yang, Q.; Groer, C.; Mohr, E.; Aires, D.J.; Axiak-Bechtel, S.M.; Flesser, B.K.; Henry, C.J.; et al. Phase I/II clinical trial of hyaluronan-cisplatin nanoconjugate in dogs with naturally occurring malignant tumors. *Am. J. Vet. Res.* 2016, 77, 1005–1016. [CrossRef]

19. Shi, M.; Fortin, D.; Paquette, B.; Sanche, L. Convection-enhancement delivery of liposomal formulation of oxaliplatin shows less toxicity than oxaliplatin yet maintains a similar median survival time in F98 glioma-bearing rat model. *Investig. New Drugs* 2016, 34, 269–276. [CrossRef]

20. Nadiradze, G.; Giger-Pabst, U.; Zieren, J.; Strumberg, D.; Solass, W.; Reymond, M.-A. Pressurized intraperitoneal aerosol chemotherapy (PIPAC) with low-dose cisplatin and doxorubicin in gastric peritoneal metastasis. *J. Gastrointest. Surg.* 2016, 20, 367–373. [CrossRef] [PubMed]

21. Demtröder, C.; Solass, W.; Zieren, J.; Strumberg, D.; Giger-Pabst, U.; Reymond, M.A. Pressurized intraperitoneal aerosol chemotherapy with oxaliplatin in colorectal peritoneal metastasis. *Colorectal Dis.* 2016, 18, 364–371. [CrossRef] [PubMed]

22. Mehta, H.J.; Jantz, M.A. Endobronchial ultrasound-guided intratumoral injection of cisplatin for the treatment of isolated mediastinal recurrence of lung cancer. *J. Vis. Exp.* 2017, 120, e54851–e54855. [CrossRef]

23. Yang, B.; He, J.-P.; Yuan, M.-L.; Li, W.; Jiao, H.; You, X.; Liu, X.-R.; Zhao, J.; Li, C.-L.; Fu, X.-B.; et al. Percutaneous intratumoral injection of gemcitabine plus cisplatin mixed with fibrin glue for advanced pancreatic carcinoma: Case Report. *Medicine* 2017, 96, e8018. [CrossRef] [PubMed]

24. Miranda, D.; Carter, K.; Luo, D.; Shao, S.; Geng, J.; Li, C.; Chitgupi, U.; Turowski, S.G.; Li, N.; Atilla-Gokcumen, G.E.; et al. Multifunctional liposomes for image-guided intratumoral chemo-phototherapy. *Adv. Healthc. Mater.* 2017, 6, 1700253. [CrossRef] [PubMed]

25. Liang, H.-K.T.; Lai, X.-S.; Wei, M.-F.; Lu, S.-H.; Wen, W.-F.; Kuo, S.-H.; Chen, C.-M.; Tseng, W.-Y.L.; Lin, F.-H. Intratumoral injection of thermogelling and sustained-release carboplatin-loaded hydrogel simplifies the administration and remains the synergistic effect with radiotherapy for mice gliomas. *Biomaterials* 2018, 151, 38–52. [CrossRef] [PubMed]

26. Gao, L.; Cai, S.; Cai, A.; Zhao, Y.; Xu, T.; Ma, Y.; Xu, Y.; Wang, Y.; Wang, H.; Hu, Y. The improved antitumor efficacy of continuous intratumoral chemotherapy with cisplatin-loaded implants for the treatment of sarcoma 180 tumor-bearing mice. *Drug Deliv.* 2019, 26, 208–215. [CrossRef] [PubMed]

27. Mori, V.; Roy, G.S.; Bates, J.H.T.; Kinsey, C.M. Cisplatin pharmacodynamics following endobronchial ultrasound-guided transbronchial needle injection into lung tumors. *Sci. Rep.* 2019, 9, 6819. [CrossRef]

28. Yu, M.; Zhang, C.; Tang, Z.; Tang, X.; Xu, H. Intratumoral injection of gels containing losartan microspheres and (PLG-g-MPEG)-cisplatin nanoparticles improves drug penetration, retention and anti-tumor activity. *Cancer Lett.* 2019, 442, 396–408. [CrossRef] [PubMed]

29. Elleaume, H.; Barth, R.F.; Rousseau, J.; Bobyk, L.; Baloso, J.; Yang, W.; Hoo, T.; Nakkula, R. Radiation therapy combined with intracerebral convection-enhanced delivery of cisplatin or carboplatin for treatment of the F98 rat glioma. *J. Neuro-Oncol.* 2020, 149, 193–208. [CrossRef] [PubMed]

30. Wang, J.L.; Barth, R.F.; Cavaliere, R.; Puduvalli, V.K.; Giglio, P.; Lonser, R.R.; Elder, J.B. Phase I trial of intracerebral convection-enhanced delivery of carboplatin for treatment of recurrent high-grade gliomas. *PLoS ONE* 2020, 15, e0244383. [CrossRef] [PubMed]

31. Jessup, J.M.; Kabbout, M.; Korokhov, N.; Joun, A.; Tollefson, A.E.; Wold, W.S.M.; Mattoo, A.R. Adenovirus and Oxaliplatin cooperate as agnostic sensitizers for immunogenic cell death in colorectal carcinoma. *Hum. Vaccines Immunother.* 2020, 16, 636–644. [CrossRef] [PubMed]

32. Zhou, Y.; Gao, Y.; Zhang, N.; Li, X.; Wang, H.; Wang, S.; Liu, J.; Gao, H.; Wang, H. Clinical effects of cisplatin plus recombiant human endostatin (rh-endostatin) intratumoral injection on malignant central airway obstruction: A retrospective analysis of 319 cases. *J. Thorac. Dis.* 2021, 13, 1100–1105. [CrossRef] [PubMed]

33. Levina, A.; Mitra, A.; Lay, P.A. Recent developments in ruthenium anticancer drugs. *Metallomics* 2009, 1, 458–470. [CrossRef] [PubMed]

34. Costa Pessoa, J.; Tomaz, I. Transport of therapeutic vanadium and ruthenium complexes by blood plasma components. *Curr. Med. Chem.* 2010, 17, 3701–3738. [CrossRef]

35. Crans, D.C.; Woll, K.A.; Prusinskas, K.; Johnson, M.D.; Norkus, E. Metal speciation in health and medicine represented by iron and vanadium. *Inorg. Chem.* 2013, 52, 12262–12275. [CrossRef] [PubMed]

36. Levina, A.; Crans, D.C.; Lay, P.A. Speciation of metal drugs, supplements and toxins in media and bodily fluids controls in vitro activities. *Coord. Chem. Rev.* 2017, 352, 473–498. [CrossRef]

37. Levina, A.; Lay, P.A. Vanadium(V)/IV-transferrin binding disruptions the transferrin cycle and reduces vanadium uptake and antiproliferative activity in human lung cancer cells. *Inorg. Chem.* 2020, 59, 16143–16153. [CrossRef] [PubMed]

38. Nunes, P.; Correia, I.; Marques, F.; Matos, A.P.; dos Santos, M.M.C.; Azevedo, C.G.; Capelo, J.-L.; Santos, H.M.; Gama, S.; Pinheiro, T.; et al. Copper complexes with 1,10-phenanthroline derivatives: Underlying factors affecting their cytotoxicity. *Inorg. Chem.* 2020, 59, 9116–9134. [CrossRef] [PubMed]

39. Nunes, P.; Correia, I.; Cavaco, I.; Marques, F.; Pinheiro, T.; Avevilla, F.; Costa Pessoa, J. Therapeutic potential of vanadium complexes with 1,10-phenanthroline ligands, quo vadis? Fate of complexes in cell media and cancer cells. *J. Inorg. Biochem.* 2021, 217, 111350. [CrossRef]
40. Costa Pessoa, J.; Correia, I. Misinterpretations in evaluating interactions of vanadium complexes with proteins and other biological targets. *Inorganics* 2021, 9, 17. [CrossRef]
41. Stone, A.T.; Dhara, V.G.; Naik, H.M.; Aliyu, L.; Lai, J.; Jenkins, J.; Betenbaugh, M.J. Chemical speciation of trace metals in mammalian cell culture media: Looking under the hood to boost cellular performance and product quality. *Curr. Opin. Biotechnol.* 2021, 71, 216–224. [CrossRef] [PubMed]
42. Yuan, S.; Zhu, Y.; Dai, Y.; Wang, Y.; Jin, D.; Liu, M.; Tang, L.; Arnesano, F.; Natile, G.; Liu, Y. 19F NMR allows the investigation of the fate of platinum(IV) prodrugs in physiological conditions. *Angew. Chem. Int. Ed.* 2022, 61, e202114250. [CrossRef] [PubMed]
43. Meggers, E. From conventional to unusual enzyme inhibitor scaffolds: The quest for target specificity. *Angew. Chem. Int. Ed.* 2011, 50, 2442–2448. [CrossRef] [PubMed]
44. Doerr, M.; Meggers, E. Metal complexes as structural templates for targeting proteins. *Curr. Opin. Chem. Biol.* 2014, 19, 76–81. [CrossRef]
45. Jaouen, G.; Vessieres, A.; Top, S. Ferrocifen type anti cancer drugs. *Chem. Soc. Rev.* 2015, 44, 8802–8817. [CrossRef] [PubMed]
46. Sasmal, P.K.; Streu, C.N.; Meggers, E. Metal complex catalysis in living biological systems. *Chem. Commun.* 2013, 49, 1581–1587. [CrossRef]
47. Liu, Z.; Sadler, P.J. Organoiridium complexes: Anticancer agents and catalysts. *Acc. Chem. Res.* 2014, 47, 1174–1185. [CrossRef]
48. Banerjee, S.; Sadler, P.J. Transfer hydrogenation catalysis in cells. *RSC Chem. Biol.* 2021, 2, 12–29. [CrossRef] [PubMed]
49. Johnstone, T.C.; Suntharalingam, K.; Lippard, S.J. The next generation of platinum drugs: Targeted Pt(II) agents, nanoparticle delivery, and Pt(IV) prodrugs. *Chem. Rev.* 2016, 116, 3436–3486. [CrossRef]
50. Renfrew, A.K.; O’Neill, E.S.; Hambley, T.W.; New, E.J. Harnessing the properties of cobalt coordination complexes for biological application. *Coord. Chem. Rev.* 2018, 375, 221–233. [CrossRef]
51. Glenister, A.; Chen, C.K.J.; Paterson, D.J.; Renfrew, A.K.; Simone, M.I.; Hambley, T.W. Warburg effect targeting Co(III) cytoxin chaperone complexes. *J. Med. Chem.* 2021, 64, 2678–2690. [CrossRef]
52. Wang, X.; Wang, X.; Jin, S.; Muhammad, N.; Guo, Z. Stimuli-responsive therapeutic metallodrugs. *Chem. Rev. 2019, 119, 1138–1192.* [CrossRef] [PubMed]
53. Chen, C.K.J.; Gui, X.; Kappen, P.; Renfrew, A.K.; Hambley, T.W. The effect of charge on the uptake and resistance to reduction of platinum(IV) complexes in human serum and whole blood models. *Metallomics* 2020, 12, 1599–1615. [CrossRef] [PubMed]
54. Carr, J.L.; Tingle, M.D.; McKeage, M.J. Rapid biotransformation of satraplatin by human red blood cells in vitro. *Cancer Chemother. Pharmacol.* 2002, 50, 9–15. [CrossRef] [PubMed]
55. Chen, C.K.J.; Zhang, J.Z.; Aitken, J.B.; Hambley, T.W. Influence of equatorial and axial carboxylato ligands on the kinetic inertness of platinum(IV) complexes in the presence of ascorbate and cysteine and within DLD-1 Cancer Cells. *J. Med. Chem.* 2013, 56, 8757–8764. [CrossRef] [PubMed]
56. Chen, C.K.J.; Kappen, P.; Gibson, D.; Hambley, T.W. *trans*-Platinum(IV) pro-drugs that exhibit unusual resistance to reduction by endogenous reductants and blood serum but are rapidly activated inside cells: 1H NMR and XANES spectroscopy study. *Dalton Trans.* 2020, 49, 7722–7736. [CrossRef] [PubMed]
57. Levina, A.; Pires Vieira, A.; Wijetungga, A.; Kaur, R.; Koehn, J.T.; Crans, D.C.; Lay, P.A. A Short-lived but highly cytotoxic vanadium(V) complex as a potential drug lead for brain cancer treatment by intratumoral injections. *Angew. Chem. Int. Ed.* 2012, 51, 18757–18764. [CrossRef] [PubMed]
58. Brown, S.B.; Brown, E.A.; Walker, I. The present and future role of photodynamic therapy in cancer treatment. *Lancet Oncol.* 2004, 5, 497–508. [CrossRef]
59. Barth, R.F.; Codreer, J.A.; Vicente, M.G.H.; Blue, T.E. Boron neutron capture therapy of cancer: Current status and future prospects. *Clin. Cancer Res.* 2005, 11, 3987–4002. [CrossRef] [PubMed]
60. Shyam, K.; Penketh, P.G.; Baumann, R.P.; Finch, R.A.; Zhu, R.; Zhu, Y.-L.; Sartorelli, A.C. Antitumor sulfonilylhydrazines: Design, structure–activity relationships, resistance mechanisms, and strategies for improving therapeutic utility. *J. Med. Chem.* 2015, 58, 3639–3671. [CrossRef]
61. Penketh, P.G.; Baumann, R.P.; Shyam, K.; Williamson, H.S. Design strategy for the EPR tumor-targeting of 1,2-bis(sulfonyl)-1-alkyhydrazines. *Molecules* 2021, 26, 259. [CrossRef]
62. Kioseoglou, E.; Petanidis, S.; Gabriel, C.; Salifoglou, A. The chemistry and biology of vanadium compounds in cancer therapeutics. *Coord. Chem. Rev.* 2015, 301–302, 87–105. [CrossRef]
63. Crans, D.C.; Yang, L.; Haase, A.; Yang, X. Health benefits of vanadium and its potential as an anticancer agent. *Met. Ions Life Sci.* 2018, 15, 251–280. [CrossRef]
64. Crans, D.C.; Koehn, J.T.; Petry, S.M.; Glover, C.M.; Wijetungga, A.; Kaur, R.; Levina, A.; Lay, P.A. Hydrophobicity may enhance membrane affinity and anti-cancer effects of Schiff base vanadium(V) catecholate complexes. *Dalton Trans.* 2019, 48, 6383–6395. [CrossRef] [PubMed]
65. Chasteen, D.N. The biochemistry of vanadium. *Struct. Bond.* 1983, 53, 105–138. [CrossRef]
66. Crans, D.C. Antidiabetic, chemical, and physical properties of organic vanadates as presumed transition-state inhibitors for phosphatases. *J. Org. Chem.* 2015, 80, 11899–11915. [CrossRef]
67. Ballhausen, C.J.; Gray, H.B. The electronic structure of the vanadyl ion. *Inorg. Chem.* 1962, 1, 111–122. [CrossRef]
68. Levina, A.; McLeod, A.I.; Lay, P.A. Vanadium speciation by XANES spectroscopy: A three-dimensional approach. *Chemistry* 2014, 20, 12056–12060. [CrossRef]
69. Althumairy, D.; Murakami, H.A.; Zhang, D.; Barisas, B.G.; Roess, D.A.; Crans, D.C. Effects of vanadium(IV) compounds on plasma membrane lipids lead to G protein-coupled receptor signal transduction. J. Inorg. Biochem. 2020, 203, 110873. [CrossRef]

70. Samart, N.; Althumairy, D.; Zhang, D.; Crans, D.C. Initiation of a novel mode of membrane signaling: Vanadium facilitated signal transduction. Coord. Chem. Rev. 2020, 416, 213286. [CrossRef]

71. Crans, D.C.; Brown, M.; Roess, D.A. Vanadium compounds promote biocatalysis in cells through actions on cell membranes. Catal. Today 2022, 388–389, 216–223. [CrossRef]

72. McLauchlan, C.C.; Peters, B.J.; Willsky, G.R.; Crans, D.C. Vanadium–phosphatase complexes: Phosphatase inhibitors favor the trigonal bipyramidal transition state geometries. Coord. Chem. Rev. 2015, 301–302, 163–199. [CrossRef]

73. Levina, A.; Lay, P.A. Metal-based anti-diabetic drugs: Advances and challenges. Dalton Trans. 2011, 40, 11675–11686. [CrossRef] [PubMed]

74. Clark, O.; Park, I.; Di Florio, A.; Cichon, A.-C.; Rustin, S.; Jugov, R.; Maeshima, R.; Stoker, A.W. Oxovanadium-based inhibitors can drive redox-sensitive cytotoxicity in neuroblastoma cells and synergise strongly with buthionine sulfoximine. Cancer Lett. 2015, 357, 316–327. [CrossRef] [PubMed]

75. Scibior, A.; Kuras, J. Vanadium and oxidative stress markers—In vivo model: A Review. Curr. Med. Chem. 2019, 26, 5456–5500. [CrossRef]

76. Sanna, D.; Micera, G.; Garrirba, E. On the transport of vanadium in blood serum. Inorg. Chem. 2009, 48, 5747–5757. [CrossRef]

77. Costa Pessoa, J.; Garrirba, E.; Santos, M.F.A.; Santos-Silva, T. Vanadium and proteins: Uptake, transport, structure, activity and function. Coord. Chem. Rev. 2015, 301–302, 49–86. [CrossRef]

78. Thompson, K.H.; Orvig, C. Vanadium in diabetes: 100 years from Phase 0 to Phase I. J. Inorg. Biochem. 2006, 100, 1925–1935. [CrossRef]

79. Crans, D.C.; Brown, M.; Roess, D.A. Developing vanadium as an antidiabetic or anticancer drug: A clinical and historical perspective. Met. Ions Life Sci. 2019, 19, 203–230. [CrossRef]

80. He, Z.; Han, S.; Zhu, H.; Hu, X.; Li, X.; Hou, C.; Wu, C.; Xie, Q.; Li, N.; Du, X.; et al. The protective effect of vanadium on cognitive impairment and the neuropathology of Alzheimer’s disease in APPSwe/PS1dE9 mice. Front. Mol. Neurosci. 2020, 13, 21. [CrossRef]

81. He, Z.; Han, S.; Wu, C.; Liu, L.; Zhu, H.; Liu, A.; Lu, Q.; Huang, J.; Du, X.; Li, N.; et al. Bis(ethylmaltolato)oxidovanadium(IV) inhibited the pathogenesis of Alzheimer’s disease in triple transgenic model mice. Metallomics 2020, 12, 474–490. [CrossRef]

82. He, Z.; You, G.; Liu, Q.; Li, N. Alzheimer’s disease and diabetes mellitus in comparison: The therapeutical efficacy of the vanadium compound. Int. J. Mol. Sci. 2021, 22, 11931. [CrossRef] [PubMed]

83. Lima, L.M.A.; Murakami, H.; Gaebler, D.J.; Silva, W.E.; Belian, M.F.; Lira, E.C.; Crans, D.C. Acute toxicity evaluation of non-innocent oxidovanadium(V) schiff base complex. Inorganics 2021, 9, 42. [CrossRef]

84. Pendergrass, J.C.; Targum, S.D.; Harrison, J.E. Cognitive impairment associated with cancer: A Brief Review. Innov. Clin. Neurosci. 2017, 15, 36–44. [PubMed]

85. Emad El-Agamy, S.; Kamal Abdel-Aziz, A.; Esmat, A.; Azab, S.S. Chemotherapy and cognition: Comprehensive review on doxorubicin-induced chemobrain. Cancer Chemother. Pharmacol. 2019, 84, 1–14. [CrossRef] [PubMed]

86. Reytmans, L.; Hochman, J.; Tshuva, E.Y. Anticancer dianimotris(phenolato) vanadium(V) complexes: Ligand-metal interplay. J. Coord. Chem. 2018, 71, 2003–2011. [CrossRef]

87. Murakami, H.A.; Uslan, C.; Haase, A.A.; Koehn, J.T.; Pires Vieira, A.; Gaebler, D.J.; Hagan, J.; Beuning, C.N.; Proschogo, N.; Levina, A.; et al. Chloro-substituted Schiff-base ligands make potential anticancer V(V) catecholate complexes more reducible, lipophilic, and water stable. JACS Au 2022, submitted.

88. Costa Pessoa, J.; Correia, I. Salan vs. salen metal complexes in catalysis and medicinal applications: Virtues and pitfalls. Coord. Chem. Rev. 2019, 388, 227–247. [CrossRef]

89. Reytmans, L.; Braithbard, O.; Hochman, J.; Tshuva, E.Y. Highly effective and hydrolytically stable vanadium(V) amino phenolato antitumor agents. Inorg. Chem. 2016, 55, 610–618. [CrossRef]

90. Le, M.; Rathje, O.; Levina, A.; Lay, P.A. High cytotoxicity of vanadium(IV) complexes with 1,10-phenanthroline and related ligands is due to decomposition in cell culture medium. J. Biol. Inorg. Chem. 2017, 22, 663–672. [CrossRef]

91. Levina, A.; Lay, P.A. Stabilities and biological activities of vanadium drugs: What is the nature of the active species? Chem.-Asian J. 2017, 12, 1692–1699. [CrossRef]

92. Irving, E.; Tagalakis, A.D.; Maeshima, R.; Hart, S.L.; Eaton, S.; Lehtonen, A.; Stoker, A.W. The liposomal delivery of hydrophobic oxidovanadium complexes imparts highly effective cytotoxicity and differentiating capacity in neuroblastoma tumour cells. Sci. Rep. 2020, 10, 16660. [CrossRef]

93. Erxleben, A. Transition metal salen complexes in bioinorganic and medicinal chemistry. Inorg. Chim. Acta 2018, 472, 40–57. [CrossRef]

94. Nunes, P.; Yildizhan, Y.; Adiguzel, Z.; Marques, F.; Costa Pessoa, J.; Acilan, C.; Correia, I. Copper(II) and oxidovanadium(IV) complexes of chormone Schiff bases as potential anticancer agents. J. Biol. Inorg. Chem. 2022, 27, 89–109. [CrossRef]

95. Chatterjee, K.K.; Favier, N.; Douglas, B.E. Copper complexes of o-hydroxy Schiff bases and the hydrolysis of the Schiff bases. J. Am. Chem. Soc. 1963, 85, 2919–2922. [CrossRef]

96. Reeves, R.L. On the mechanism, substituent effects, and intramolecular catalysis in Schiff base hydrolysis. J. Org. Chem. 1965, 30, 3129–3135. [CrossRef]
97. Sagasser, J.; Ma, B.N.; Baeker, D.; Salcher, S.; Herrmann, M.; Lamprecht, J.; Angerer, S.; Obexer, P.; Kircher, B.; Gust, R. A new approach in cancer therapy: Discovery of chlorido[N,N′-disalicylidene-1,2-phenylenediamine]iron(III) complexes as ferroptosis inducers. J. Med. Chem. 2019, 62, 8053–8061. [CrossRef]

98. Baeker, D.; Ma, B.N.; Sagasser, J.; Schultz, L.; Hoerschlaeger, C.; Weinreich, M.; Steiner, L.; Kircher, B.; Gust, R. Amide and ester derivatives of chlorido[4-carboxyl-1,2-disalicylideneaminobenzene]iron(III) as necroptosis and ferroptosis inducers. Dalton Trans. 2020, 49, 6842–6853. [CrossRef] [PubMed]

99. Bouche, M.; Hognon, C.; Grandemange, S.; Monari, A.; Gros, P.C. Recent advances in iron-complexes as drug candidates for cancer therapy: Reactivity, mechanism of action and metabolites. Dalton Trans. 2020, 49, 11451–11466. [CrossRef]

100. Baecker, D.; SESli, Ö.; Knabl, L.; Huber, S.; Orth-Höller, D.; Gust, R. Investigating the antibacterial activity of salen/salophene metal complexes: Induction of ferroptosis as part of the mode of action. Eur. J. Med. Chem. 2021, 209, 112907. [CrossRef] [PubMed]

101. Cosials, E.; El Hage, R.; Dos Santos, L.; Gong, C.; Mehrpour, M.; Hamai, A. Ferroptosis: Cancer stem cells rely on iron until “to die for” it. Cells 2021, 10, 2981. [CrossRef] [PubMed]

102. Benjamin-Rivera, J.A.; Cardona-Rivera, A.E.; Vazquez-Maldonado, A.L.; Dones-Lassalle, C.Y.; Pabon-Colon, H.L.; Rodriguez-Rivera, H.M.; Rodriguez, I.; Gonzalez-Espriet, J.C.; Pazol, J.; Perez-Rios, J.D.; et al. Exploring serum transferrin regulation of nonferric metal therapeutic function and toxicity. Inorganics 2020, 8, 48. [CrossRef]

103. Levina, A.; Lay, P.A. Transferrin cycle and clinical roles of citrate and ascorbate in improved iron metabolism. ACS Chem. Biol. 2019, 14, 893–900. [CrossRef] [PubMed]

104. Gaur, K.; Perez Otero, S.C.; Benjamin-Rivera, J.A.; Rodriguez, I.; Loza-Rosas, S.A.; Vazquez Salgado, A.M.; Akam, E.A.; Hernandez-Matias, L.; Sharma, R.K.; Alicea, N.; et al. Iron chelator transmetallative approach to inhibit human ribonucleotide reductase. JACS Au 2021, 1, 865–878. [CrossRef]

105. Mueller, I.A.; Keppler, B.K.; Berger, W. Challenges and chances in the preclinical to clinical translation of anticancer metallodrugs. RSC Metallobiol. 2019, 22, 381–409. [CrossRef] [PubMed]

106. Singh, R.B.; Ishii, H. Analytical potentialities of thiosemicarbazones and semicarbazones. Anal. Chim. Acta 2017, 94, 120–126. [CrossRef]

107. Heffeter, P.; Pape, V.F.S.; Enyedy, E.A.; Keppler, B.K.; Szakacs, G.; Kowol, C.R. Anticancer thiosemicarbazones: Chemical properties, interaction with iron metabolism, and resistance development. Antioxid. Redox Signal. 2019, 30, 1062–1082. [CrossRef] [PubMed]

108. Carcelli, M.; Tegoni, M.; Bartoli, J.; Marzano, C.; Pelosi, G.; Salvalaio, M.; Rogolino, D.; Gandin, V. In vitro and in vivo anticancer activity of tridentate thiosemicarbazone copper complexes: Unravelling an unexplored pharmacological target. Eur. J. Med. Chem. 2020, 194, 112266. [CrossRef]

109. Vieites, M.; Buccino, P.; Otero, L.; Gonzalez, M.; Piro, O.E.; Sanchez Delgado, R.; Sant’Anna, C.M.R.; Barreiro, E.J.; Cerecetto, H.; Gambino, D. Chemo-selective hydrolysis of the iminic moiety in salicylaldehyde semicarbazone promoted by ruthenium. Inorg. Chim. Acta 2005, 358, 3065–3074. [CrossRef]

110. Kopf, H.; Kopf-Maier, P. Titanocene dichloride—The first metalocene with cancerostatic activity. Angew. Chem. Int. Ed. 1979, 18, 477–478. [CrossRef] [PubMed]

111. Mattern, J.; Keppler, B.; Volm, M. Preclinical evaluation of diethoxy(1-phenyl-1,3-butanedionato)titanium(IV) in human tumor xenografts. Arzneim.-Forsch. 1984, 34, 1289–1290. [CrossRef]

112. Tshuva, E.Y.; Miller, M. Coordination complexes of titanium(IV) for anticancer therapy. Met. Ions Life Sci. 2018, 18, 219–250. [CrossRef] [PubMed]

113. Poetsch, I.; Baier, D.; Keppler, B.K.; Berger, W. Challenges and chances in the preclinical to clinical translation of anticancer metallo-drugs. RSC Metallodrugs. 2019, 14, 308–347. [CrossRef] [PubMed]

114. Cini, M.; Bradshaw, T.D.; Woodward, S. Using titanium complexes to defeat cancer: The view from the shoulders of titans. Chem. Soc. Rev. 2017, 46, 1040–1051. [CrossRef] [PubMed]

115. Tinoco, A.D.; Saxena, M.; Sharma, S.; Noinaj, N.; Delgado, Y.; Quinones Gonzalez, E.P.; Conklin, S.E.; Zambrana, N.; Loza-Rosas, S.A.; Parks, T.B. Unusual synergism of transferrin and citrate in the regulation of Ti(IV) speciation, transport, and toxicity. J. Am. Chem. Soc. 2016, 138, 5695–5669. [CrossRef] [PubMed]

116. Loza-Rosas, S.A.; Vazquez-Salgado, A.M.; Rivero, K.I.; Negron, L.J.; Delgado, Y.; Benjamin-Rivera, J.A.; Vazquez-Maldonado, A.L.; Parks, T.B.; Munet-Colon, H.L.; Tinoco, A.D. Expanding the therapeutic potential of the iron chelator deferasirox in the development of aqueous stable Ti(IV) anticancer complexes. Inorg. Chem. 2017, 56, 7788–7802. [CrossRef] [PubMed]

117. Saxena, M.; Loza-Rosas, S.A.; Gaur, K.; Sharma, S.; Perez Otero, S.C.; Tinoco, A.D. Exploring titanium(IV) chemical proximity to iron(III) to elucidate a function for Ti(IV) in the human body. Coord. Chem. Rev. 2018, 363, 109–125. [CrossRef] [PubMed]

118. Serrano, R.; Martinez-Aregido, I.; Fernandez-Sanchez, M.; Pacheco-Linan, P.; Bravo, I.; Cohen, B.; Calero, R.; Ruiz, M.J. New titanocene derivative with improved stability and binding ability to albumin exhibits high anticancer activity. J. Inorg. Biochem. 2021, 223, 111562. [CrossRef] [PubMed]

119. Fernandez-Vega, L.; Silva, V.A.R.; Dominguez-Gonzalez, T.M.; Claudio-Betancourt, S.; Toro-Maldonado, R.E.; Maso, L.C.C.; Ortiz, K.S.; Perez-Verdejo, J.A.; Gonzalez, J.R.; Rosado-Fratelli, G.T.; et al. Evaluating ligand modifications of the titanocene and auranofin moieties for the development of more potent anticancer drugs. Inorganics 2020, 8, 10. [CrossRef]

120. Nahari, G.; Braithbard, O.; Larush, L.; Hochman, J.; Tshuva, E.Y. Effective oral administration of an antitumorogenic nanoformulated titanium complex. ChemMedChem 2021, 16, 108–112. [CrossRef] [PubMed]
121. Sweeney, N.J.; Mendoza, O.; Mueller-Bunz, H.; Pampillon, C.; Rehmann, F.-J.K.; Strohfeldt, K.; Tacke, M. Novel benzyl substituted titanocene anti-cancer drugs. J. Organomet. Chem. 2005, 690, 4537–4544. [CrossRef]
122. Meker, S.; Bratibard, O.; Hall, M.D.; Hochman, J.; Tishuva, E.Y. Specific design of titanium(IV) phenolato chelates stable and accessible, effective and selective anticancer agents. Chem. – Eur. J. 2016, 22, 9986–9995. [CrossRef] [PubMed]
123. Lui, G.Y.L.; Kovacevic, Z.; Richardson, V.; Merlot, A.M.; Kalinowski, D.S.; Richardson, D.R. Targeting cancer by binding iron: Dissecting cellular signaling pathways. Oncotarget 2015, 6, 18746–18779. [CrossRef] [PubMed]
124. Chitambar, C.R. Gallium and its competing roles with iron in biological systems. Biochim. Biophys. Acta- Mol. Cell Res. 2016, 1863, 2044–2053. [CrossRef] [PubMed]
125. Chitambar, C.R. Gallium complexes as anticancer drugs. Met. Ions Life Sci. 2007, 790, 44, 1722–1727. [CrossRef]
126. Peng, X.-X.; Gao, S.; Zhang, J.-L. Gallium (III) complexes in cancer chemotherapy. Inorg. Chem. 2018, 57, 119, 5269–5295. [CrossRef] [PubMed]
127. Krakoff, I.H.; Newman, R.A.; Goldberg, R.S. Clinical toxicologic and pharmacologic studies of gallium nitrate. Cancer 1979, 44, 1722–1727. [CrossRef]
128. Glickson, J.D.; Pitner, T.P.; Webb, J.; Gams, R.A. Hydrogen-1 and gallium-71 nuclear magnetic resonance study of gallium citrate in aqueous solution. J. Am. Chem. Soc. 1975, 97, 1679–1683. [CrossRef]
129. O’Brien, P.; Salacinski, H.; Motevalli, M. The X-ray single crystal structure of a gallium complex (NH4)[Ga(C6H5O7)2], 4H2O. J. Am. Chem. Soc. 1997, 119, 12695–12696. [CrossRef]
130. El Hage Chahine, J.-M.; Hemadi, M.; Ha-Duong, N.-T. Uptake and release of metal ions by transferrin and interaction with receptor I. Biochim. Biophys. Acta. Subj. 2007, 1800, 334–347. [CrossRef] [PubMed]
131. Hummer, A.A.; Bartel, C.; Arion, V.B.; Jakupec, M.A.; Meyer-Klaucke, W.; Geraki, T.; Quinn, P.D.; Keppler, B.K.; El Hage Chahine, J.-M.; Hemadi, M.; Ha-Duong, N.-T. Uptake and release of metal ions by transferrin and interaction with receptor I. Biochim. Biophys. Acta. Subj. 2007, 1800, 334–347. [CrossRef] [PubMed]
132. Nguyen, A. Biological Speciation of Therapeutic Gallium Drugs. Ph.D. Thesis, The University of Sydney, Sydney, Australia, 2012.
133. Wood, M.L. Investigating Uptake and Biochemical Effects of Gallium Anti-Cancer Agents. Ph.D. Thesis, The University of Sydney, Sydney, Australia, 2014.
134. Tardito, S.; Barilli, A.; Bassanetti, I.; Tegoni, M.; Bussolati, O.; Franchi-Gazzola, R.; Mucchino, C.; Marchio, L. Copper-dependent cytotoxicity of 8-hydroxyquinoline derivatives correlates with their hydrophobicity and does not require caspase activation. J. Med. Chem. 2012, 55, 10448–10459. [CrossRef] [PubMed]
135. Bockman, R.S.; Boskey, A.L.; Blumenthal, N.C.; Alcock, N.W.; Warrell, R.P., Jr. Gallium increases bone calcium and crystallite perfection of hydroxyapatite. Calciif. Tissue Int. 1986, 39, 376–381. [CrossRef] [PubMed]
136. Wang, Y.; Han, B.; Xie, Y.; Wang, H.; Wang, R.; Xia, W.; Li, H.; Sun, H. Combination of gallium(III) with acetate for combating antibiotic resistant Pseudomonas aeruginosa. Chem. Sci. 2019, 10, 6099–6106. [CrossRef] [PubMed]
137. Bastos, R.W.; Rossato, L.; Valero, C.; Lagrou, K.; Colombo, A.L.; Goldman, G.H. Potential of gallium as an antifungal agent. Front. Cell. Infect. Microbiol. 2019, 9, 414. [CrossRef]
138. Neill, C.; Harris, S.; Goldstone, R.; Lau, E.; Henry, T.B.; Yu, H.H.P.; Smith, D. Antibacterial activities of gallium Ga(III) against E. coli are substantially impacted by ferric Fe(III) uptake systems and multidrug resistance (MDR) in combination with oxygen stress response (evgS) protect Escherichia coli from killing by gallium nitrate, an antimicrobial candidate. Antimicrob. Agents Chemother. 2021, 65, e01595. [CrossRef]
139. Wang, Z.; Li, J.; Benin, B.M.; Yu, B.; Bunge, S.D.; Abeydeera, N.; Huang, S.D.; Kim, M.-H. Lipophilic Ga complex with broad-spectrum antimicrobial activity and the ability to overcome gallium resistance in both Pseudomonas aeruginosa and Staphylococcus aureus. J. Med. Chem. 2021, 64, 9381–9388. [CrossRef]
140. Pandey, A.; Smilowicz, D.; Boros, E. Gallobloxacin: A xenometal-antibiotic with potent in vitro and in vivo efficacy against S. aureus. Chem. Sci. 2021, 12, 14546–14556. [CrossRef]
141. Visaggio, D.; Frangipani, E.; Hijazi, S.; Pirolo, M.; Leoni, L.; Rampioni, G.; Imperi, F.; Bernstein, L.; Sorrentino, R.; Ungaro, F.; et al. Variable susceptibility to gallium compounds of major cystic fibrosis pathogens. ACS Infect. Dis. 2022, 8, 78–85. [CrossRef]
142. King, M.M.; Kayastha, B.B.; Franklin, M.J.; Patrauchan, M.A. Calcium regulation of bacterial virulence. Adv. Exp. Med. Biol. 2020, 1131, 827–855. [CrossRef] [PubMed]
143. Zeng, J.; Wu, L.; Liu, Z.; Lv, Y.; Feng, J.; Wang, W.; Xue, Y.; Wang, D.; Li, J.; Drlica, K.; et al. Gain-of-function mutations in acid stress response (evgS) protect Escherichia coli from killing by gallium nitrate, an antimicrobial candidate. Antimicrob. Agents Chemother. 2021, 65, e01595. [CrossRef]
144. Seo, S.K.; Liu, C.; Dadwal, S.S. Infectious disease complications in patients with cancer. Crit. Care Clin. 2021, 37, 69–84. [CrossRef] [PubMed]
145. Chang, S.W.; Lewis, A.R.; Prosser, K.E.; Thompson, J.R.; Gladkich, M.; Bally, M.B.; Warren, J.J.; Walsby, C.J. CF3 Derivatives of the anticancer Ru(III) complexes KP1019, NKP-1339, and their imidazole and pyridine analogues show enhanced lipophilicity, albumin interactions, and cytotoxicity. Inorg. Chem. 2016, 55, 4850–4863. [CrossRef] [PubMed]
146. Alessio, E. Thirty years of the drug candidate NAMI-A and the myths in the field of ruthenium anticancer compounds: A personal perspective. Eur. J. Inorg. Chem. 2017, 2017, 1549–1560. [CrossRef]
148. Alessio, E.; Messori, L. NAMI-A and KP1019/1339, two iconic ruthenium anticancer drug candidates face-to-face: A case story in medicinal inorganic chemistry. *Molecules* **2019**, *24*, 1995. [CrossRef] [PubMed]

149. Thota, S.; Rodrigues, D.A.; Crans, D.C.; Barreiro, E.J. Ru(II) compounds: Next-generation anticancer metallotherapeutics? *J. Med. Chem.* **2018**, *61*, 5805–5821. [CrossRef]

150. Trondil, R.; Heffeter, P.; Kowol, C.R.; Jakupc, M.A.; Berger, W.; Keppler, B.K. NKp-1339, the first ruthenium-based anticancer drug on the edge to clinical application. *Chem. Sci.* **2014**, *5*, 2925–2932. [CrossRef]

151. Wernitznig, D.; Kiakos, K.; Del Favero, G.; Harner, N.; Machat, H.; Osswald, A.; Jakupc, M.A.; Wernitznig, A.; Sommergruber, W.; Keppler, B.K. First-in-class ruthenium anticancer drug (KP1339/IT-139) induces an immunogenic cell death signature in colorectal spheroids in vitro. *Metallomics* **2019**, *11*, 1044–1048. [CrossRef]

152. Neuditschko, B.; Gerner, C.; Legin, A.A.; Bäier, D.; Schintlmeister, A.; Wagner, M.; Reipert, S.; Keppler, B.K.; Berger, W.; Meier-Menches, S.M. Interaction with ribosomal proteins accompanies ER stress-induction of the anticancer metallodrug BOLD-100/KP1339. *Angew. Chem.* **2021**, *60*, 5063–5068. [CrossRef]

153. Baier, D.; Schoenhacker-Alte, B.; Rusz, M.; Pirker, C.; Mohr, T.; Mendrina, T.; Kirchhofer, D.; Meier-Menches, S.M.; Hohenwallner, K.; Schauer, M.; et al. The anticancer ruthenium compound BOLD-100 targets glycolysis and generates a metabolic vulnerability towards glucose deprivation. *Pharmaceutics* **2022**, *14*, 238. [CrossRef]

154. Jones, M.R.; Mu, C.; Wang, M.C.P.; Webb, M.I.; Walsby, C.J.; Storr, T. Modulation of the Aβ peptide aggregation pathway by KP1019 limits Aβ-associated neurotoxicity. *Metallomics* **2015**, *7*, 129–135. [CrossRef] [PubMed]

155. Yawson, G.K.; Will, M.F.; Huffman, S.E.; Strandquist, E.T.; Bothwell, P.J.; Oliver, E.B.; Apuzzo, C.F.; Platt, D.C.; Weitzel, C.S.; Jones, M.A.; et al. A dual-pronged approach: A ruthenium(III) complex that modulates amyloid-β aggregation and disrupts its formed aggregates. *Inorg. Chem.* **2021**, *60*, 2733–2744. [CrossRef] [PubMed]

156. Liu, M.; Lim, Z.J.; Gwee, Y.Y.; Levine, A.; Lay, P.A. Characterization of a ruthenium(III)/NAMI-A adduct with bovine serum albumin that exhibits a high anti-metastatic activity. *Angew. Chem. Int. Ed.* **2010**, *49*, 1661–1664. [CrossRef]

157. Cetinbas, N.; Webb, M.I.; Dubland, J.A.; Walsby, C.J. Serum-protein interactions with anticancer Ru(III) complexes KP1019 and KP418 characterized by EPR. *JBC J. Biol. Inorg. Chem.* **2010**, *15*, 131–145. [CrossRef] [PubMed]

158. Webb, M.I.; Chard, R.A.; Al-Jobory, Y.M.; Jones, M.R.; Wong, E.W.Y.; Walsby, C.J.; Pyridine analogues of the antimetastatic Ru(III) complex NAMI-A targeting non-covalent interactions with albumin. *Inorg. Chem.* **2012**, *51*, 954–966. [CrossRef]

159. Webb, M.I.; Wu, B.; Jang, T.; Chard, R.A.; Wong, E.W.Y.; Song, M.Q.; Yapp, D.T.T.; Walsby, C.J. Increasing the bioavailability of Ru(III) anticancer complexes through hydrophobic albumin interactions. *Chem. Eur. J.* **2013**, *19*, 17031–17042. [CrossRef]

160. Levine, A.; Aitken, J.B.; Gwee, Y.Y.; Lim, Z.J.; Liu, M.; Singhary, A.M.; Wong, P.F.; Lay, P.A. Biotransformations of anticancer ruthenium(III) complexes: An X-ray absorption spectroscopic study. *Chem.-Eur. J.* **2013**, *19*, 3609–3619. [CrossRef]

161. Casini, A.; Temperini, C.; Gabbiani, C.; Supuran, C.T.; Messori, L. The X-ray structure of the adduct between NAMI-A and carbonic anhydrase provides insights into the reactivity of this metallodrug with proteins. *ChemMedChem* **2010**, *5*, 1989–1994. [CrossRef]

162. Messori, L.; Merlino, A. Ruthenium metathelation of proteins: The X-ray structure of the complex formed between NAMI-A and hen egg white lysozyme. *Dalton Trans.* **2014**, *43*, 6128–6131. [CrossRef]

163. Chiniadis, L.; Giastas, P.; Bratsos, I.; Papakyriakou, A. Insights into the protein ruthenation mechanism by antimetastatic metallo drugs: High-resolution X-ray structures of the adduct formed between hen egg-white lysozyme and NAMI-A at various time points. *Inorg. Chem.* **2021**, *60*, 10729–10737. [CrossRef]

164. Bijelic, A.; Theiner, S.; Keppler, B.K.; Rompel, A. X-ray structure analysis of indazolium trans-[tetrachlorobis(1H-indazole)ruthenate(III)] (KP1019) bound to human serum albumin reveals two ruthenium binding sites and provides insights into the drug binding mechanism. *J. Med. Chem.* **2016**, *59*, 5894–5903. [CrossRef] [PubMed]

165. Munteanu, A.-C.; Uivarosi, V. Ruthenium complexes in the fight against pathogenic microorganisms. an extensive review. *Pharmaceutics* **2021**, *13*, 874. [CrossRef] [PubMed]

166. Huffman, S.E.; Yawson, G.K.; Fisher, S.S.; Bothwell, P.J.; Platt, D.C.; Jones, M.A.; Hamaker, C.G.; Webb, M.I. Ruthenium(III) complexes containing thiazole-based ligands that modulate amyloid-β aggregation. *Metallomics* **2020**, *12*, 491–503. [CrossRef] [PubMed]

167. Riccardi, C.; Musumeci, D.; Trifuoggi, M.; Irace, C.; Paduano, L.; Montesarchio, D. Anticancer ruthenium(III) complexes and Ru(III)-containing nanoformulations: An update on the mechanism of action and biological activity. *Pharmaceuticals* **2019**, *12*, 146. [CrossRef] [PubMed]

168. Englinger, B.; Pirker, C.; Heffeter, P.; Terenzi, A.; Kowol, C.R.; Keppler, B.K.; Berger, W. Metal drugs and the anticancer immune response. *Chem. Rev.* **2019**, *119*, 1519–1624. [CrossRef]

169. Sen, S.; Won, M.; Levine, M.S.; Noh, Y.; Sedgwick, A.C.; Kim, J.S.; Sessler, J.L.; Arambula, J.F. Metal-based anticancer agents as immunogenic cell death inducers: The past, present, and future. *Chem. Soc. Rev.* **2022**, in press. [CrossRef]

170. Champliau, T.; Tseliakas, L.; Farhane, S.; Raoulit, T.; Texier, M.; Lanoy, E.; Massard, C.; Robert, C.; Ammari, S.; De Baere, T.; et al. Intratumoral immunotherapy: From trial design to clinical practice. *Clin. Cancer Res.* **2021**, *27*, 665–679. [CrossRef]

171. Cai, S.; Xie, Y.; Bagby, T.R.; Cohen, M.S.; Forrest, M.L. Intralymphatic chemotherapy using a hyaluronan–cisplatin conjugate. *J. Surg. Res.* **2008**, *147*, 247–252. [CrossRef]
