

Transition metal compounds as cancer radiosensitizers

Martin R. Gill^{*a} and Katherine A. Vallis^a

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The concomitant administration of ionising radiation (IR) in the form of external beam radiotherapy or targeted radionuclide therapy (TRT) alongside radiosensitizing small molecules is a highly successful strategy for the treatment of cancer. The major clinical impact of the radiosensitizing platinum(II) drug cisplatin has encouraged the design of many transition metal coordination or organometallic complexes and their assessment as anticancer candidates. The growing recognition that metallodrugs halt cancer cell proliferation through mechanisms other than DNA damage and the recent expansion of the range of their potential biological targets has further stimulated the field. A central aim is to generate new therapeutic candidates with improved anticancer activity, distinct mechanisms of action(s) and reduced cross-resistance compared to existing drugs. The question arises of how lead candidates should be combined with other treatment modalities, particularly radiotherapy. In this review, work is highlighted that specifically examines transition metal complexes in combination with IR with an emphasis on complexes that function as radiosensitizers. The chemical design principles and cellular mechanisms of action to achieve synergistic or additive effects in cancer cell killing are outlined. Finally, we discuss emerging applications in this area of research, such as the combination of metallo-compound radiosensitizers with radionuclides and within drug delivery approaches.

1 Introduction

Cancer remains one of humanity's greatest challenges. According to the World Health Organisation (WHO) it is the second leading cause of death globally with approximately 14 million new cases in 2012 and a predicted rise in new cases of 70% over the next 2 decades.¹ Radiotherapy is a critically important component of oncologic practice, with roughly a half of all cancer patients receiving this form of treatment in the modern era.² External beam radiotherapy (EBRT) uses high-energy electrons, γ -, X-rays or, more recently, charged particles as hadrontherapy, to target localised tumour sites. Targeted radionuclide therapy (TRT), on the other hand, is used to treat widespread disease.³ Despite the great success and central role of radiotherapy in cancer medicine, side effects and resistance remain on-going challenges. For external beam sources damage to healthy organs that lie adjacent to tumours can be particularly challenging. To minimise adverse effects, careful treatment planning is employed to optimise the radiation absorbed dose distribution. The benefits of radiotherapy can sometimes be enhanced by delivering it in combination with other treatment modalities and a particularly successful strategy is the use of concomitant chemoradiotherapy for the treatment of solid tumours.^{4,5} A key aspect of the clinical rationale for combining radiation with small molecule therapeutics is the ability of some to function as radiosensitizers, where synchronous exposure results in an

additive or supra-additive (i.e. synergistic) impact on radiation-induced cell killing compared to either treatment in isolation. Many established chemotherapy drugs such as gemcitabine and fluorouracil (5FU) are known to be radiosensitizers and are frequently used in concurrent chemoradiotherapy regimens to sensitize tumour cells to IR.⁶ However, DNA-damaging chemotherapeutics present a "double-edged sword", inevitably resulting in toxicity due to their detrimental effects on normal cells, while the genotoxicity associated with these agents may result in the emergence of treatment-induced cancers.⁷ As such, the identification of efficient radiosensitizers with minimal side-effects remains highly desirable. Deeper understanding of the genetic basis of cancers has facilitated the development of targeted therapeutics that act on specific signalling pathways that cancer cells rely on for proliferation and avoidance of cell death. Many such targeted therapeutics have radiosensitizing properties and are being explored in combination with IR in clinical trials.⁸ To date, small organic molecules make up the majority of radiosensitizers that have been used in the clinic or are under investigation.⁹ However, more recently heavy-metal materials such as gold nanoparticles have been explored in this context.¹⁰

There has also been considerable recent interest in the anticancer activity of "cold" (i.e. non-radioactive) transition metal organometallic or coordination compounds and many biologically-active transition metal scaffolds have now been described.^{11,12} Transition metal chemistry presents attractive features for the design of biologically-active small molecules, where since judicious selection of metal centre, coordination number, labile groups, and bioactive or ancillary ligands may result in distinct biological mechanisms of action.¹³ Moreover,

^a CRUK/MRC Oxford Institute for Radiation Oncology, Department of Oncology, University of Oxford, Oxford, UK. Email: martin.gill@oncology.ox.ac.uk

as transition metal centres may possess coordination numbers greater than carbon's limit of four bonds, increased "chemical space" is thereby available compared to pure organics. This provides chemical diversity for drug design, which in turn, it is hoped, will translate to novel biomolecular binding interactions and downstream cellular responses.¹⁴ The best example of a clinical metal-based anticancer drug is cisplatin, or cis-diamminedichloridoplatinum(II), a chemically reactive platinum(II) complex that forms platinum-DNA cross-links by coordinate bond generation between the Pt(II) centre and the nitrogen atoms of DNA base pairs, particularly guanine. Based on the success of cisplatin, numerous reactive metal halide complexes have been investigated for cytotoxicity while, more recently, substitutionally inert complexes have come under greater scrutiny. Many complexes have been selected for study on the basis of cell-free DNA binding interactions and marked *in vitro* cytotoxicity, however, recent efforts have been made to expand the range of biological targets of these agents and to generate distinct biological mechanisms of action.

In addition to their effects as single-agents, the role of transition metal complexes as photosensitizers for photodynamic therapy (PDT) or as light-activated pro-drugs within photoactivated chemotherapy (PACT) has been explored extensively.^{15–17} These applications rely upon the photosensitizer generating cytotoxic singlet oxygen (PDT) or dissociating into cytotoxic species upon exposure to light (PACT). As PDT and PACT combine non-cytotoxic light with a non-cytotoxic photosensitizer, substantial therapeutic enhancement in a highly localised environment can be achieved. However, a substantial limitation of PDT and PACT for treating cancers is the limited penetrative depth of light in tissue: typically less than 5 mm, even for near-infrared (NIR) light.¹⁸ For this reason, the use of PDT is limited to superficial tumours or those that are accessible by endoscopy, although light sources with greater penetrative depth and the localised delivery of light are being explored to address this issue.¹⁹ In contrast, high energy X-, γ -rays or ion beams have the advantage of a far greater penetrative depth than light (>10 cm, Fig. 1a) and can therefore target *any* region within the body. In addition to IR from an external beam source, radionuclides can be used to provide localised delivery of radiotoxic IR as either brachytherapy or TRT. The selection of a particular radionuclide for a given clinical situation is based on the type of emission (α , β particles as well as Auger electrons or γ -rays, or combinations of these) as well as their energy and path length (Fig. 1b).²⁰

In contrast to the extensive research into metal complexes as single-agents or photosensitizers, the possible use of metal complexes as radiosensitizers or in combination with IR has been largely ignored, despite the widespread use of cisplatin alongside radiotherapy. This deficit is highlighted by a widely-cited review on both topics which dedicates only a short section to radiosensitization.²¹ In this review, the general mechanisms of radiosensitization of cancer cells by small molecules will be outlined while work to date that has specifically examined transition metal complexes in this capacity is summarised. Finally, suggestions for future directions that this potentially promising area of research may take are provided.

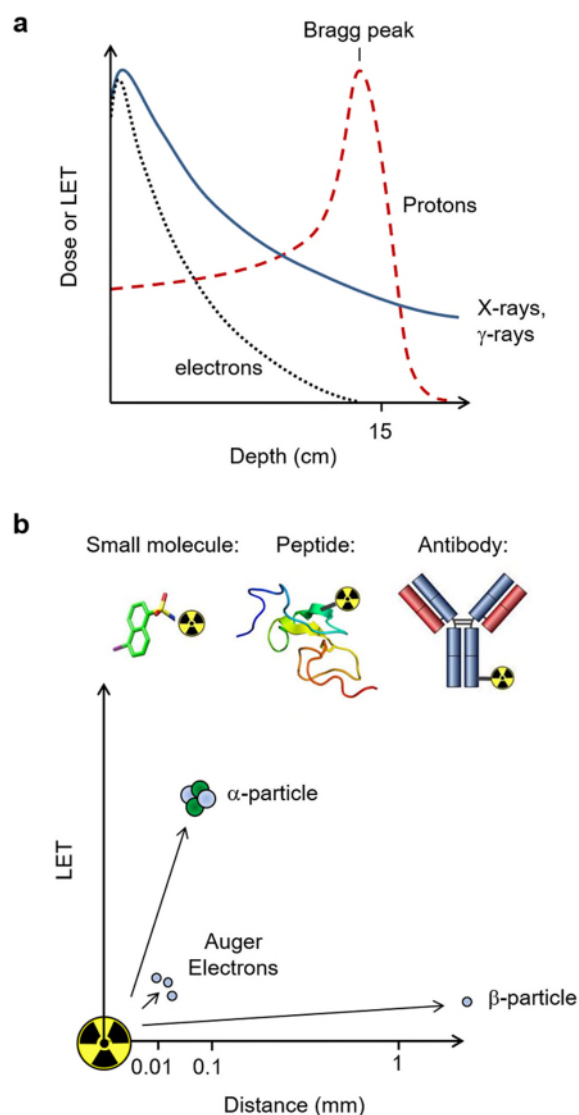


Fig. 1 (a) Approximate depth range of selected external beam ionising radiation (IR) sources in tissue. Adapted from reference 22. (b) Depiction of classes of targeted radionuclide therapeutics (top) and LET versus distance for common radionuclide decay products (bottom). LET = linear energy transfer. Adapted from data within reference 20.

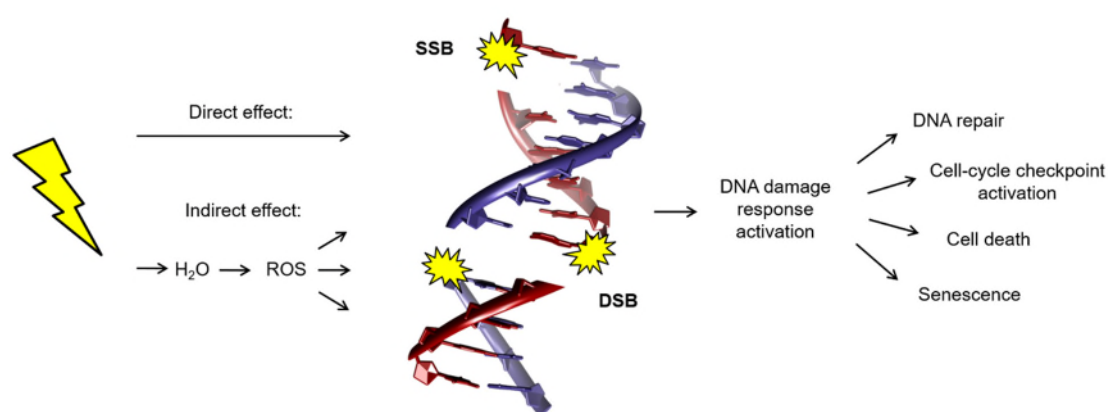


Fig. 2 Simplified representation of cellular responses to DNA damage lesions induced by IR. ROS = reactive oxygen species, SSB = single strand break, DSB = double strand break.

2 Radiotherapy

The toxic effects of radiotherapy are primarily attributed to the generation of DNA damage which, if unrepaired, results in cell death or permanent growth arrest. Thus the uncontrolled proliferation of cancer cells and tumour growth are impeded when tumours are exposed to IR. DNA lesions may result from direct damage, following the interaction of photons or ionizing particles with DNA itself, or indirect damage, which occurs via the generation of reactive oxygen species (ROS), particularly hydroxyl radicals formed by the radiolysis of water, which may then react with DNA (Fig. 2). A variety of DNA lesions are generated by IR, most notably single-strand breaks (SSBs) and double-strand breaks (DSBs), where the number of SSBs per cell generated are substantially (25-fold) greater than DSBs.²³ Although less frequent than SSBs, DSBs are more lethal, triggering cell death pathways such as mitotic catastrophe or apoptosis.²⁴ The success of external beam radiotherapy relies upon the ability to focus the radiation to the tumour as well as the generally reduced DNA repair capacity of cancer versus normal tissues. However, collateral irradiation of nearby healthy cells results in toxicity whilst mis-repaired or unrepaired DSB damage may result in mutagenesis and malignant cell transformation. A further challenge to the use of radiotherapy is hypoxia; regions within tumours that are marked by low oxygen content, where IR is less effective due to lower ROS generation.

2.1 Chemoradiotherapy

Radiotherapy is used as the principal curative treatment modality for a number of cancers when the extent of disease is limited to the primary cancer or to the primary cancer plus regional lymph nodes and when surgery is not possible for technical or other reasons. In many situations this high-dose (radical) use of radiotherapy has been found to be most effective when combined with concurrent or immediately sequential chemotherapy. In many other clinical situations, such as following surgery for early breast cancer, lower dose

radiotherapy is delivered to eliminate any residual microscopic amounts of disease. When radiotherapy is used in this way, as a post-operative adjuvant therapy, outcomes are excellent and simultaneous chemotherapy usually unnecessary.

Cancers for which there is randomised clinical trial evidence of benefit from chemo-radiotherapy regimens include certain stages of non-small cell lung cancer, squamous cell carcinoma of the head and neck and cancers of the urinary bladder, oesophagus, anal canal and uterine cervix. In almost all these cases the chemoradiotherapy protocol includes cisplatin or a closely related drug. One justification for combining chemotherapy with radiotherapy is that local delivery of radiation eradicates the primary tumour while systemic chemotherapy eliminates occult micro-metastases. In addition to this 'spatial cooperation' between the two modalities, a major advantage of combination protocols is the radiosensitizing effect of chemotherapy whereby the interaction between drug and radiation in the irradiated volume results in enhanced cancer cell kill.⁴

2.2 Radiosensitizers

As experimental therapeutics progress through pre-clinical studies and clinical trials, it is often beneficial to determine their effect in combination with IR at an early stage to facilitate integration into existing treatment regimens. If the combination results in a biological response greater than would have been expected from the predicted additive effect of IR treatment and the therapeutic, it is a synergistic relationship and the agent is classified as a radiosensitizer. Many potent radiosensitizers employed in the clinic are DNA-damaging agents and prominent examples include the nucleoside analogue gemcitabine, the antimetabolite 5FU (fluorouracil) and alkylating agent temozolomide (Fig. 3a).

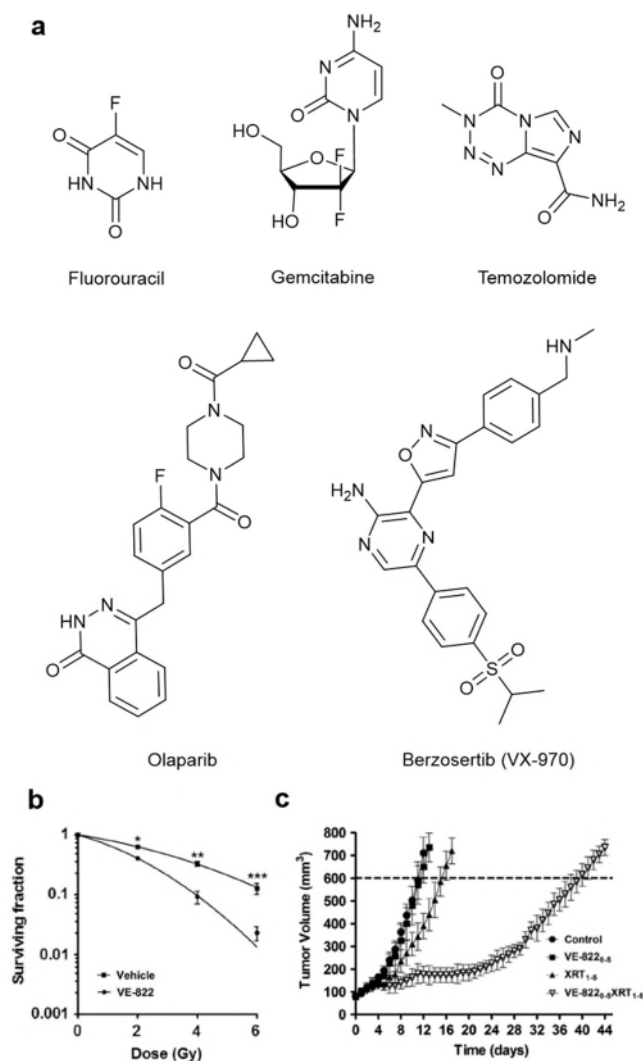


Fig. 3 (a) Selected examples of organic radiosensitizers currently used in the clinic or in late-stage clinical development. (b) Clonogenic survival of PSN-1 pancreatic cancer cells after exposure to VE-822 (VX-970) (80 nM), demonstrating efficient radiosensitization by the decrease in surviving fraction in compound-treated versus vehicle control. (c) Enhancement of tumour response to IR by VE-822 in PSN-1 xenografts. Mice bearing PSN-1 xenograft tumours ($n=4$) were treated with vehicle (control), VE-822 (60 mg/kg) from days 0 to 5 (VE-822₀₋₅), fractionated IR using five daily doses of 2 Gy from day 1 to day 5 (XRT₁₋₅) or the combination of VE-822 fractionated IR (VE-822₀₋₅XRT₁₋₅). Note: VE-822 = VX-970 (now Berzosertib) and XRT = IR. Figures 3b,c adapted from reference 27 under the terms of the Creative Commons Attribution-NonCommercial-No Derivative Works 3.0 Unported License (<http://creativecommons.org/licenses/by-nc-nd/3.0/>). Published by Springer Nature.

2.2.1 Mechanisms of radiosensitization Radiosensitization is most commonly established *in vitro* in clonogenic survival assays, which provide an accurate measure of irreversible inhibition of proliferation by IR. Compound-treated cells are exposed to an IR dose range and resultant surviving fractions are compared to a control group treated in the absence of compound. By employing a sub-cytotoxic therapeutic dose of the compound or normalising data to account for single-agent effects, a supra-additive or synergistic relationship may be

differentiated from additivity, thereby demonstrating the compound is a radiosensitizer (Fig. 3b). *In vivo* studies in animal models of cancer may confirm a synergistic relationship and determine optimum dosage and timing of the combination (Fig. 3c).

Radiosensitizers operate by several non-exclusive mechanisms to exert their radiation-enhancing effects on cancer cells, which are summarised below. In early work, Fowler and others categorized five possible mechanisms of radiosensitization:²⁵

(1) Enhancement of IR-generated DNA damage. As DNA is regarded as the critical target of IR, small molecules capable of enhancing DNA damage at sub-cytotoxic doses will cause decreased cell survival in IR-treated cells.

(2) Inhibition of post-irradiation cellular repair. Based on the identification of DNA damage as the critical target for IR, it follows that pharmacological or genetic inhibition of DNA damage repair pathways will act to increase IR-induced DNA damage, thereby increasing radiotoxicity. Prominent examples include the PARP inhibitor olaparib²⁶ and the ATR inhibitor VX-970 (Berzosertib, Fig. 3a),²⁷ which each act to prevent repair of SSB breaks and to exacerbate IR-induced DNA damage. These agents are currently undergoing clinical trials in combination with radiotherapy.

(3) Cell-cycle dysregulation. Cells in late G1 or G2/M phases of the cell cycle are relatively sensitive to the effects of IR while cells in S phase are the most resistant. Accordingly, compounds that induce cell cycle blocks at specific points in the cell-cycle are often investigated as radiosensitizers. Many of the current portfolio of targeted therapeutics act to inhibit specific cell-cycle proteins required for cell-cycle progression and have shown promise in this capacity.⁸

(4) Activity enhancement in hypoxic cells. As IR mainly acts by generating ROS which cause DNA damage, hypoxic cancers are often radioresistant. It follows that if ROS production is artificially increased, the effectiveness of RT will be improved. Molecular oxygen, for example, is a potent radiosensitizer and oxygen-mimetic compounds such as nitroimidazoles, amongst others, have been examined for radiosensitization of hypoxic cancers.²⁸

(5) Radiolysis of the sensitizer to form cytotoxic molecular fragments. Although examples are relatively rare, radiation-activated prodrugs have been developed, such that their exposure to IR results in release of cytotoxic components and cell death.^{29,30}

This early classification of the properties of radiosensitizers has since been expanded:

(6) Inhibition of prosurvival/radioresistance pathways and abrogation of rapid tumour cell repopulation.⁴ Observations of differential gene expression in determining radiation sensitivity of a particular cancer compared to normal tissue has paved the way for inhibitors of signal transduction pathways to be examined alongside radiotherapy. For example, the epidermal growth factor receptor (EGFR) antibody cetuximab significantly increased overall survival when applied alongside radiotherapy for treating head and neck cancer,³¹ although the precise molecular basis for this effect is not clearly understood.

(7) Modulating the tumour microenvironment and amelioration of IR damage to healthy tissues.^{4,8} Although a challenge to treat with IR, hypoxic regions in cancers present an attractive target for radiosensitizer design as they are tumour specific. Efforts in this area include hypoxia-activated pro-drugs³² or molecules that act to modulate the aberrant vasculature commonly encountered in tumours.³³

(8) Finally, cellular internalisation of high atomic number (Z) materials before exposure to IR have also been documented to generate Auger effects resulting from inner-shell ionization.³⁴ The resultant Auger cascade may generate cytotoxic DNA damage if it occurs in close proximity to DNA. This is perhaps best demonstrated by stereotactic synchrotron radiotherapy (SSR), which involves loading a tumour with a high-Z number atom such as iodine before irradiation.³⁵ By tuning the X-rays to the energy of the K-shell electrons of the sensitizer, enhanced absorbed dose following irradiation may be achieved in tumour cells. This phenomenon may enhance the radiobiological effects of IR and potentially explains sensitization by heavy-metal nanomaterials such as gold nanoparticles.

3 Transition metal compounds as radiosensitizers

Transition metal chemistry presents attractive features for the design of biologically-active small molecules and many have been explored as anti-cancer agents.¹¹ For a given compound, bioactivity may result from biomolecular binding interactions, which may be covalent or non-covalent, metal- or ligand-based in origin, the generation of bioactive species through ligand dissociation or electron transfer reactions (i.e. the complex functions as a pro-drug) or through biorthogonal redox/osmotic/catalytic activity which may result in biomolecule degradation or organelle dysfunction. Metal complexes additionally possess many attractive properties for determining bioactivity: the luminescent properties of many coordination and organometallic complexes presents a convenient method by which to examine their cellular uptake and localisation.³⁶ Furthermore, the presence of a metal atom in the molecular structure makes mass spectrometry-based target validation approaches convenient while analytical techniques such as ICP-MS (inductively coupled plasma mass spectrometry) may be employed to obtain quantitative data on subcellular localisation and *in vivo* biodistribution. In this section we provide a review of anti-cancer transition metal complexes that have been examined in combination with IR, outlining the chemical and molecular basis for successful additive or synergistic relationships.

3.1 Platinum complexes

3.1.1 FDA-approved platinum drugs Initially identified by Rosenberg and co-workers as a potent anti-tumour agent,³⁷ the square planar Pt(II) complex cisplatin (cis-diamminedichloridoplatinum(II), CDDP, Fig. 4) was approved by the FDA in 1978 for the treatment of testicular cancer and is a broad-scope chemotherapeutic employed today in the management of many types of cancer, including testicular,

ovarian, lung, bladder and oesophageal cancers.³⁸ In addition to cisplatin, FDA-approved platinum drugs include carboplatin and oxaloplatin while nedaplatin has been approved for use in Japan (Fig. 4a). In addition to square planar Pt(II) complexes, octahedral Pt(IV) complexes such as Satraplatin are under investigation as orally-active pro-drugs (Fig. 4b). The mechanism of action of cisplatin and derivatives is through ligand substitution and the formation of inter- and intrastrand DNA adducts via coordinate bond formation to the Pt metal centre (Fig. 5a). The resultant Pt-DNA adducts act to distort DNA structure, inhibiting replication and transcription, typically resulting in G1 cell-cycle arrest and triggering apoptosis in proliferating cells.³⁹ In addition to binding DNA, cisplatin has also been reported to form Pt-protein adducts⁴⁰ and endoplasmic reticulum stress, mitochondrial stress and oxidative stress have all been documented in response to the drug.⁴¹ Although highly effective in many cases, acquired or intrinsic resistance to cisplatin limit its clinical use, as do associated serious side effects such as nephro-, neuro and ototoxicity. Resistance may result from decreased drug uptake, increased efflux, increased inactivation by sulfhydryl molecules, upregulated repair of Pt-DNA adducts by nucleotide excision repair (NER) or deficient activation of apoptotic pathways in response to cisplatin-induced DNA damage.³⁹

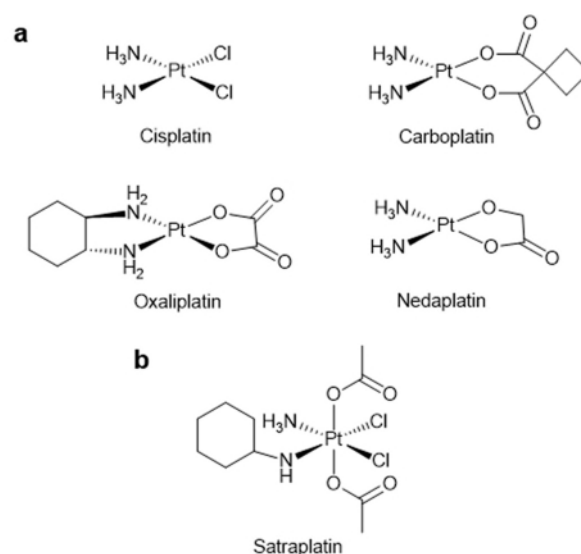


Fig. 4 (a) FDA-approved platinum(II) drugs cisplatin, carboplatin and oxaloplatin. Nedaplatin is approved for use in Japan. (b) Experimental Pt(IV) pro-drug Satraplatin.

As well as being an effective single-agent chemotherapeutic, cisplatin is a potent radiosensitizer that is active at nanomolar concentrations and is one of the drugs used most frequently in chemoradiotherapy regimens.⁴ Despite this, surprisingly few studies have examined the mechanisms by which cisplatin radiosensitizes cancer cells. Two main nonexclusive mechanisms have been proposed, both of which are based on direct platination of DNA rather than downstream cellular effects of cisplatin treatment, where the resultant cell-cycle

deregulation and increased percentage of cells in G1 phase would also be predicted to enhance radiosensitivity. Turchi *et al.* examined the biochemical mechanism of cisplatin-IR synergy in non-small cell lung cancer cells, and concluded that the observed cell death is a direct effect of interference with repair by non-homologous end-joining (NHEJ) when Pt-DNA lesions occur near DSB sites generated by IR. As a result, DSB damage, sustained DNA damage response (DDR) signalling and increased cell death ensues in NHEJ-proficient cells (Fig. 5b).^{42–44} In addition to the inhibition of DSB repair, Sanche and colleagues proposed that the reactive oxygen species generated in cells on exposure to IR cause greater damage in the presence of pre-existing cisplatin-DNA adducts. Employing low drug ratios (<1 Pt per 500 base pairs), which they suggest are similar to those achieved *in vivo*, they demonstrated in cell-free studies that SSB and DSB induced by low energy electrons (LEEs) are substantially enhanced when cisplatin is covalently bonded to DNA (Fig. 5c).^{45,46} As LEEs are major secondary products of high-energy radiation in biological material, they hypothesised that this phenomenon contributes to radiosensitization by cisplatin. Carboplatin and oxaliplatin have also been shown to be effective radiosensitizers *in vitro*.^{47,48} Although mechanisms of radiosensitization for carboplatin and oxaliplatin have not been defined, similarities with cisplatin would be predicted based on their similar ability to form Pt-DNA adducts. Interestingly, in cell-free studies conducted by Rezaee *et al.*, carboplatin and oxaliplatin-modified DNA resulted in greater sensitization to

LEEs than did DNA alterations associated with cisplatin. It was concluded that carboplatin should be considered a more efficient radiosensitizer than cisplatin for an equal number of Pt-adducts.⁴⁹

Although the high Z-number of the platinum core has been postulated to contribute towards the radiosensitizing effects of cisplatin and carboplatin,³⁴ this effect is likely minor in comparison to the biological effects of the drugs outlined above. Employing an *in vitro* and an *in vivo* brain tumour model, Rousseau *et al.* found the additive effect to be independent of the X-ray beam energy and concluded the production of Auger electrons (emitted through relaxation of excited platinum atoms following IR) do not contribute to the radiosensitizing effects of either platinum drug.⁵⁰

3.1.2 Experimental platinum therapeutics In addition to FDA-approved platinum drugs, the radiosensitizing effects of several chloroplatinate complexes was examined by Tiecher *et al.*^{51,52} This work featured the coordination of nitroaromatic heterocyclic compounds which function as radiosensitizers due to their ability to mimic oxygen. Similarly, Skov, Farrell and colleagues examined platinum complexes coordinated to nitroimidazole ligands, finding that the reactive Pt centre targeted these radiosensitizing groups to DNA.^{53–56} Disadvantages of these complexes were the relatively high concentrations (typically 100 or 200 μM) required for radiosensitization.

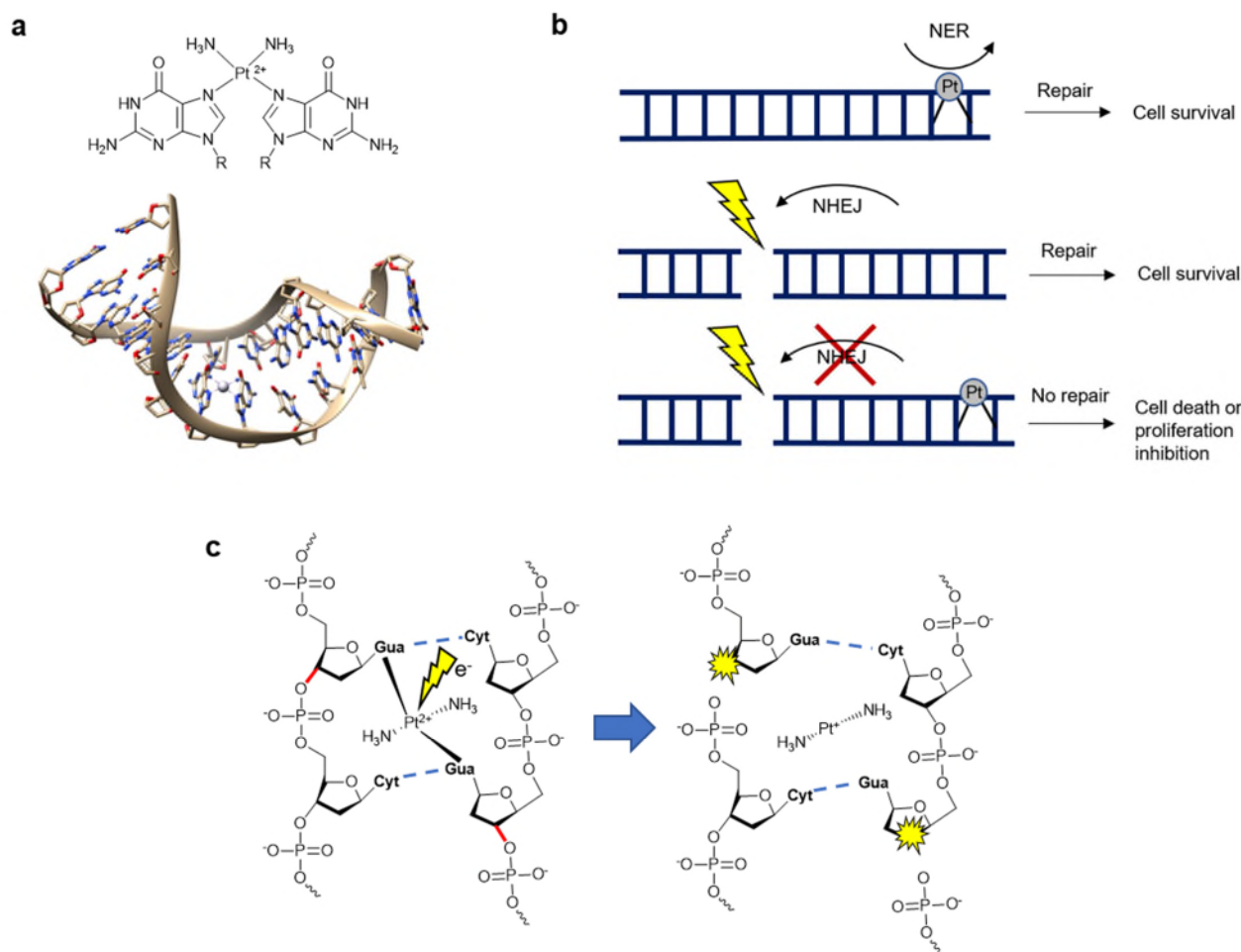


Fig. 5 (a) Chemical (top) and 2D NMR (bottom) structure of Pt(II)-DNA crosslink (NDB ID: 1A84). (b) Schematic of IR and platinum synergy, where Pt-DNA lesions interfere with NHEJ-mediated repair of IR-induced DSBs. References 42–44. NER = nucleotide excision repair, NHEJ = non-homologous end-joining. (c) Enhancement of DNA damage by IR in the presence of pre-existing cisplatin-DNA adducts. Adapted from references 45,46.

3.2 Ruthenium complexes

After platinum-containing compounds, ruthenium-based complexes represent the group of metallo complexes that have been most heavily-studied for anticancer activity. Attractive features for Ru pharmacophore design include distinct octahedral molecular geometries along with a wide variety of binding ligands or leaving group(s) for the construction of closely-related molecular libraries. Reactive Ru(II) or Ru(III) halides were the initial focus of research while, more recently, attention has turned to substitutionally inert Ru(II) complexes.

Notably, two ruthenium(III)-halide complexes NAMI (imidazolium trans-[tetrachlorido(1H-imidazole)(S-dimethyl sulfoxide)ruthenate(III)]) and KP1019 (indazolium trans-[tetrachloridobis(1H-indazole)ruthenate(III)]) have been investigated in phase I clinical trials^{57,58} while the PDT complex TLD-1433 [Ru(dmb)₂(LL')]²⁺ where dmb = 4,4'-dimethyl-2,2'-bipyridine and LL' = 2-(2',2'':5'',2'''-terthiophene)-imidazo[4,5-f][1,10] phenanthroline) has completed a Phase 1 clinical trial for treating bladder cancer with PDT (ClinicalTrials.gov Identifier: NCT03053635) (Fig. 6).

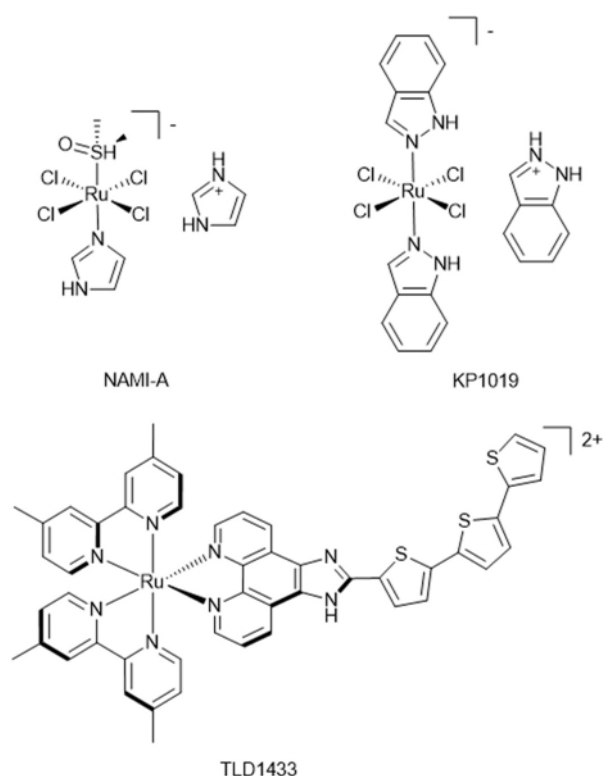


Fig. 6 Ruthenium complexes that have been tested in Phase I clinical trials.

3.2.1 Labile ruthenium complexes A large number of Ru(II)- or Ru(III)-halide coordination or organometallic compounds have been tested for bioactivity systematically examining the impact of ancillary ligand(s) and leaving group(s) on relative potency.⁵⁹ Many early candidates were designed to target DNA through the formation of ruthenium-DNA coordinate bonds in a manner analogous to canonical Pt(II) drugs.⁶⁰ However, DNA targeting is not a requirement for bioactivity. The aforementioned NAMI-A exerts an extra-cellular mechanism to inhibit cell motility⁶¹ while KP1019 possesses relatively mild *in vitro* cytotoxicity and is not believed to target DNA.⁶² Other noteworthy preclinical examples include the ruthenium(II)-arene bis-chlorido RAPTA compounds (general formula: $[\text{Ru}(\eta^6\text{-arene})(\text{PTA})(\text{Cl})_2]$ where $\text{PTA} = 1,3,5\text{-triaz-7-phosphatricyclo-[3.3.1.1]decane}$),⁶³ which demonstrate anti-metastatic and antiangiogenic effects⁶⁴ and the organometallic Ru(II) complex $[\text{Ru}(\text{phen})(\text{ppy})(\text{NCCH}_3)_2]\text{PF}_6$ ($\text{phen} = 1,10\text{-phenanthroline}$, $\text{ppy} = 2\text{-phenyl-pyridine}$) that inhibited the growth of U87 human glioblastoma tumours in xenograft models by inducing CHOP-mediated ER stress in addition to a mild DNA damage response.⁶⁵ Early work in this area examined a range of $[\text{RuCl}_2(\text{DMSO})_2(\text{N})_2]$ complexes where $\text{N} = \text{substituted 4-nitroimidazoles}$ in combination with IR, finding $\text{N} = 4\text{-nitroimidazole}$ was able to radiosensitize hypoxic CHO cells.^{66,67} Similar to work employing Pt(nitroimidazole) complexes described above, high concentrations (200 μM) were required for radiosensitization. Sadler and co-workers have conducted intensive investigations of Ru(II) arene monohalide complexes (general formula: $[\text{Ru}(\eta^6\text{-arene})(\text{en})(\text{Cl})]^+$ where $\text{en} = \text{H}_2\text{NCH}_2\text{CH}_2\text{NH}_2$ or derivatives thereof).⁶⁸ These complexes coordinate DNA through ligand substitution and form Ru(II)-DNA adducts with N7 of guanine bases.⁶⁹ While this is similar to cisplatin, these compounds possess a single leaving group compared to the two of traditional platinum-based drugs and so cannot cross-link DNA. In addition to this difference, the bulky arene ligand provides noncovalent hydrophobic interactions with DNA, including intercalation.⁷⁰ Most promisingly, a lead candidate developed by the group, $[\text{Ru}(\text{C}_6\text{H}_5\text{C}_6\text{H}_5)(\text{en})(\text{Cl})]\text{PF}_6$, demonstrates reduced cross-resistance in cisplatin-resistant ovarian cancer cells *in vitro* and in mouse xenograft models.⁷¹ The cellular mechanism of action for a representative molecule from this class was found to be G1 arrest accompanied by activation of p53/p21 DNA damage signalling pathways⁷² and the majority of complexes induce high levels of apoptosis at low micromolar doses. Although it was speculated by the authors that there was an altered cellular response compared to cisplatin, temporal activation of DDR signalling in a similar manner to cisplatin was apparent. Thus the cellular response to the Ru-DNA lesions would appear to bare close similarity to the cellular processing of Pt-DNA adducts.

Carter *et al.* screened the radiosensitizing capability of 14 ruthenium-arene complexes developed by the Sadler group, and found that five complexes functioned as radiosensitizers of DLD1 colorectal cancer cells in MTT cell proliferation assays.⁷³ Clonogenic survival assays confirmed radiosensitization for two complexes, AH54 $[\text{Ru}(\eta^6\text{-flu})(\text{en})(\text{Cl})]\text{Cl}$ and AH63 $[\text{Ru}(\eta^6\text{-dihyphen})(\text{en})(\text{Cl})]\text{Cl}$ ($\text{flu} = \text{fluorene}$ and $\text{dihyphen} = 9,10\text{-dihydrophenanthrene}$, Fig 7a,b). Wildtype p53 was required for radiosensitization in HCT116 colorectal cancer cells, implying activation of this pathway is necessary for radiosensitization by these two agents. As for $[\text{Ru}(\text{C}_6\text{H}_5\text{C}_6\text{H}_5)(\text{en})(\text{Cl})]\text{PF}_6$, both AH54 and AH63 retained their effectiveness as single-agents towards oxaliplatin- and cisplatin-resistant cancer cells.

Deng *et al.* examined the selenium-containing Ru(II) complex $[\text{Ru}(\text{phtpy})(\text{phenSe})(\text{Cl})](\text{ClO}_4)$ ($\text{phtpy} = 4\text{-phenyl-2,20:60,200-terpyridine}$, $\text{phenSe} = 2\text{-selenicimidazole[4,5-f]1,10-phenanthroline}$) in combination with IR (Fig. 7c).⁷⁴ In this work, the novel strategy of introducing Se into a Ru complex to increase lipophilicity was tested and resulted in enhanced cellular uptake. The authors examined the combination of this complex with X-rays (8 Gy) in A375 melanoma cells, and found a greater impact on cell viability (by MTT assay) for the combination than for radiation alone. Although a synergistic impact (and therefore radiosensitization) on cell survival was not shown, an additive effect of the compound and X-rays was apparent with increased intracellular ROS levels and G2/M arrest following the combined treatment. Encouragingly, a more modest effect was observed in non-malignant HK-2 human kidney cells, likely due to the lower cellular uptake and corresponding single-agent cytotoxicity of the compound in this cell line.

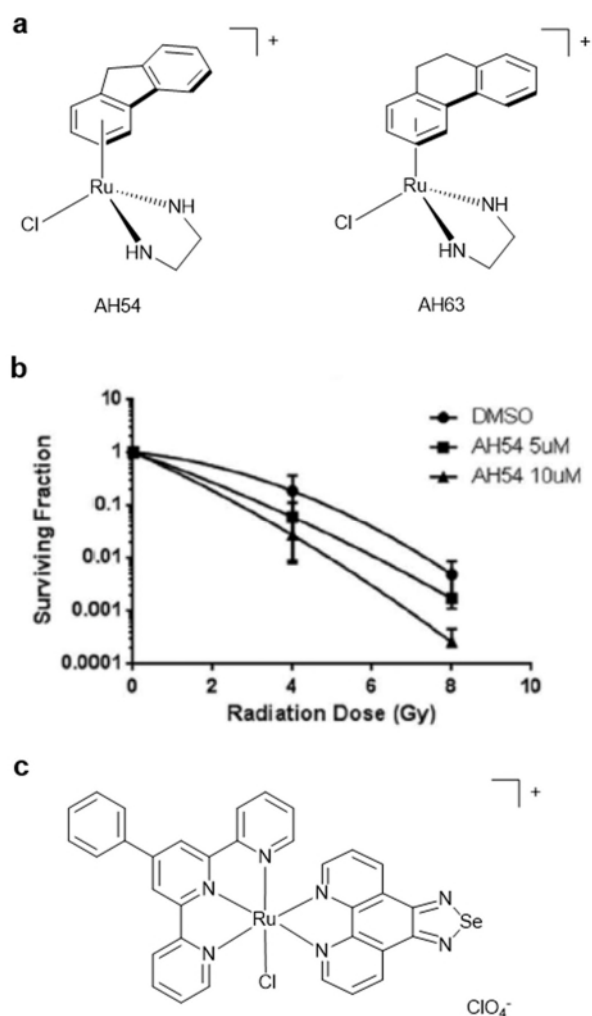


Fig. 7 (a) Ru(II) arene “piano stool” complexes AH54 $[\text{Ru}(\eta^6\text{-flu})(\text{en})(\text{Cl})]^+$ and AH63 $[\text{Ru}(\eta^6\text{-dihyphen})(\text{en})(\text{Cl})]^+$ (b) Clonogenic survival of p53 wildtype HCT116 cells pre-treated with AH54 (5 or 10 μM) for 6 hrs before 0–8 Gy ^{137}Cs - γ -rays. Medium was refreshed 24 hours after radiation. Adapted from reference 73 under the terms of the Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>). Published by Springer Nature Limited. © The Author 2016. (c) Selenium-containing Ru(II) complex $[\text{Ru}(\text{phtpy})(\text{phenSe})(\text{Cl})]^+$. Featured in reference 74.

3.2.2 Substitutionally inert ruthenium complexes In addition to reactive ruthenium-halide complexes, substitutionally inert ruthenium complexes have also been investigated for anticancer activity. These are perhaps best exemplified by Ru(II) polypyridyl complexes (RPCs), which typically employ bi- or tridentate polypyridyl ligands and may incorporate organometallic or coordination bonding. Originally developed as site- and structure-specific reversible DNA-binding agents,⁷⁵ the distinctive increase in MLCT (metal-to-ligand charge transfer) emission that occurs when these agents bind DNA has provoked substantial interest in their cellular uptake, biomolecule targeting and anticancer properties.⁷⁶ Bio-activity is primarily determined by ligand-biomolecule interactions and cellular uptake and localisation is influenced by charge and

overall hydrophobicity. RPCs such as TLD-1433 are excellent photosensitizers, with excitation into $^3\text{MLCT}$ or $^3\text{LLCT}$ (ligand-to-ligand charge transfer) states by visible light facilitating the generation of cytotoxic $^1\text{O}_2$.¹⁵ Non-PDT complexes possess a range of intracellular targets and mechanisms of action and we direct readers to two excellent recent reviews on this subject.^{77,78} Of particular significance is work employing the dinuclear DNA groove-binder $[(\text{phen})_2\text{Ru}(\text{tatpp})\text{Ru}(\text{phen})_2](\text{Cl})_4$ (tatpp = 9,11,20,22-tetraazatetrapyrido[3,2- α :2',3'-c:3'',2''-1:2''',3'''-n]-pentacene) that is able to generate catalytic generation of DNA damage and has proven effective in stopping the growth of hypoxic lung cancer cells, including in mouse xenograft models.^{79,80}

We recently demonstrated that $[\text{Ru}(\text{dppz})_2(\text{PIP})]^{2+}$ (dppz = dipyrido[3,2- α :2',3'-c]phenazine, PIP = (2-(phenyl)imidazo[4,5-f][1,10]phenanthroline), a hydrophobic multi-intercalator, acts to rapidly stall DNA replication forks and induce a cell-cycle block at the G1-S phase transition (Fig. 8a,c).⁸¹ Importantly, $[\text{Ru}(\text{dppz})_2(\text{PIP})]^{2+}$ is able to radiosensitize HeLa cervical cancer cells in addition to its single agent effects and the addition of $[\text{Ru}(\text{dppz})_2(\text{PIP})]^{2+}$ to IR results in an increase in IR-induced DNA damage, as shown by the increased expression of the DNA damage marker γH2AX . Interestingly, a close structural derivative, $[\text{Ru}(\text{dppz})_2(\text{p-HPIP})]^{2+}$ (p-HPIP = (2-(4-hydroxyphenyl)imidazo[4,5-f][1,10]phenanthroline), demonstrates a relatively modest radiosensitizing effect. As the latter complex operates via a DNA damage-independent mechanism, we concluded that the impact of $[\text{Ru}(\text{dppz})_2(\text{PIP})]^{2+}$ on DNA replication was a requirement for efficient radiosensitization. Subsequent examination of $[\text{Ru}(\text{phen})_2(\text{tpphz})]^{2+}$ (Fig. 8b), a hydrophilic mono-intercalator previously reported to demonstrate high nuclear uptake and to image DNA,⁸² revealed this complex likewise acts to stall DNA replication fork progression, activating the replication stress and DSB DDR pathways upon fork collapse (Fig. 8c).⁸³ Moreover, the generation of metaphase chromosome spindle attachment failure by $[\text{Ru}(\text{phen})_2(\text{tpphz})]^{2+}$ results in blockade of mitotic progression, thereby resulting in preferential activity towards rapidly-proliferating cancer cells with elevated mitotic indices (Fig. 8d). In a panel of p53-deficient oesophageal cancer cells, effective radiosensitization at sub-cytotoxic doses (2 μM) of $[\text{Ru}(\text{phen})_2(\text{tpphz})]^{2+}$ was observed (Fig. 8e). Dose modification factors (DMF = ratio of radiation doses with or without the compound, causing the same level of effect (in this case, the same reduction in SF)) at $\text{S.F.} = 0.1$ were comparable or greater than cells treated with cisplatin. A substantial increase in the expression of the DNA damage marker γH2AX were observed in radiosensitizing conditions at an early timepoint by both immunofluorescence and immunostaining techniques, indicating DNA damage enhancement to be the primary mechanism of radiosensitization (Fig. 8f). The absence of induced apoptosis despite high levels of DNA replication stress distinguishes the mechanisms of action of both $[\text{Ru}(\text{dppz})_2(\text{PIP})]^{2+}$ and $[\text{Ru}(\text{phen})_2(\text{tpphz})]^{2+}$ from the majority of DNA-damaging agents and is likely responsible for their low cytotoxicity towards non-cancer cell lines.

In other work, Deng *et al.* examined three Ru(II) complexes containing bis-benzimidazole derivatives.⁸⁴ Cytotoxicity enhancement in A375 human skin melanoma cells was observed with the addition of X-rays where the most efficient complex effectively sensitized A375 cells to 8 Gy X-ray

irradiation. An increase in ROS and DNA damage in dual-treated cells compared to single-agent conditions was demonstrated, which the authors suggested was responsible for the radiosensitizing properties of the complex.

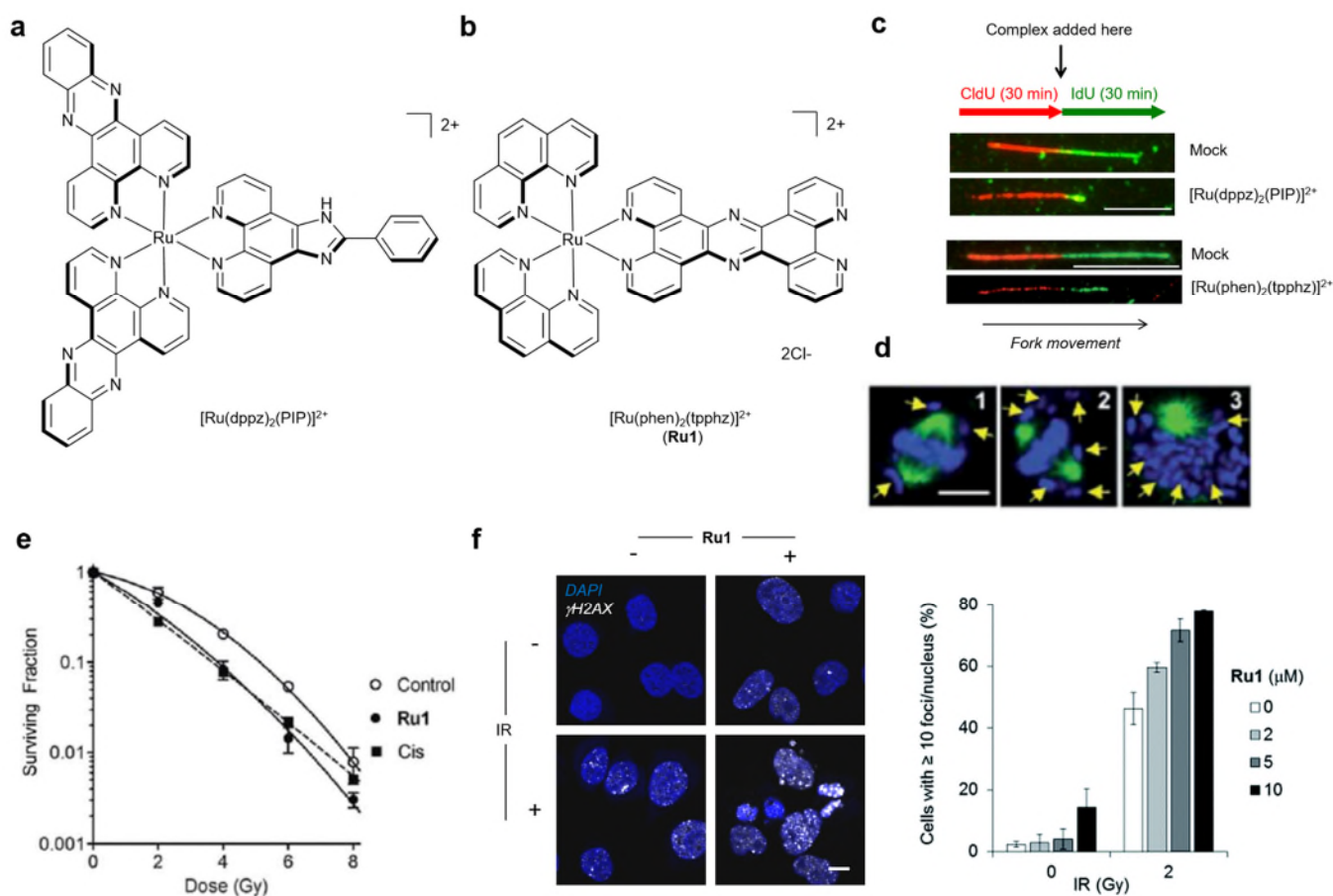


Fig. 8 (a,b) Structures of Ru(II) polypyridyl radiosensitizers $[\text{Ru}(\text{dppz})_2(\text{PIP})]^{2+}$ and $[\text{Ru}(\text{phen})_2(\text{tpphz})]^{2+}$ (**Ru1**). (c) Stalling of DNA replication forks by $[\text{Ru}(\text{dppz})_2(\text{PIP})]^{2+}$ and **Ru1** in HeLa and OE21 cells respectively, as shown by DNA fibre assay. (d) Chromosome misalignments in metaphase OE21 oesophageal cancer cells treated with **Ru1**. Blue = DAPI. Green = α -tubulin. (e) Radiosensitization of OE33 oesophageal cancer cells to ^{137}Cs - γ -rays by **Ru1** (2 μM , 24 h treatment before IR exposure), as determined by clonogenic survival assay. (f) DNA damage enhancement by **Ru1** in combination with IR (2 Gy) in OE21 cells. Left, immunofluorescence staining with anti- γH2AX antibody (white) provides visualisation of DSB damage per nucleus (DAPI, blue). Right, quantification of γH2AX foci/nucleus in control (white bars) and **Ru1**-treated cells (grey) +/- IR. Scale bars = 10 μm . Figure 8c, top, adapted from reference 81 under the terms of the Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>). Published by Springer Nature Limited. © The Author 2016. Figures 8c, bottom, and 8d-g adapted from reference 83 under the terms of the Creative Commons Attribution 3.0 Unported Licence (<https://creativecommons.org/licenses/by/3.0/>). Published by The Royal Society of Chemistry. © The Author 2018.

3.3 Cobalt complexes

The range of cobalt oxidation states and coordination numbers are attractive properties for the construction of substitutionally-labile complexes or the intracellular release of bioactive species.⁸⁵ For example, the reduction of Co(III) to the more kinetically labile Co(II) can release cytotoxic ligands in hypoxic conditions.⁸⁶ This effect has been explored utilising IR for radiolysis. This may be demonstrated by the Co(III) complex containing the potent DNA minor groove alkylator azachloromethylbenzindoline (Fig. 9a). Irradiation of the parent complex was then able to generate a potent cytotoxin when reduced by either IR or cells under hypoxic conditions.⁸⁷ Earlier work in the early by Teicher *et al.* examined several Co(III)

complexes coordinated to a varying number of NO_2 ligands as potential radiosensitizers towards normoxic and hypoxic EMT6 mouse breast cancer cells.^{88,89} Several complexes demonstrated effective radiosensitization with a DMF of 2.4 and the trans-tetramminedinitro complex $[\text{Co}(\text{NH}_3)_4(\text{NO}_2)_2]\text{CH}_3\text{CO}_2$ showed a DMF of 1.6 in a murine tumour model (Fig. 9b). A relatively high concentration of Co(III) complex (100 μM) was required for efficient *in vitro* radiosensitization and was also required for *in vivo* studies (5 x 200 mg/kg).

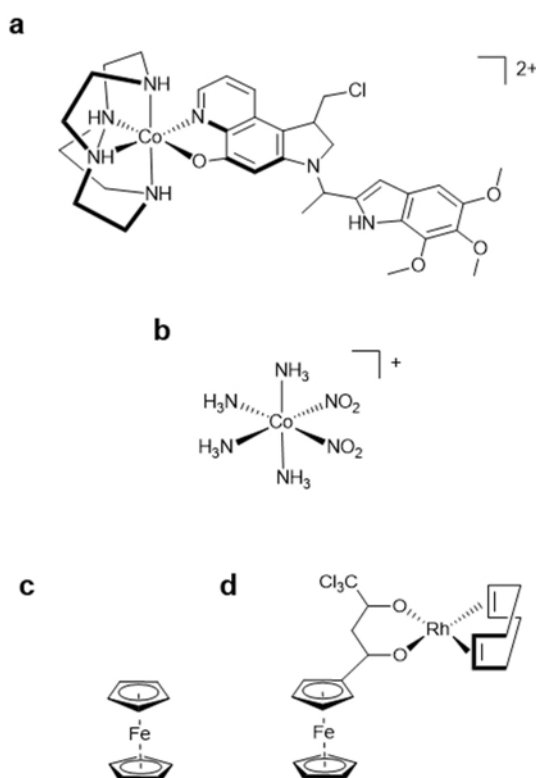


Fig. 9 (a,b) Examples of cobalt radiosensitizers. (c) Ferrocene and a ferrocene-Rh conjugate radiosensitizer (d). References 87– 89, 91 and 98.

3.4 Iron complexes

Ferrocene (Fig. 9c), one of the most distinctive organometallic complexes, and its derivatives have been investigated for their anticancer and anti-bacterial properties.⁹⁰ However, despite the large number of ferrocene derivatives that have been prepared and examined for bioactivity, there are surprisingly few studies examining this class of molecule in combination with IR. Yet evidence for the radiosensitizing properties of ferrocene was provided by Teicher *et al.*⁸⁸ and a detailed study by Joy *et al.* examined two ferricenium salts as radiosensitizers of hypoxic cells *in vitro* and of KHT murine sarcoma cells *in vivo*.⁹¹ A DMF of 2 for sensitization of hypoxic V79 Chinese hamster lung fibroblast cells by 10 μ M ferrocene was achieved.

3.5 Rhodium complexes

Rhodium(III) or iridium(III) complexes exhibit a variety of properties that make them interesting as prospective anticancer drugs. As for Ru(II) complexes, reactivity and cellular uptake are strongly dependent on their ligand combination and coordination geometry. In addition they offer a high level of stability due to their relative chemical inertness. As such, a growing number of rhodium or iridium scaffolds have been examined for biological activity.⁹² This includes work by the Barton group utilising rhodium-based polypyridyl complexes to target mismatch sites of DNA.^{93–95} This class of compound

selectively target these sites over matched DNA where they bind via metallo-insertion with ejection of the mismatched base pairs. These complexes are preferentially active towards MMR-deficient cancer cells, where DNA lesions generated by metallo-insertion activate Chk1 and γ H2AX DNA damage response signalling pathways,⁹⁶ indicating both replication stress and DSB damage are induced. Similar to work using Ru(II) polypyridyl metallo-intercalators,^{81,83} the authors excluded apoptosis as a major response to DNA damage to lesions generated by metallo-insertion and concluded cell-cycle arrest to be the predominant mechanism of proliferation inhibition.

The potential for Rh complexes as radiosensitizers is shown in work on Rh(II) nitroimidazole, where the Rh(II) complexes displayed greater sensitization than the comparable Pt(II) complexes.⁹⁷ More recently, two potent ferrocene-derived anticancer agents [Rh(fcta)(cod)] and [Rh(fctca)(cod)] (fcta = ferrocenoylacetonato-4,4,4-trifluoro, fctca = ferrocenoyl-4,4,4-trichloro- and cod = 1,5-cyclooctadiene) were examined in combination with IR.⁹⁸ The rhodium (I) complex [Rh(fcta)(cod)] (Fig. 9d) was found to have a comparable DMF to cisplatin (1.93 and 1.99 respectively). While Ir(III) complexes are commonly encountered as photosensitizers,¹⁷ their interaction with IR is currently unknown.

3.6 Gadolinium complexes

Extending our examples to include lanthanides as well as transition metals, gadolinium (Gd)-containing porphyrin-based complexes have also been assessed as radiosensitizers. Frequently employed as contrast agents for MRI (magnetic resonance imaging), their preferential uptake by tumour cells compared to normal tissue, high nuclear accumulation,⁹⁹ and ability to inhibit antioxidant proteins such as thioredoxin reductase¹⁰⁰ are seen as strong advantages in this capacity. Young *et al.* presented results showing two gadolinium(III) Texaphyrin complexes radiosensitized a murine mammary carcinoma model *in vitro* and *in vivo*.¹⁰¹ A follow-up phase I trial showed selective localisation of Motexafin gadolinium (also known as Gadolinium(III) texaphyrin, MGd, Fig. 10) in tumours¹⁰² and a phase III trial showed MGd provided modest improvement in time to neurological progression in patients with brain metastases from lung cancer when it was combined with whole brain radiotherapy.¹⁰³ MGd is also being evaluated in ongoing or completed clinical trials involving radiotherapy for other central nervous system tumours such as glioblastoma multiforme and pediatric brainstem gliomas, as well as various carcinomas including pancreaticobiliary, non-small cell lung, and head and neck cancers.¹⁰⁴ The molecular basis for radiosensitization by MGd remains controversial,¹⁰⁵ although increased oxidative stress as a consequence of futile redox cycling¹⁰⁶ or presence of high-Z number Gd atoms⁹⁹ have been suggested as explanations.

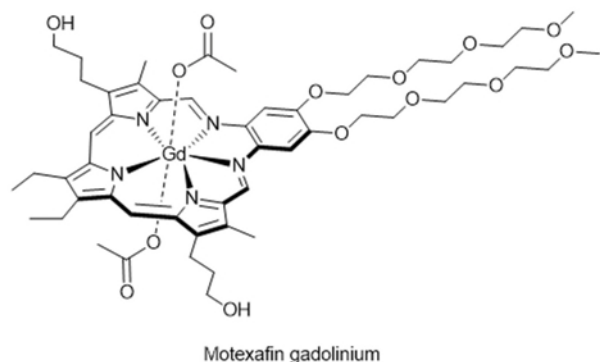


Fig. 10 Structure of motexafin gadolinium.

4 Hadrontherapy

Computational and imaging advances introduced over the last two decades allow modern photon radiotherapy to be delivered with a high level of precision, conforming in 3D to the tumour volume. However, beams must pass through normal tissues both before and after having reached deep-seated tumours. In addition, to account for uncertainties in the radiotherapy planning and delivery process, a rim of normal tissue surrounding a tumour often receives a moderate dose. Therefore, it remains a challenge to target the radiation to sites of disease in sufficient dose to halt tumour growth whilst minimising damage to healthy tissue. Hadrontherapy, that is the use of beams of charged particles, is typified by the “Bragg peak”, an abrupt release of energy at the end to the particle track (Fig. 1a). This phenomenon means that there is relatively little absorption of energy in tissues lying proximal to the tumour, and a sharp fall off in dose after the Bragg peak.²² The most widely used form of hadrontherapy employs proton beams, although the use of helium, lithium or carbon ions are also under investigation.¹⁰⁷ It has been speculated that the higher LET of “heavy ion” beams such as carbon result in altered radiobiology compared to low LET X- or γ -rays and is therefore being explored in cancers resistant to low LET radiation, such as hypoxic tumours.¹⁰⁸

4.1 FDA-approved platinum drugs

Clinical trials of cisplatin in combination with proton beam therapy for cervical (NCT01019278), oesophageal¹⁰⁹ and lung¹¹⁰ cancers are ongoing. The basis for an additive/synergistic combination of proton beam radiotherapy with FDA-approved platinum drugs is yet to be examined: a surprising omission considering there may be altered biological effects between proton and photon irradiations.¹¹¹

Nakano and co-workers examined the radiosensitizing effects of platinum drugs on carbon-ion beam irradiation. Kubo *et al.* showed the successful sensitization to carbon ions of H460 human non-small-cell lung cancer cells by carboplatin (Fig. 11a), where increased levels of the apoptotic markers cleaved caspase 3 and BAX indicated enhanced irradiation-induced

apoptosis due to carboplatin treatment (Fig. 11b).¹¹² Carboplatin treatment resulted in a reduced sensitizer enhancement ratio (SER, defined as the ratio of radiation doses required to obtain a SF (in this case a SF of 0.1) in the absence or presence of the drug) for carbon ions compared to X-rays (1.21 and 1.41 at the dose giving a 10% survival fraction for carbon ions and X-rays, respectively). For comparison, cells treated in parallel with the microtubule-stabilising agent paclitaxel showed consistent SER values for both beams (Fig. 11a). The same group showed in a panel of 20 human cancer cell lines that sensitization to carbon ions by carboplatin was weaker compared to an equal-but not isoeffective-dose (4 Gy) of X-rays.¹¹³ The authors suggested this indicates potentially different target mechanisms between X-rays and carbon ions and this work also showed high levels of mitotic catastrophe is generated by carbon ions, suggesting this is the primary mode of cell death. A related study showed carbon ion beam (average LET 50 keV/ μ m) suppressed malignant mesothelioma cell viability and the addition of cisplatin significantly enhanced its action.¹¹⁴ However, an additive rather than synergistic relationship was indicated by the majority of assays employed, potentially due to the relatively high concentrations (25 μ M) of cisplatin used.

4.2 Experimental platinum complexes

Le Cech and co-workers examined the Pt(II) complex [Pt(terpy)Cl](Cl) as a sensitizer for He²⁺ and C⁶⁺ ions in Chinese hamster ovary (CHO) cells (Fig. 11c), finding the presence of platinum increases the efficiency of both these ion beams in inducing cell death.¹¹⁵ Interestingly, the specific contribution of the heavy atom to cell death is increased at lower LET values and the most successful sensitization was observed for He²⁺ ions (LET = 2 keV/ μ m). Although this molecule is also able to metallo-intercalate DNA,¹¹⁶ NANO-SIMS showed a cytosolic localisation of the Pt complex. As this Pt(II) complex is non-cytotoxic, even at the maximum dose of 500 μ M, this supplies evidence that molecules containing high-Z atoms might efficiently enhance the cell death rate of hadrontherapy due to cellular internalisation rather than bioactivity.

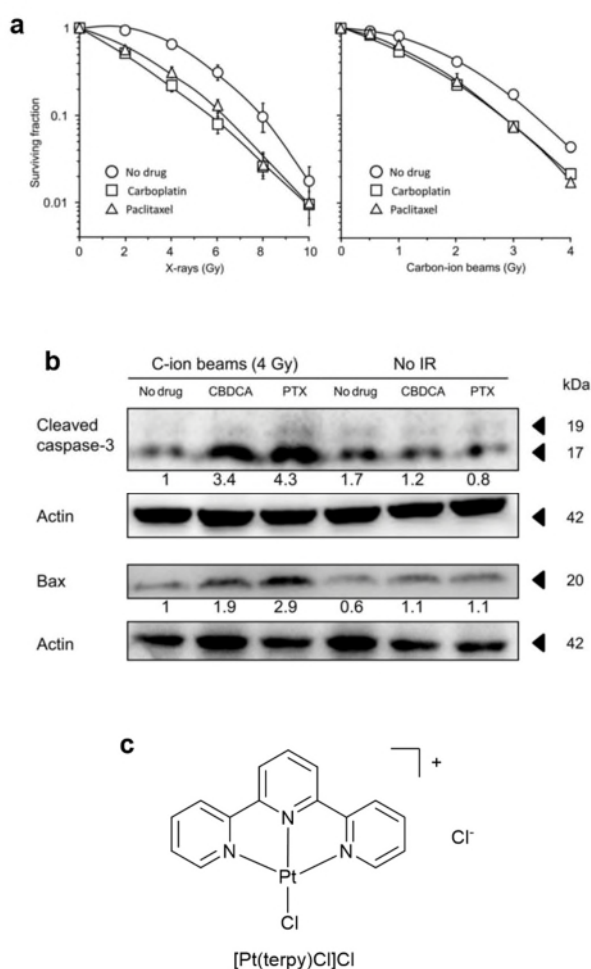


Fig. 11 (a) Sensitization of H460 human non-small-cell lung cancer cells to X-rays (left) or carbon ions (right) by carboplatin (7.3 μ M) or paclitaxel (8.3 nM). (b) Increased levels of apoptotic markers cleaved caspase 3 and BAX in H460 cells pre-treated with carboplatin (CBDCA) or Paclitaxel (PAX). (a,b) Adapted from reference 112 under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>). © The Author 2015. Published by Oxford University Press on behalf of The Japan Radiation Research Society and Japanese Society for Radiation Oncology. (c) Structure of non-cytotoxic Pt(II) complex [Pt(terpy)Cl]Cl that functions as a sensitizer for He^{2+} and carbon ions. Featured in reference 115.

5 Radionuclide therapeutics and brachytherapy

In addition to IR from an external beam source, radionuclides can be used to provide localised delivery of radiotoxic IR. This may be in the form of brachytherapy, which involves the radioactive source being placed directly into or near the site of disease. A variety of medical devices for administering brachytherapy exist, such as SIR-Spheres® (^{90}Y resin microspheres) for the treatment of liver metastases. In addition to this, conjugation of a radionuclide to a targeting vector allows molecularly-targeted, systemic delivery of IR to tumours.⁵ This latter form of treatment, TRT (targeted radionuclide therapy), has the advantage of being able to target metastatic sites of disease in addition to primary tumours, provided the molecular marker is expressed in these regions. This form of treatment, TRT, has the advantage that it can target multiple sites of

disease. The large number of vector–radionuclide pairings examined to date has resulted in several radiopharmaceuticals approved for clinical use or in late-stage clinical trials.³ A prominent recent example is the success of ^{177}Lu -Dotatate (dodecanetetraacetic acid-tyrosine-3-octreotate) in a Phase 3 trial for the treatment of neuroendocrine tumours.¹¹⁷

Several on-going clinical trials exploring TRT alongside cisplatin-based chemotherapy have provided encouraging results for combining radiosensitizing chemotherapy with TRT in a similar manner as for EBRT (external beam radiotherapy).⁵ In addition to this, concurrent cisplatin and brachytherapy has been used in the treatment of early or locally advanced cervical cancer.¹¹⁸ Although mechanisms of radiosensitization have not been examined in detail, for low LET β -emitting radionuclides such as ^{177}Lu similarities with EBRT would be predicted.

In a recent study Bai *et al.* utilised ^{125}I seeds for brachytherapy to enhance sensitivity of cancer cells towards the RPC L-[Ru(bpy)₂(o-tFMPIP)](ClO₄)₂ (o-tFMPIP = 20-trifluoromethylphenyl)imidazo [4,5-f][1,10]phenanthroline).¹¹⁹ Continuous, low-dose radiation with ^{125}I applied alongside this RPC decreased the inhibitory activities of the complex against HepG2 and SW480 cells 5.4 and 1.4-fold respectively, indicating at least an additive relationship, and the authors suggested enhanced DNA damage to accompany this finding. This work demonstrated an additive effect of the RPC and ^{125}I when applied together, however, further work would be required to establish radiosensitizing effects of the compound.

6 Delivery of radiosensitizing metallodrugs

The radiosensitizing effects of a metal complex may be enhanced and prolonged through use of an appropriate drug delivery vector. Numerous materials have been explored as drug delivery vehicles for cancer, including liposomes, nanoparticles, micelles, dendrimers, polymersomes and metal-organic frameworks (MOFs), with different drug release properties, substitution chemistry and pharmacokinetic profiles. Drug delivery vehicles may improve the effective bioavailability of an agent, increase the circulation time and/or improve tumour uptake compared to the free drug. The addition of surface ligands may provide cell-specific uptake, while encapsulation of high payloads of one or multiple drug(s) is possible. While discussion of progress in this area of research is beyond the scope of this review, we direct readers to a well-balanced review for further information.¹²⁰

6.1 Single agent drug delivery

Liposomes, spherical vesicles composed of phospholipids and functionalised with peptides or polymers, are one leading class of drug delivery vehicle. Harrington *et al.* examined pegylated liposome encapsulated cisplatin against KB head and neck squamous cell cancer xenograft tumours in nude mice, finding the addition of the liposomal drug formulation at low dose (2 mg/kg) enhanced the effect of both single-fraction (one dose of 4.5 Gy) and fractionated (9 Gy administered in 3 \times 3 Gy fractions over three days) radiotherapy.¹²¹ Zhang *et al.* explored the *in*