Metallo-Drugs: Development and Action of Anticancer Agents (Metal Ions in Life Sciences Book 18)

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Astrid Sigel, Helmut Sigel, Eva Freisinger, Roland K.O. Sigel

Metal Ions in Life Sciences 18

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Edited by

Astrid Sigel, Helmut Sigel, Eva Freisinger and Roland K.O. Sigel

Volume 18

Editors
Astrid Sigel and Helmut Sigel

Department of Chemistry

Inorganic Chemistry

University of Basel

Spitalstrasse 51

CH-4056 Basel

Switzerland

<astrid.sigel@unibas.ch>

<helmut.sigel@unibas.ch> Eva Freisinger and Roland K. O. Sigel

Department of Chemistry

University of Zürich

Winterthurerstrasse 190

CH-8057 Zürich

Switzerland

<freisinger@chem.uzh.ch>

<roland.sigel@chem.uzh.ch>

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imidazole residue of histidine-105 from human serum albumin. This figure is an expanded version of Figure 3(A) in Chapter 13; it was prepared by Christian Hartinger and coworkers based on PDB ID 4S1Y and modified for the cover by Fabio Steffen and Roland Sigel from the University of Zürich.

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About the Editors

Astrid Sigel(University of Basel) has studied languages; she was an editor of the Metal Ions in Biological Systems series (until Volume 44) and also of the "Handbook on Toxicity of Inorganic Compounds" (1988), the "Handbook on Metals in Clinical and Analytical Chemistry" (1994; both with H. G. Seiler), and on the "Handbook on Metalloproteins" (2001; with Ivano Bertini). She is also an editor of the MILS series from Volume 1 on and she co-authored about 40 papers on topics in Bioinorganic Chemistry.

Helmut Sigel is Emeritus Professor (2003) of Inorganic Chemistry at the University of Basel, Switzerland, and a previous editor of the MIBS series until Volume 44. He served on various editorial and advisory boards, published over 350 articles on metal ion complexes of nucleotides, coenzymes, and other ligands of biological relevance, and lectured worldwide. He was named Protagonist in Chemistry (2002) by ICA (issue 339); among further honors are the P. Ray Award (Indian Chemical Society, of which he is also an Honorary Fellow), the Alfred Werner Prize (Swiss Chemical Society), a Doctor of Science honoris causa degree (Kalyani University, India), appointments as Visiting Professor (e.g., Austria, China, Japan, Kuwait, UK) and Endowed Lectureships; he is also a Honorary Member of SBIC (Society of Biological Inorganic Chemistry).

After participating in the edition of three Handbooks (see above at A.S.) and 44 volumes in the previous series Metal Ions in Biological Systems as well as in the first 18 volumes (including this one) of our new series Metal Ions in Life Sciences, it is time to step down as an Editor. However, I am very pleased to hand over the burden to Eva Freisinger, my daughter-in-law. I am very fortunate that a most competent bioinorganic colleague is replacing myself. Hence, I welcome Eva as an Editor of the MILS series and wish her much success and satisfaction in her new editorial job.

H.S.

Eva Freisinger is an independent group leader at the Department of Chemistry at the University of Zürich, Switzerland. She obtained her doctoral degree (2000) from the University of Dortmund, Germany, working with Bernhard Lippert. Thereafter she spent three years as a postdoc at SUNY Stony Brook, USA, with Caroline Kisker. Since 2003 she performs independent research at the University of Zürich where she held a Förderungsprofessur of the Swiss National Science Foundation from 2008 to 2014. In 2014 she received her Habilitation in Bioinorganic Chemistry. Her research is focused on the study of plant metallothioneins and the sequence-specific modification of nucleic acids. She serves on a number of Advisory Boards for international conference series; since 2014 she is the Secretary of the European Bioinorganic Chemistry Conferences (EuroBICs). She is one of the Editors of the Bioinorganic Chemistry section of the International Journal of Molecular Sciences (IJMS) and now also of the MILS series from Volumes 18 on.

Roland K. O. Sigel is Full Professor (2016) of Chemistry at the University of Zürich, Switzerland. In the same year he became Vice Dean of Studies (BSc/ MSc) and in 2017 Dean of the Faculty of Science. From 2003 to 2008 he was endowed with a Förderungsprofessur of the Swiss National

Science Foundation and he is the recipient of an ERC Starting Grant 2010. He received his doctoral degree summa cum laude (1999) from the University of Dortmund, Germany, working with Bernhard Lippert. Thereafter he spent nearly three years at Columbia University, New York, USA, with Anna Marie Pyle (now Yale University). During the six years abroad he received several prestigious fellowships from various sources, and he was awarded the EuroBIC Medal in 2008 and the Alfred Werner Prize (SCS) in 2009. His research focuses on the structural and functional role of metal ions in ribozymes, especially group II introns, regulatory RNAs, and on related topics. He was also an editor of Volumes 43 and 44 of the MIBS series and of the MILS series from Volume 1 on.

Historical Development and Perspectives of the Series Metal Ions in Life Sciences[1]

It is an old wisdom that metals are indispensable for life. Indeed, several of them, like sodium, potassium, and calcium, are easily discovered in living matter. However, the role of metals and their impact on life remained largely hidden until inorganic chemistry and coordination chemistry experienced a pronounced revival in the 1950s. The experimental and theoretical tools created in this period and their application to biochemical problems led to the development of the field or discipline now known as Bioinorganic Chemistry, Inorganic Biochemistry, or more recently also often addressed as Biological Inorganic Chemistry.

By 1970 Bioinorganic Chemistry was established and further promoted by the book series Metal Ions in Biological Systems founded in 1973 (edited by H. S., who was soon joined by A. S.) and published by Marcel Dekker, Inc., New York, for more than 30 years. After this company ceased to be a family endeavor and its acquisition by another company, we decided, after having edited 44 volumes of the MIBS series (the last two together with R. K. O. S.) to launch a new and broader minded series to cover today's needs in the Life Sciences. Therefore, the Sigels new series is entitled

Metal Ions in Life Sciences.

After publication of 16 volumes (since 2006) with various publishers during the past 10 years, we are happy to join forces (from Volume 17 on) in this still growing endeavor with Walter de Gruyter GmbH, Berlin, Germany, a most experienced Publisher in the Sciences.

The development of Biological Inorganic Chemistry during the past 40 years was and still is driven by several factors; among these are (i) attempts to reveal the interplay between metal ions and hormones or vitamins, etc., (ii) efforts regarding the understanding of accumulation, transport, metabolism and toxicity of metal ions, (iii) the development and application of metal-based drugs, (iv) biomimetic syntheses with the aim to understand biological processes as well as to create efficient catalysts, (v) the determination of high-resolution structures of proteins, nucleic acids, and other biomolecules, (vi) the utilization of powerful spectroscopic tools allowing studies of structures and dynamics, and (vii), more recently, the widespread use of macromolecular engineering to create new biologically relevant structures at will. All this and more is reflected in the volumes of the series Metal Ions in Life Sciences.

The importance of metal ions to the vital functions of living organisms, hence, to their health and well-being, is nowadays well accepted. However, in spite of all the progress made, we are still only at the brink of understanding these processes. Therefore, the series Metal Ions in Life Sciences links coordination chemistry and biochemistry in their widest sense. Despite the evident expectation that a great deal of future outstanding discoveries will be made in the interdisciplinary areas of science, there are still "language" barriers between the historically separate spheres of chemistry, biology, medicine, and physics. Thus, it is one of the aims of this series to catalyze mutual "understanding".

It is our hope that Metal Ions in Life Sciences continues to prove a stimulus for new activities in the fascinating “field” of Biological Inorganic Chemistry. If so, it will well serve its purpose and be a rewarding result for the efforts spent by the authors.

Astrid Sigel and Helmut Sigel

Department of Chemistry, Inorganic Chemistry

University of Basel, CH-4056 Basel, Switzerland

Roland K. O. Sigel

Department of Chemistry

University of Zürich, CH-8057 Zürich, Switzerland

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Preface to Volume 18 Metallo-Drugs: Development and Action of Anticancer Agents

Platinum-based anticancer drugs are among the most widely used of all chemotherapeutic cancer treatments. Three FDA-approved platinum(II) anticancer drugs, i.e., cisplatin, carboplatin, and oxaliplatin, have been in the clinic for many years to treat testicular, ovarian, cervical, head and neck, colorectal, and other cancers. These breakthroughs are the result of the serendipitous discovery of the anticancer activity of cisplatin, cis-diamminodichloroplatinum(II), more than 50 years ago. Meanwhile an understanding of its medicinal properties has developed, allowing for improved treatment regimens reducing somewhat the side effects, including nephrotoxicity, myelosuppression, peripheral neuropathy, ototoxicity, and nausea. All this and more is covered in Chapter 1 which focuses on Cisplatin and Oxaliplatin.

Polynuclear Platinum Complexes (PPCs) were developed to combat cisplatinresistant cancers. PPCs represent a discrete structural class of DNA-binding agents: The use of at least two platinum-coordinating units makes multifunctional binding modes possible. Proof of principle of this hypothesis was achieved by the advance to the clinic (Phase II trials) of triplatin, a charged trinuclear, bifunctional, DNA-binding agent with two terminal arms, –NH2-Pt(NH3)2Cl. Chapter 2 emphasizes the structural diversity and reactivity of PPCs.

Another approach to avoid resistance and side effects centers on octahedral and kinetically inert Pt(IV) Prodrugs (Chapter 3) They can be reduced in cancer cells to active square-planar Pt(II) complexes, e.g., by intracellular reducing agents such as glutathione or by photoexcitation. The additional axial ligands in Pt(IV) complexes, which are released on reduction, allow bioactive molecules to be delivered, which can act synergistically with the Pt(II) species in killing the cancer cells. Pt(IV) complexes are likely to be stable under the highly acidic conditions in the stomach and therefore suitable for oral administration.

Chapter 4 introduces the concept of Metalloglycomics, that is, the interaction of metal ions with biologically relevant oligosaccharides, in particular glycosaminoglycans (GAGs) such as heparin and heparan sulfate. Their structure and conformation and the role of various metal ions during their interaction with proteins and enzymes are reviewed. Cleavage of heparan sulfate proteoglycans by heparanase modulates tumor-related events. Heparan sulfate is identified as a ligand receptor for polynuclear platinum complexes defining a new mechanism of cellular accumulation.

The structure of the ruthenium(III) drug candidates KP1019 and NAMI-A is deceptively similar, i.e., trans-RuCl4(X,Y)–, as discussed in Chapter 5, yet, surprisingly they have markedly different macroscopic pharmacological activities: KP1019 behaves rather as a classical antitumor compound (with the advantage of being active also against platinum(II)-resistant tumors), whereas NAMI-A has a more unconventional activity that affects metastases and not the primary tumor. The complicated in vivo chemistry (no clearly identified target) is affecting negatively their further clinical development after initial progress (Phase II).

Organometallic ruthenium-arene complexes (Chapter 6) have risen to prominence as a pharmacophore due to the success of other ruthenium drug candidates in clinical trials. Ru(arene) complexes are almost exclusively octahedral, low-spin d6 Ru(II) species. Mononuclear Ru(arene) complexes have therapeutic properties against cancer in vitro and in vivo, therefore researchers began exploiting these potentially therapeutic entities for higher-order multinuclear Ru(arene) complexes.

The Medicinal Chemistry of Gold Anticancer Metallodrugs is described in Chapter 7. Since ancient times gold and its complexes have been used as therapeutics against different diseases. In modern medicine gold drugs are applied for the treatment of rheumatoid arthritis, but recently they also serve as antiparasitic, antibacterial, and antiviral agents. The exciting findings on gold(I) and gold(III) complexes as antitumor agents are summarized and warrant the discussion of the relevant aspects of their modes of action.

Titanium(IV) complexes represent attractive alternatives to Pt(II)-based anticancer drugs because of their low toxicity (Chapter 8). The pioneering compounds titanocene dichloride and budotitane were the first to enter clinical trials. Yet, despite the high efficacy and low toxicity observed in vivo, they failed, mainly because of formulation complications, their rapid hydrolysis, the difficulty of isolating and identifying the particular active species and the precise cellular target. The following generation of phenolato-based complexes came three decades later and exhibited high activity and improved stability, with no signs of toxicity to the treated animals. The mechanistic insights gained so far include the interaction with DNA and the induction of apoptosis; hence, these Ti(IV) complexes are highly promising for future clinical development.

Vanadium compounds have been known for long to have beneficial therapeutic properties (Chapter 9), but it was not until 1965 when it was discovered that these effects could be extended to treating cancer due to the similarities in some metabolic pathways that are utilized by both diabetes and cancer. The links between these diseases emerged through epidemiological investigations which suggest that the incidence of pancreatic, liver, and endometrial cancers are associated with diabetes though the links are not yet fully understood.

The antineoplastic activity of gallium nitrate, Ga(NO3)2, was recognized over three decades ago and several clinical trials (Phase I and II) have confirmed this in patients with lymphoma and bladder cancer (Chapter 10). Ga(III) shares chemical characteristics with Fe(III) and these enable it to interact with iron-binding proteins and to disrupt iron-dependent tumor cell growth. Beyond the first generation of gallium(III) salts (parenterally administered) a new generation of complexes such as tris(8-quinolinato)gallium(III) with oral bioavailability, has emerged and is now evaluated in the clinic while other ligands for Ga(III) are in preclinical development.

Non-covalent Metallo-Drugs: Using Shape to Target DNA and RNA Junctions and Other Nucleic Acid Structures is the title of Chapter 11. This shape specificity contrasts with the most effective class of anticancer drugs in clinical use, the Pt(II) agents, which act by binding to duplex B-DNA in a sequence-specific manner, but duplex B-DNA is not DNA in its active form. The chapter describes how large cationic metallo-supramolecular structures can be used to bind to less common, yet active, nucleic acid structures like Y-shaped forks and 4-way junctions, and thus, possibly display

high cytotoxicity and inhibit cancer.

Chapter 12 deals with Nucleic Acid Quadruplexes and Metallo-Drugs. Guanine-rich sequences of DNA can readily fold into tetra-stranded helical assemblies, known as G-quadruplexes (G4). It has been proposed that these structures play important roles in transcription, translation, replication, and telomere maintenance. Therefore they receive attention as potential drug targets for small molecules including metal complexes. Indeed, G4s have been identified as potential drug targets, in particular for cancer.

Anticancer platinum-based drugs are widely used in the treatment of a variety of tumoric diseases. They target DNA and thereby induce apoptosis in cancer cells. Their reactivities with other biomolecules have often been associated with side effects during chemotherapy. The development of metal compounds that target proteins rather than DNA has the potential to overcome or to reduce these disadvantages. New compounds on track toward clinical application are highlighted in Chapter 13, Antitumor Metallodrugs that Target Proteins.

Chapter 14, entitled Metallointercalators and Metalloinsertors deals with their structural requirements for DNA recognition and anticancer activity. The focus is on the non-covalent recognition of the highly structured DNA surface by substitutionally inert metal complexes (mostly of Ru(II) and Rh(III) with low-spin 4d6) capable of either sliding in between the normal base pairs (metallointercalators) or flipping out thermodynamically destabilized mispaired nucleobases (metalloinsertors). New structural insights enable the development of novel DNA binding modes and thus, new anticancer drug candidates.

The last three chapters of this volume deal with essential metal ions. First, Iron and Its Role in Cancer Defence: A Double-Edged Sword is discussed (Chapter 15). Iron is vital for many biological functions including electron transport, DNA synthesis, detoxification, and erythropoiesis. Interactions between Fe(II/III) and O2can result in the generation of reactive oxygen species. Excess iron may cause oxidative damage resulting in cell death, but DNA damage may also lead to permanent mutations. Hence, iron is carcinogenic and may initiate tumor formation and growth; however, Fe(II/III) can also contribute to cancer defence by initiating specific forms of cell death, which will benefit cancer treatment. Furthermore, Fe-binding and Fe-regulatory proteins, such as heme oxygenase-1, ferritin, and iron-sulfur clusters can display antitumor properties in certain cancer types. Consequently, very specific and selective drugs that target Fe metabolism in tumors are promising candidates for the prevention and therapy of cancer.

Copper is another essential micronutrient required for fundamental biological processes in all organisms (Chapter 16). It is a redox-active metal able to shift between reduced (Cu+) and oxidized (Cu2+) states. Free copper ions can generate highly reactive oxygen species (ROS) and damage lipids, proteins, nucleic acids, and other biomolecules. Hence, copper homeostasis is tightly regulated to ensure sufficient copper for cuproprotein biosynthesis, while limiting oxidative stress and toxicity. Over the last century copper complexes have been developed as antimicrobials and for treating special diseases which now also include cancer because copper has been recognized as a limiting factor for multiple aspects of cancer progression including growth, angiogenesis, and metastasis. Consequently, ‘old copper complexes’ (e.g., tetrathiomolybdate and clioquinol) have been repurposed for cancer therapy and have demonstrated anticancer activity in vitro and in preclinical models. Likewise, with tailer-made copper complexes considerable progress has been made in understanding their pharmacological requirements and human clinical trials continue.

Zinc(II) is gaining momentum as a potential target for cancer therapy since it has been recognized as a second messenger (Chapter 17). It is able to activate many signalling pathways within a few minutes by an extracellular stimulus which leads to the release of zinc(II) from intracellular stores.This zinc(II) release inhibits tyrosine phosphatases preventing the inactivation of tyrosine

kinases, etc. These signalling pathways are commonly considered the main driving force in aberrant cancer growth. These insights position zinc(II) signalling as a particularly important new target to prevent aggressive cancer growth.

To conclude, this volume, devoted to Metallo-Drugs: Development and Action of Anticancer Agents, is rich on specific information. MILS-18 updates our knowledge not only on platinum(II) and related platinum complexes, but it provides also deep insights on the new research frontiers dealing with the next generation of anticancer drugs. It is a must for all researchers working in medicinal chemistry and beyond as well as for teachers giving courses on this topic.

Astrid Sigel

Helmut Sigel

Eva Freisinger

Roland K. O. Sigel

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Contributors to Volume 18

Numbers in parentheses indicate the pages on which the authors' contributions begin.

Enzo AlessioDepartment of Chemical and Pharmaceutical Sciences, University of Trieste, Via L. Giorgieri 1, I-34127 Trieste, Italy <alessi@units.it= (141)

Wee Han AngDepartment of Chemistry, National University of Singapore, Block S15, Level 5, 3 Science Drive 2, Singapore 117543 <chmawh@nus.edu.sg= (171)

Maria V. BabakDepartment of Chemistry, National University of Singapore, Block S15, Level 5, 3 Science Drive 2, Singapore 117543 <phamari@nus.edu.sg= (171)

Viktor BrabecInstitute of Biophysics, Academy of Sciences of the Czech Republic, Královopolská 135, CZ-61265 Brno, Czech Republic

<brabec@ibp.cz= (43)

Sue Berners-PriceInstitute for Glycomics, Griffith University, Gold Coast Campus, Southport, QLD 4222, Australia <s.berners-price@griffith.edu.au= (109)

Lucia CardoSchool of Chemistry, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK < l.cardo@bham.ac.uk= (303)

Angela CasiniSchool of Chemistry, Cardiff University, Park Place, Cardiff, CF10 3AT, UK < casinia@cardiff.ac.uk= (199)

Michael A. CaterCentre for Cellular and Molecular Biology, School of Life and Environmental Sciences, Deakin University, Burwood, VIC 3125, Australia, Department of Pathology, The University of Melbourne, Parkville, Victoria, Australia <mcater@unimelb.edu.au= and Rural Clinical School, The University of New South Wales, Port Macquerie, New South Wales, Australia

<m.cater@unsw.edu.au= (469)

Christopher R. ChitambarDepartment of Medicine, Hematology and Oncology Division, Medical College of Wisconsin and Froedtert Clinical Cancer Center, 9200 W. Wisconsin Avenue, Milwaukee, WI 53226, USA <cchitamb@mcw.edu= <chitambr@mcw.edu= (281)

Sharnel A. S. ClatworthyCentre for Cellular and Molecular Biology, School of Life and Environmental Sciences, Deakin University, Burwood, Victoria, Australia (469)

Debbie C. CransDepartment of Chemistry and Cell and Molecular Biology, Colorado State University, Fort Collins, CO 80523, USA

<debbie.crans@colostate.edu= (251)

Delphine DenoyerCentre for Cellular and Molecular Biology, School of Life and Environmental Sciences, Deakin University, Burwood, Victoria, Australia <d.denoyer@deakin.edu.au= (469)

Nicholas P. FarrellDepartment of Chemistry, Virginia Commonwealth University, 1001 West Main Street, P.O.B. 842006, Richmond, VA 23284–2006, USA <npfarrell@vcu.edu= (43, 109)

Anil K. GorleInstitute for Glycomics, Griffith University, Gold Coast Campus, Southport, QLD 4222, Australia (109)

Allison HaaseDepartment of Chemistry and Cell and Molecular Biology, Colorado State University, Fort Collins, CO 80523, USA (251)

Michael J. HannonSchool of Chemistry, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK <m.j.hannon@bham.ac.uk= (303)

Christian G. HartingerSchool of Chemical Sciences, University of Auckland, Private Bag 92019, NZ-1142 Auckland, New Zealand

<c.hartinger@auckland.ac.nz= (351)

Hannah U. HoltkampSchool of Chemical Sciences, University of Auckland, Private Bag 92019, NZ-1142 Auckland, New Zealand (351)

Jana KasparkovaInstitute of Biophysics, Academy of Sciences of the Czech Republic, Královopolská 135, CZ-61265 Brno, Czech Republic (43)

Stephen J. LippardDepartment of Chemistry, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139-4307, USA

<lippard@mit.edu= (1)

Vijay MenonDepartment of Chemistry, Virginia Commonwealth University, 1001 West Main Street, P.O.B. 842006, Richmond, VA 23284-2006, USA (43)

Luigi MessoriDepartment of Chemistry ‘Ugo Schiff’, University of Florence, Via della Lastruccia 3–13, I-50019 Sesto Fiorentino, Italy <luigi.messori@unifi.it= (141)

Maya MillerInstitute of Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel (219)

Olivia OgleSchool of Pharmacy and Pharmaceutical Sciences, College of Biomedical and Life Sciences, Cardiff University, Redwood Building, King Edward VIIth Avenue, Cardiff, CF10 3NB, UK (507)

Ingo OttInstitute of Medicinal and Pharmaceutical Chemistry, Technical University Braunschweig, Beethovenstr. 55, D-38106 Braunschweig, Germany <ingo.ott@tu-bs.de= (199)

Erica J. PetersonDepartment of Chemistry, Virginia Commonwealth University, 1001 West Main Street, P.O.B. 842006, Richmond, VA 23284–2006, USA (109)

Imogen A. RiddellDepartment of Chemistry, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139–4307, USA (1)

Peter J. SadlerDepartment of Chemistry, University of Warwick, Coventry CV4 7AL, UK < p.j.sadler@warwick.ac.uk= (69)

Ulrich SchatzschneiderInstitut für Anorganische Chemie, Julius-Maximilians-Universität Würzburg, Am Hubland, D-97074 Würzburg, Germany <ulrich.schatzschneider@uni-wuerzburg.de= (387)

Matthew P. SullivanSchool of Chemical Sciences, University of Auckland, Private Bag 92019, NZ-1142 Auckland, New Zealand (351)

Raymond Wai-Yin SunGuangzhou Lee & Man Technology Co. Ltd., Guangzhou, Guangdong, P. R. China and Department of Chemistry, Shantou University, 243 Daxue Road, Shantou, Guangdong 515063, P. R. China

<rwysun@leemanchemical.com= (199)

Kathryn M. TaylorSchool of Pharmacy and Pharmaceutical Sciences, College of Biomedical and Life Sciences, Cardiff University, Redwood Building, King Edward VIIth Avenue, Cardiff, CF10 3NB, UK < taylorkm@cardiff.ac.uk= (507)

Frank ThévenodZBAF (Centre for Biomedical Education and Research), Department of Medicine, Faculty of Health, Private University of Witten/Herdecke, Stockumer Str. 12, D-58453 Witten, Germany

<frank.thevenod@uni-wh.de= (437)

Edit Y. TshuvaInstitute of Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel < edit.tshuva@mail.huji.ac.il= (219)

V. VenkateshDepartment of Chemistry, University of Warwick, Coventry CV4 7AL, UK < venka7@gmail.com= (69)

Ramon VilarDepartment of Chemistry, RCS1–315, Imperial College London, South Kensington Campus, London, SW7 2AZ, UK <r.vilar@imperial.ac.uk= (325)

Lining YangDepartment of Chemistry and Cell and Molecular Biology, Colorado State University, Fort Collins, CO 80523, USA and College of Pharmacy, Xi'an Medical University, Xi'an, Shaanxi 710021, China (251)

Xiaogai YangDepartment of Chemical Biology, School of Pharmaceutical Sciences, Peking University, Beijing 100191, China (251)

Silvia ZiliottoSchool of Pharmacy and Pharmaceutical Sciences, College of Biomedical and Life Sciences, Cardiff University, Redwood Building, King Edward VIIth Avenue, Cardiff, CF10 3NB, UK (507)

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Imogen A. Riddell and Stephen J. Lippard

Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

<lippard@mit.edu>

Abstract: Following the serendipitous discovery of the anticancer activity of cisplatin over 50 years ago, a deep understanding of the chemical and biochemical transformations giving rise to its medicinal properties has developed allowing for improved treatment regimens and rational design of second and third generation drugs. This chapter begins with a brief historical review detailing initial results that led to the worldwide clinical approval of cisplatin and development of

the field of metal anticancer agents. Later sections summarize our understanding of key mechanistic features including drug uptake, formation of covalent adducts with DNA, recognition and repair of Pt-DNA adducts, and the DNA damage response, with respect to cisplatin and oxaliplatin. The final section highlights known shortcomings of classical platinum anticancer agents, including problems with toxicity and mutagenicity, and the development of resistance and enrichment of cancer stem cells brought about through treatment. Instances where specific differences in the response or mechanism of action of cisplatin versus oxaliplatin have been demonstrated are discussed in the text. In this manner the chapter provides a broad overview of our current understanding of the mechanism of action of platinum anticancer agents, providing a framework for improving the rational design of better Pt-based anticancer agents.

Keywords: anticancer • DNA damage response • DNA repair • mechanistic understanding • platinum

1.INTRODUCTION

Following Barnett (Barney) Rosenberg's appointment to Michigan State University in 1961 he began to investigate the possibility that cell division might be affected by electric fields having noted that the arrangement of the mitotic spindle in dividing cells resembled that of iron filings within a magnetic field [1, 2]. Initial experiments performed with Escherichia coli (E. coli) bacterial cells grown in ammonium chloride-buffered solutions stimulated with platinum electrodes showed distinctive changes in morphology [3]. Elongation of the bacterial cells was ultimately determined to arise from inhibition of cellular division while cell growth was maintained, allowing filamentous strands to grow up to 300 times their normal length. Further studies determined that it was not the electric field, but platinum compounds generated at 1–10 ppm concentrations under the experimental conditions, that brought about the morphological changes [4, 5]. Subsequent experiments probing the effect of cis-dichlorodiammineplatinum(II) (cisplatin or CDDP) and cis-tetrachlorodiammineplatinum(IV) on sarcoma 180 and leukemia L1210 mouse models confirmed the potent anticancer activity of cisplatin and led to a collaboration with the National Cancer Institute (NCI) [6]. The NCI had over the previous 15 years screened approximately 140,000 compounds to assess their anticancer activity [5], however, inorganic compounds had ceased to be of interest to the community and only a handful of the compounds evaluated contained metals.

Despite the predisposition against metal compounds as chemotherapeutic agents at the time, cisplatin displayed broad-spectrum activity against transplantable, carcinogenically-induced, and virally-generated tumors. In addition, experiments revealed that even highly advanced stage tumors could be treated successfully with cisplatin [5] and, appropriately, the clinical development of cisplatin was fast-tracked with initial clinical trials being run in 1971 [7, 8] and Platinol, the trade name for cisplatin, was brought to the market by Bristol Laboratories (later Bristol-Myers Squibb) in 1978. Initially, Food and Drug Administration (FDA) approval was given for the treatment of advanced testicular, ovarian, and bladder cancers. Since then, however, the spectrum of cancers for which cisplatin has received approval was significantly extended to include cervix, head and neck, esophageal, and small cell lung cancers as well as some pediatric malignancies among many others.

Although it was clear from preclinical studies [9] onwards that cisplatin was a very potent anticancer agent with broad-spectrum activity, it suffered from certain toxic side effects. Preclinical studies indicted platinum concentration in the excretory organs and that it could persist in the body for up to four months after treatment. Nephrotoxicity reported in beagle dogs continued to be a major concern throughout Phase I studies. However, the discovery that high-volume fluid hydration [10] and forced diuresis could prevent renal damage combined with unprecedented cures of testicular germ cell tumors ensured continued development of the drug candidate [2]. Similarly potent antiemetics including the 5HT3 antagonists [11–13] were developed to overcome acute and delayed emesis as a result of cisplatin treatment.

In addition to research for the development of protective compounds able to ameliorate the undesired side effects of cisplatin, another key area of research was the discovery of less toxic cisplatin analogs. Several second-generation compounds containing dicarboxylate leaving groups in place of the more labile chloride ions of the parent compound cisplatin were investigated in the 1980s based on the hypothesis that platinum(II) diammine compounds containing more stable leaving groups would retain the desired anticancer properties while imparting lower toxicity and more predictable pharmokinetics [2, 14]. This hypothesis turned out to be correct for carboplatin, which was granted FDA approval in 1989, and is now widely used in clinics primarily in the treatment of ovarian cancer and where patients have recurrent, platinum-resistant disease. Research indicated that the adducts formed by carboplatin are identical to those of cisplatin, but the rate of adduct formation is 10-fold slower, which necessitates 20- to 40-fold higher concentrations of carboplatin to produce the same number of adducts [15]. Four other analogs, namely, enloplatin (1,1-cyclobutane dicarboxylato-O′,O′ tetrahydro-4H pyran-4,4-dimethylamine-N′,N′ platinum(II)), zeniplatin (2,2-bis aminomethyl-1,3-propandiol-N-N′ 1,1-cyclobutane dicarboxylate-O′,O′ platinum(II)), NK-121/C1–973 (cis-1,1-cyclobutane dicarboxylato(2R)-2-methyl-1,4-butanediamine platinum(II)), and miboplatin (R-2-amino methyl pyrrolidine 1,1-cyclobutane dicarboxylate platinum(II)) also entered clinical trials in the 1980s [12, 16], but with the exception of miboplatin they all suffered from loss of anticancer activity and/or dose-limiting toxicity. In contrast, miboplatin performed well, and reached Phase III clinical trials in Japan. Ultimately, however, miboplatin was abandoned despite its good anticancer activity, for it showed no clear advantage over cisplatin [12].

In addition to lowering toxicity, significant research has been invested into improving the spectrum of activity of platinum anticancer agents, with oxaliplatin (trans-L-diaminocyclohexane oxalate platinum(II)) emerging as the prominent third-generation drug. Oxaliplatin was originally proposed as a potential anticancer agent in the late 1970s [17] but did not receive FDA approval until 2002 [18]. Early murine leukemia studies indicated that oxaliplatin performed better than cisplatin while showing reduced side effects [12]. Moreover, in vitro screening of oxaliplatin against the NCI-60 human cancer cell panel [19] indicated that oxaliplatin might provide a suitable treatment for cancers including colon cancer that do not respond to cisplatin treatment. Following four large Phase III trials in the early 2000s that demonstrated the potential of oxaliplatin treatment in combination with 5-fluorouracil and leucovorin for the treatment of metastatic colon cancer [14], oxaliplatin was given FDA approval. Prior to oxaliplatin approval it was widely accepted that colon cancer did not respond to treatment with platinum agents, with response rates reported as low as 19 % [20] and 22 % [21], respectively, following cisplatin treatment. In contrast, colorectal cancer response rates were significantly improved, with oxaliplatin-based chemotherapy raising this value to 50 % [22].

In addition to cisplatin, carboplatin, and oxaliplatin, which have received worldwide approval, nedaplatin (diammine[hydroxyacetato-O,O′]platinum(II)) was approved for treatment in Japan, lobaplatin ([2-hydroxypropanoato-O1,O2][1,2-cyclobutanedimethanamine-N,N′]platinum(II)) for treatment in China, and heptaplatin ([propanedioato-O,O′][2-(1-methylethyl)-1,3-dioxolane-4,5-dimethanamine-N,N′]platinum(II)) for treatment in the Republic of Korea (Figure 1).

With the advent of platinum drugs and altered behavioral patterns, cancer prognosis improved significantly over the last fifty years. Approval of cisplatin and subsequent optimization in treatment regimes for platinum drugs, combined with an increased understanding of the factors that promote cancer, have ultimately reduced cancer incidence and provided cures. In particular, successes in treatment of testicular cancer and lung cancer across this period are clearly identified from cancer statistics [23]. Prior to approval of cisplatin, testicular cancer was only curable in its earliest stages through a combination of aggressive surgery and radiation, but for patients in the later stage of the disease a diagnosis was almost always fatal [8]. In stark contrast, cisplatin

treatment offers an overall 90 % cure rate, which, when coupled with public education campaigns highlighting the importance of early detection, has resulted in a sharp decline in cancer-related deaths in men. Similarly, public education campaigns and governmental policies restricting the sale of tobacco have significantly lessened the incidence of lung cancer. The American Cancer Institute reports cancer-related deaths in the US have decreased by 23 % since 1991, a value that corresponds to 1.7 millions deaths being averted since 2012 [23]. Continued research to identify improved anticancer agents able to target resistant cancer cell lines, or combat recurrent disease in combination with increased understanding of the mechanistic actions of platinum agents offers the possibility to further improve these statistics. In particular, as we move into the era of personalized medicine, detailed mechanistic studies are expected to be invaluable in guiding clinicians to the best treatment regimens, optimized to take advantage of an individual's biochemistry and/or genetics. Figure 1. Platinum drugs that have received clinical approval for cancer treatment in at least one country. Cisplatin, carboplatin, and oxaliplatin have all received worldwide approval, while nedaplatin is approved in Japan, heptaplatin in the Republic of Korea, and lobaplatin in China.

The present chapter provides a broad summary of our current knowledge of the mechanisms of action of cisplatin and oxaliplatin, detailing mechanisms by which platinum agents pass through cell membranes (Section 2), form covalent adducts with biological nucleophiles (Section 3), and are processed by cellular machinery (Sections 4 and 5). Finally, we conclude by highlighting specific challenges that the medicinal inorganic community is yet to overcome. For many of these challenges non-classical anticancer agents, including monofunctional, multi-nuclear, or non-platinum based anticancer agents that are discussed in later chapters of this book have been postulated to further exceed the potency of the approved platinum drugs. 2.CELLULAR UPTAKE AND EFFLUX/MEMBRANE TRANSPORT 2.1.Overview of Platinum Transport Processes

The anticancer activity of both cisplatin and oxaliplatin arises from the ability of these complexes to generate covalent adducts with nuclear DNA, which ultimately triggers cell death [24]. In order to generate platinum-DNA adducts, the platinum agents must avoid deactivation in the bloodstream and be internalized by the cell. Once inside the cell platinum agents will undergo aquation due to the reduced concentration of chloride ions in the cytosol compared with the extracellular chloride concentration, and then, following localization in the nucleus, bind covalently at the most nucleophilic sites on DNA. Cellular uptake thus serves as a critical step in the mechanism of action of platinum anticancer agents. Of equal importance is the ability of cells to effectively export platinum agents, with platinum efflux being a demonstrated mechanism by which cells can become resistant to platinum-based chemotherapy [25]; export also mediates toxic side effects associated with platinum-based chemotherapy [26].

Both active and passive processes have been implicated in platinum agent membrane transport [27] (Figure 2), the difference being that active transport employs a receptor that utilizes energy to convey the platinum agent, whereas passive transport occurs without the need for energy input. Passive diffusion refers to processes by which platinum agents diffuse through the lipid membrane to enter cells and requires neither energy input nor a transporter for platinum uptake [28]. Figure 2. Summary of the proposed platinum transport processes including passive diffusion driven by reduced intracellular chloride concentration, and the postulated transporter-mediated processes including uptake by copper transporters Ctr1 and Ctr2 and the polyspecific organic cation transporters OCT1, OCT2, and MATE, and export via P-type ATPases ATP7A and ATP7B.

Early research examining the cellular accumulation of cisplatin and its analogs concluded that passive diffusion was the main route by which cisplatin accumulates in cells [29]. Support for passive uptake of cisplatin was provided by the observations that (i) cellular uptake depended

linearly on cisplatin concentration and (ii) that structural analogs of cisplatin did not inhibit uptake, as expected if active transport were implicated. More recently the possibility that passive diffusion might be operative for cisplatin uptake was reinvestigated, providing compelling evidence that protein-mediated uptake is not an absolute requirement for cisplatin accumulation [30]. These observations, combined with the growing skepticism surrounding mechanisms of active transport, call into question whether passive transport is the dominant mechanism for cellular uptake of cisplatin.

To test the hypothesis that cisplatin is internalized through passive diffusion, the permeability coefficient, Pd, of cisplatin was monitored as a function of the chloride concentration of the medium using unilamellar DOPC vesicles. Here, Pd is defined by Equation (1):

where kobs is the rate of cisplatin uptake, me asured by stopped-flow spectroscopy, and r is the average vesicle radius, as measured by dynamic light scattering. These studies showed a gradual increase in Pd as a function of chloride concentration up to approximately 100 μM, at which point the system was saturated, consistent with passive diffusion of cisplatin into the vesicles. The authors argue that the internalization and accumulation of cisplatin in cells through passive diffusion is not surprising given the speciation of cisplatin intra- and extracellularly. Outside of the cell cisplatin has an overall neutral charge and is therefore not subjected to the large Born energy barrier that prevents many small, hydrophilic ions from crossing the plasma membrane. However, once internalized, cisplatin will form a mixture of mono- and dicationic species that will have to pay a large energy penalty (100–300 kJmol–1) to passively diffuse out of the cell. These differential energy requirements for passive diffusion of cisplatin into and out of the cell are believed to account for the cellular accumulation observed for cisplatin [30].

Extensive research into active transporters expressed on the cell surface has also been undertaken and a detailed discussion of the role of the copper transporters, organic cation transporters, and multidrug and toxin extrusion protein is given below. Unlike passive diffusion, utilizing membrane transporters for drug uptake offers opportunities for selective targeting of tumor cells and reduced toxic side effects in instances where the transporter is preferentially expressed in specific tissues.

2.2.Copper Transporters

The copper transporters Ctr1, Ctr2, ATP7A, and ATP7B as well as the copper chaperone ATOX1 have all been implicated in the regulation of cisplatin in mammalian cells [31, 32]; however, the mechanism by which these transporters interact with cisplatin remains unknown. Therefore, speculation as to which of the transporters is the most influential and indeed, whether or not these transporters actually play a critical role in import and export of cisplatin and its analogs, remains unknown [33]. Furthermore, recent findings support the hypothesis that reduction of cellular copper sensitizes cells to cisplatin treatment [34], adding to the debate around whether copper transporters facilitate cisplatin transport across the membrane.

2.2.1.Copper Import Transporters 1 and 2

The copper transporter proteins 1 and 2, (Ctr1; SLC31A1 and Ctr2; SLC31A2, respectively), share considerable structure homology while varying in their amino acid sequences, with only 41 % conserved residues [32]. They are surface receptors generated from homotrimers of the monomer unit. Their primary function is copper homeostasis, providing a mechanism by which copper can enter cells, but in recent years they have received attention as a transporter for cisplatin.

Initial studies in yeast correlated intracellular platinum concentrations with yCtr1 expression levels [35, 36]. Subsequent work confirmed an equivalent correlation between human Ctr1 expression and cisplatin uptake, when hCtr1 was forcibly overexpressed [37]. Several research groups have also reported reduced Ctr1 expression in cisplatin resistant cell lines [38, 39] and downregulation of Ctr1 in response to cisplatin treatment [40]. Reports that the Ctr1 transporter is internalized in

response to cisplatin treatment [41] led to the suggestion that resistance may arise from reduced cisplatin uptake by Ctr1 transporters. A recent clinical study also identified two single nucleotide polymorphisms present in the Ctr1 genes of patients with non-small cell lung cancer who did not respond well to platinum chemotherapy [42].

However, as Ivy and Kaplin [33] point out, all of these data are 'correlative and not causative' and there is a growing body of evidence that supports Ctr1 being unable to import cisplatin into a cell. Recently [33], experiments that were initially put forward as evidence of passive diffusion of cisplatin into cells confirmed that uptake does not saturate at biologically relevant concentrations. This result indicates that cisplatin uptake is most likely not protein-mediated. In the same publication the authors also evaluated cisplatin and copper uptake in HEK cells expressing hCtr1 under the influence of a tetracycline-sensitive promotor. There was growth in the presence of tetracycline that increased the copper uptake by 8- to 10-fold, but cisplatin uptake was unchanged irrespective of whether or not tetracycline was present. Similarly, by comparing Ctr1 (C/C) and Ctr1(–/–) mouse embryonic fibroblasts (MEFs), the authors saw no difference in the rate of cisplatin uptake and no difference in uptake in Ctr1(C/C) MEFs when copper was added, indicating that copper does not compete with cisplatin for the mechanism of uptake. Recently, work using the CRISPR-Cas9 genome editing system to systematically knockout CTR1, CTR2, ATOX1 or CCS from HEK-293T cells [43] revealed that, following knockdown, none of the cell lines exhibited greater cisplatin sensitivity than the variance in the parental populations. The results indicate that neither Ctr1 nor Ctr2 is implicated in the mechanism of cisplatin uptake.

Structural modification of Ctr1 has also provided insight into the potential mechanism by which the copper transporter may interact with cisplatin. Deletion of the N-terminal extracellular domain of hCTR1 reduced cisplatin uptake while completely eliminating copper uptake [44]. Modification of Met150, Met154 or His139, the three residues involved in the transchelation mechanism invoked for copper uptake, disabled copper uptake but increased cisplatin accumulation [45]. These results together with the limited pore size of Ctr1 (estimated to be 8 Å), smaller than the estimated radius of cisplatin (9.57 Å), suggest that if Ctr1 is involved in cisplatin uptake the mechanism by which cisplatin is internalized by Ctr1 must be significantly different from that of copper [33].

Less is known about the function of mammalian Ctr2 than Ctr1, but due to structural similarity between the transporters the effect of Ctr2 on cisplatin uptake was evaluated [46, 47]. Ctr2 binds copper albeit with a lower affinity than Ctr1 (Km ~1.7 μM versus ~6–10 μM, respectively), but the differential localization of Ctr1 and Ctr2 indicates that they may play different roles in copper homeostasis and interact differentially with cisplatin [32]. Ctr1 is localized exclusively to the membrane, whereas Ctr2 is found in late endosomes, lysomes, and the nucleus. Initial studies indicated that, unlike Ctr1, knockdown of Ctr2 enhances platinum uptake and the platinum sensitivity of cells [46]. Following Ctr2 knockdown in MEFs, cisplatin and carboplatin uptake increased by 2- to 3-fold, independent of Ctr1 expression levels. The increased platinum concentration was attributed to an initial influx of platinum and not from decreased efflux of platinum agents from the cells or increased concentration of platinum in intracellular vesicles. Similar results were observed in mouse xenograft models. When Ctr2 was knocked down, there was a 9.1-fold increase in platinum at the tumor site relative to the parent cell line [48]. However, in the most recent paper where Ctr2 was knocked out by using CRISPR-Cas9, the same authors acknowledge that the 2- to 5-fold changes in cisplatin sensitivity do not exceed the variance observed in the parental populations, thereby negating a role for Ctr2 in cisplatin uptake [43]. Further work is required to establish a relevance of Ctr1 and Ctr2 expression in cancer treatment, either as a prognostic marker or as a harbinger of successful treatment.

2.2.2.P-Type Export Transporters

The p-type proteins, ATP7A and ATP7B, function primarily to sequester and extrude excess copper. These transporters share 65 % amino acid sequence and homologous structures comprising eight

transmembrane domains [27] and a conserved CxxC domain [49], but are differentiated by their tissue expression and interaction with platinum agents. ATP7A is expressed preferentially in the intestine, choroid plexus in smooth muscle cells, vascular endothelial cells, aorta, and cerebrovascular endothelial cells, whereas ATP7B is primarily expressed in liver and brain [26].

Both p-type proteins have been linked with cisplatin resistance, although the mechanism by which resistance occurs appears to differ subtly. Both transporters mediate platinum sequestration in intracellular vesicles, but cells expressing ATP7B appear to promote trafficking and extracellular efflux of the platinum to a far greater extent than those expressing ATP7A. Cells expressing ATP7A typically show increased intracellular platinum accumulation despite having high cisplatin and oxaliplatin resistance [50]. In contrast, overexpression of ATP7B has been correlated with reduced cisplatin uptake and an increased rate of platinum efflux [51]. Employing fluorescently tagged ATP7B and a fluoresceinlabeled cisplatin analog, it was observed that intracellular platinum and ATP7B colocalize within vesicles that subsequently move toward the cell surface [50].

In particular, ATP7B has been more closely linked with cisplatin resistance than either Ctr1 or ATP7A [52]. Therapeutically, a strong correlation exists between patients having a poor outcome for oxaliplatin treatment of colorectal cancer and those displaying increased levels of ATP7B [53]. Recent studies evaluating codelivery of an ATP7B silencing siRNA alongside cisplatin [53] have shown great promise, increasing the human oral squamous cell line OSC-19-R sensitivity by 10.6-fold over cells not transfected with siRNA [52]. The dual therapy reduces cancer cell proliferation and angiogenesis while increasing tumor cell apoptosis.

2.3.Polyspecific Organic Cation Transporters

The organic cation transporters (OCT1-3; SLC22A1-3) and multidrug and toxin extrusion antiporters (MATE; SLC47) are termed polyspecific because they transport a broad range of compounds, both endogenous and exogenous, having different sizes and molecular structures. By contrast, the majority of membrane transporters are metabolite- or nutrient-specific and are termed oligospecific [54–56]. Figure 3. Vectorial movement of platinum agents from the blood to urine or bile, through the basolateral then apical membrane of a polarized epithelial cell. The differential uptake of cisplatin and oxaliplatin by OCTs and MATEs is highlighted.

Both OCTs and MATEs are highly expressed in excretory organs where they work sequentially to move organic cations from the blood to either the bile in the liver or to urine in the kidneys [54]. In the initial step, OCTs transfer substrates through the basolateral membrane of polarized epithelial cells before they interact with MATEs localized in the apical plasma membrane (Figure 3).

Given the wide variety of substrates transported by polyspecific transporters, large variations in the binding affinity and rate of transport for different species are expected and, critically, not all substrates transported by OCTs are good substrates for MATEs. This differential binding (Figure 3) is intimately linked with drug toxicity and provides a strong rationale for why some platinum agents and not others are nephrotoxic [26, 54].

2.3.1.SLC22A1-3 Transporters

The organic cation transporters, OCT1-3, are expressed in different locations and it is important to note for translational purposes that variations in distribution between human and rodent orthologs have been reported [57]. hOCT1 is preferentially expressed in the liver, hOCT2 in the kidney and brain, specifically in dopamine-rich regions of the pyramidal cells of the cerebral cortex and the hippocampus, and hOCT3 in a broad range of tissues including brain, heart, liver, skeletal muscle, placenta, and kidney [56]. Despite their differential expression, all OCTs function as bidirectional transporters for small hydrophobic compounds ~60–350 Da in size [55]. Research indicates that both cisplatin and oxaliplatin are substrates for hOCTs, and because the mechanism of transport relies on both an electrical and concentration gradient [54], the intracellular platinum

concentration may exceed the extracellular platinum concentration.

Renal tubular epithelial cell toxicity was linked with cisplatin nephrotoxicity for many years; however, the molecular mechanisms of action giving rise to this toxicity did not become apparent until the discovery of OCT1 in 1994 [58]. Subsequent studies with OCT1/2 knockout mice confirmed the role of OCTs in excretion of organic cations [59]. Direct evidence for the role of OCTs in cisplatin uptake came in 2005 when two groups [60, 61] transfected HEK293 cells with rat and human orthologs of OCT2, respectively, and observed increased cellular uptake of platinum, correlating with increased cell death. Subsequent studies employing inhibitors of OCT2 [62] and OCT2 knockout mice [63] confirmed the initial observations that OCT2 is a key determinant of cisplatin-induced nephrotoxicity. Clinical data also support use of cimetidine, a known OCT2 substrate, as a protective agent for the kidneys of patients undergoing cisplatin treatment [64, 65].

Following the observation that OCT2 is expressed in the outer hair cells and the stria vascularis cells of the cochlea [66], the role of OCTs in ototoxicity has also been investigated. Reduced or completely eliminated ototoxicity was observed following genetic deletion of OCT1 and OCT2 [63], and co-treatment of cisplatin with cimetidine in mice [66] confirmed the conclusion that cisplatin uptake by OCTs is linked with both nephro- and ototoxicity.

In contrast to cisplatin, oxaliplatin exhibits low nephrotoxicity. Therefore, based on the hypothesis above it might be expected that oxaliplatin is not transported by OCTs. Indeed, when carboplatin and nedaplatin, widely regarded to have low nephrotoxicity, were evaluated for uptake in OCTs, no evidence for their transport was recorded [67]. Oxaliplatin, however, interacts strongly with hOCT2 as demonstrated through cell studies [67, 68] and visual inspection of renal slices having collapsed non-perfused lumens [69]. The nephrotoxic patterns of these slices resemble those of cisplatin and, therefore, the hypothesis was drawn that platinum uptake via OCTs is non-toxic when coupled with an effective efflux transporter [67]; this transporter was later identified as the hMATE transporter (see below, Section 2.3.2).

Variations in OCT substrate specificity and the implications thereof are highlighted by uptake data for hOCT3. The observation that oxaliplatin but not cisplatin is a substrate for hOCT3, which is expressed among other locations in the intestine, led researchers to postulate that the cytotoxicity of oxaliplatin against colon cancer cells arises at least in part from the expression of hOCT3 in this location [67]. Moreover, recent studies have indicated a correlation between hOCT3 expression in cancerous versus non-cancerous colon and rectal tissues, with a 9.7-fold higher mRNA level being reported for patient-derived colon cancer tissues over their non-cancerous counterparts [70].

2.3.2. Multidrug and Toxin Extrusion Antiporters

As noted above hMATEs including hMATE1 and hMATE2-K are widely accepted to be efflux transporters that protect cells by transferring organic cations to the bile and urine. Experiments in mice with genetic deletion of hMATE support this postulate through increased incidence of nephrotoxicity [71]. As with the OCTs, MATE tissue distribution varies between species with hMATE1 being highly expressed in the kidney, liver, heart, skeletal muscle, and other locations, whereas hMATE2-K is kidney-specific [56].

Contradictory results have been reported for the uptake of cisplatin by hMATE1, in part due to the H+/organic cation antiporter nature of the transporter, which requires pretreatment of cells with ammonium chloride to activate the H+ gradient across the plasma membrane [26]. However, good substrate specificity has been demonstrated for MATE2-K, with oxaliplatin being readily taken up by this transporter while cisplatin is not [67]. This specificity, combined with hMATE2-K localization in the kidneys, has led to the hypothesis that the differential nephrotoxicity of oxaliplatin and cisplatin arises from their interaction with hOCT2 and hMATE2-K, facilitating their passage into the

waste stream. If, like cisplatin, a platinum complex interacts with only the import transporter hOCT2 it will most likely exhibit toxic side effects due to renal accumulation [72] (Figure 3).

3.COVALENT ADDUCTS GENERATED WITH PLATINUM AGENTS

3.1.Cytosolic Transformations of Platinum Drugs

It is widely accepted that when platinum agents pass into a cell they readily undergo aquation in response to the significant decrease in chloride concentration between the extracellular blood plasma (~100 mM) and the intracellular cytosol [24, 25, 30]. The chloride ion concentration within a cell is typically reported at 4 mM based on application of the Nernst equation to passive diffusion of chloride across the plasma membrane in muscle and nerve cells [73], however, direct measurements within a range of cells including cancer cells reveal actual intracellular chloride concentrations in the 12–55 mM range [73]. Diffusion of aquated, cationic platinum complexes out of the cell is unfavorable, and instead they react with a variety of cellular components, including DNA, RNA, proteins, phospholipids, and thiol complexes, via competing pathways [74–77]. The thermodynamics and kinetics of many of these pathways have been extensively studied as platinum speciation is intimately linked with the efficacy and toxicity of platinum agents.

For cisplatin it has been shown that the neutral parent complex cis-[Pt(NH3)2Cl2] (1; Figure 4) fails to react directly with typical nitrogen and oxygen donor biological nucleophiles; however, complexes activated by replacement of chloride ligands with labile water ligands (t1/2 ~2 h) are 10–70 × m ore reactive than their parent starting material and react indiscriminately with biological nucleophiles [73, 77]. The rate of reaction for complex 2 with biological nucleophiles (t1/2 ~0.1 h) occurs on a suf ficiently fast timeframe that 4 is not observed in appreciable concentrations in vivo [74].

In addition to aquation, deprotonation of coordinated water molecules may occur to generate the hydroxo complexes cis-[Pt(NH3)2Cl(OH)] (3) and cis-[Pt(NH3)2(OH)2] (6) that are considerably less reactive than their corresponding aqua species 2 and 4 owing to the relative inertness of the Pt–OH bond. Furthermore, formation of hydroxide-bridged oligomers [74] of the form [{Pt(NH3)2(μ-OH)}n]nC have been reported under specific conditions. The extent of deprotonation of the aqua ligands (Ka) is governed by its pKa and the pH of the aqueous sol ution. Recent studies employing 15N-edited 1H and [1H,15N] heteronuclear multiple quantum interference NMR spectroscopy [78] provide the most accurate measurements of the pKa values for cisplatin. In particular, this met hodology overcomes complications associated with fitting potentiometric curves comprising multiple overlapping events and reduction in signal due to formation of hydroxo bridged dimers. pKa values of 6.41 for cis -[PtCl(H2O)(NH3)2]+ and 5.37 and 7.21 for cis-[Pt(H2O)2(NH3)2]+, were reported, in agreement with previous reports.

Single crystal X-ray diffraction [79, 80] and 195Pt NMR spectroscopic studies [81] support the formation of the dimers, trimers, and higher oligomers when the pH of the solution is within two pKa units of the Pt–OH2 bond (pKa ± 2). Formation of hydroxide-bridged dimers is undesired because they remove active drug from solution and may modulate toxicity [82]. Detailed studies indicate that, if the mononuclear diammineplatinum(II) complex is injected into a patient, the platinum concentration in vivo will be sufficiently low to prevent oligomer formation. However, should a hydroxo-bridged oligomer be injected it would most likely persist [74, 83, 84], highlighting the importance of properly storing and administering the drug.

Figure 4. Structures and equilibria of species derived from cisplatin in aqueous solution.

Cisplatin may also react with other biological nucleophiles including sulfur atom donor species [76] and carbonate [85]. The high affinity of thiolate anions for platinum complexes has been cited as a major cause of platinum drug resistance [25], with increased intracellular glutathione and overexpression of glutathione S-transferase [86, 87] being correlated strongly with resistance.

Experimental evidence indicates that sulfur nucleophiles react directly with cisplatin without the need for activation via aquation [88]. Furthermore, recent research has indicated that Pt-guanosine adducts may be generated more rapidly in the presence of sulfur nucleophiles [89], but the importance of this reaction pathway in platinum treatment regimens has yet to be determined.

Like cisplatin, oxaliplatin forms adducts with a variety of biological nucleophiles following aquation. In vivo, biotransformation studies were performed where the plasma ultrafiltrate of five patients who received oxaliplatin by infusion at 130 mg/m2 for 2 h was analyzed, and the [Pt(DACH)(Cl)(OH2)]C (DACH = trans-R,R-1,2-diaminocyclohexane) derivative was identified as the major species (31–100 %) by HPLC. Methionine (8–24 %), diaqua (2–26 %), monochlorocreatinine (2–11 %), and glutathione (1 %) adducts were also tentatively identified [90].

3.2.Speciation of DNA-Platinum Adducts

Within the cell, cisplatin forms adducts with both nuclear and mitochondrial DNA [91]. Aside from its localization, mitochondrial DNA differs from nuclear DNA in its lack of histones and slower kinetics of cisplatin intrastrand cross-link repair [92] (discussed below). A larger percentage of Pt-DNA adducts [93] (4-to 6-fold [94]) have been reported for mitochondrial DNA over nuclear DNA, and this difference has been attributed to higher initial binding rates and inefficient removal of the major adducts by repair processes [95].

Most research has focused on the ability of cisplatin to modify nuclear DNA, and it is widely accepted that platinum reaching the nucleus without being deactivated primarily forms covalent adducts at the N7 position of the purine bases [91, 96]. The monoaqua adduct of cisplatin (t1/2 = ~2 hours) reacts rapidly to generate cov alent adducts with the N7 positions on adenine and guanine bases (t1/2 = 0.1 h), after which the sec ond chloride ligand is aquated to facilitate formation of a bifunctional cross-link [24, 77]. Cross-links are designated as 1,2-or 1,3-intra- or interstrand cross-links, where the numeric designation specifies whether two modified nucleotides are adjacent to each other (1,2-d(GpG)) or separated by an unmodified nucleotide N (1,3-d(GpNpG)). For intrastrand cross-links both modified nucleotides are on the same strand of the DNA, whereas interstrand cross-links have one modified nucleotide on each strand of duplex DNA. Numerous enzymatic degradation and acid hydrolysis experiments have been performed to elucidate the distribution of Pt-DNA adducts with nuclear DNA. The consensus is that approximately 60–65 % of Pt-DNA adducts are 1,2-intrastrand d(GpG) links, 25–30 % are 1,2-intrastrand d(ApG) links, 5–10 % are 1,3-intrastrand d(GpNpG) linkages, and 1–3 % of Pt-DNA adducts are inter-strand cross-links (ICLs) [97, 98]. Comparable Pt-DNA adduct profiles are reported for oxaliplatin [99].

Each platinum adduct distorts and unwinds the structure of double-stranded DNA to which it is bound in a unique manner [100]. Single crystal X-ray structures (Figure 5) of platinum adducts of double-stranded DNA have provided insight into the structural basis of DNA processing events that are influenced by platinum binding. Analysis of the solid state structure of the major cisplatin 1,2-d(GpG) intrastrand cross-link [101] (Figure 5A) reveals that the Pt adduct forms hydrogen bonds with the DNA backbone on the 5′ side of the lesion and unwinds the duplex DNA by ~25°. In the solid state, a global bend of 35–40° in the DNA occurs, whereas NMR data [102, 103] suggest that, in solution, this bend angle may be significantly increased, up to 60–70°. The 1,3-d(GpNpG) minor adduct [104] (Figure 5B) is globally bent by about 30°, but the region around the platinated nucleotide is more severely distorted. In contrast to both the intrastrand cross-links, crystal structures of a cisplatin interstrand cross-link [105] (Figure 5C) reveal that the duplex is bent at a 47° angle toward the minor instead of the major groove, and that the duplex is unwound by 110° overall. Finally, analysis of the crystal structure of the oxaliplatin adduct on duplex DNA [106] (Figure 5D) highlights the effects of exchanging a cis-diammine(II) [80] moiety for a cis-{Pt(DACH)}2+ group, mainly involving a change in hydrogen bonding around the lesions. In

contrast to cisplatin, structural characterization of oxaliplatin DNA adducts supports hydrogen bond formation between the platinum adduct and the DNA backbone on the 3′ side of the cross-link [107]. Only the biologically active R,R-isomer, and not the S,S-isomer, can generate 3′ hydrogen bonds. This conformational difference between cisplatin and oxaliplatin adducts is proposed to interfere with the recognition of the damaged DNA by cellular components, contributing to the differential properties of the two drug molecules [100].

Figure 5. X-ray crystal structures of platinum lesions on duplex DNA. (A) 1,2-d(GpG) cisplatin lesion (1AIO [101]); (B) 1,3-d(GpG) cisplatin lesion (1DA4 [104]); (C) inter-strand cisplatin lesion (1A2E [105]); (D) 1,2-d(GpG) oxaliplatin lesion (1IHH [106]). Cisplatin and oxaliplatin are shown in space-filling representation; platinum(grey), nitrogen(blue), and carbon (beige). 3.3.Effects on Chromatin

DNA in the nucleus is packaged as nucleosomes, which consist of a core of eight histone proteins (H2aH2bH3H4)2 around which genomic DNA (146 bp dup lex) is wrapped in a shallow, left-handed helix. The nucleosomes are separated by strings of linker DNA (typically 10–50 bp [108]) that are not tightly bound to proteins. Because the positioning of the genome on the nucleosome influences gene expression [109], and it has been proposed that platination of nucleosomal DNA may alter its positioning [110], several groups have investigated chromatin modification by cisplatin and related platinum agents.

Early studies [111] confirmed that the formation of cisplatin adducts with DNA occurred to a similar extent in the presence and absence of core proteins, with an average of ~45 platinum atoms per core particle being reported upon saturation after 40 hours. Additionally, in contrast to the trans isomer (trans-dichlorodiammineplatinum(II)), no significant histone-histone or histone-DNA cross-links were observed. Later studies sought to evaluate whether cisplatin preferentially formed covalent adducts with linker versus nucleosomal DNA [112–114], and it was concluded that platinum adducts preferentially form in the linker region [113]. Measurement of the rate of platinum adduct formation with chromatin, core particle, and DNA as a function of platinum concentration indicated that the platinum/chromatin ratio was equivalent to the platinum/free DNA ratio and differed significantly from the platinum/core particle ratio, which was significantly less. Subsequent experiments with chromatin extracted from human cells [115] and reconstituted chromatin [114] confirmed that DNA within the core particles was protected from cisplatin damage through direct visualization of the platination sites using a polymerase stop assay. Experiments also showed that direct platination of histones prevents nucleosome core particle formation, but platination of nuclear DNA prior to nucleosome formation does not affect core formation [114].

Given that most (75–90 %) nuclear DNA is wrapped around nucleosomes [116], significant research has been performed to elucidate the effect of platination on the translational and rotational settings of the nucleosome core particles. Experiments [108] in which nucleosome core particles were directly treated with cisplatin and oxaliplatin indicate that lesions formed on assembled core particles do not significantly affect their positioning but instead are generated at intrinsically preferred sites. Complementary experiments [117, 118] in which site-specifically platinated DNA was assembled with histone proteins indicate that platination overrides the predefined rotational setting of the nucleosomes. In both cases [108, 117, 118] the platinum lesions were directed inward, facing the histone octamer core, thus shielding their recognition and repair by DNA damage recognition proteins [119] (see Section 4.3.1). Additionally, platination of nucleosomal DNA reduces the dynamic nature of the nucleosome [108]. 4.CELLULAR PROCESSING OF PLATINUM DNA ADDUCTS 4.1.Inhibition of DNA Synthesis

Based on prior observations that the organic anticancer agent hydroxyurea causes E.coli to elongate and inhibits division of mammalian cells and DNA synthesis, it was postulated that cisplatin might also block DNA synthesis [96]. Quantitation of the rate of incorporation of

3H-thymidine, 3H-uridine, and 3H-L-leucine in the presence of cisplatin confirmed that, at concentrations of □5 μM, DNA synthesis was selectively inhibited by cisplatin in AV3 cells, whereas at concentrations of >25 μM, DNA, RNA, and protein synthesis were all blocked [96]. Subsequent experiments evaluated the roles of purified, individual DNA polymerases in processing both intra- and interstrand cross-links, revealing that DNA replication polymerases are inhibited by bifunctional lesions ~90 % of the time [24, 98, 120]. In contrast to these results, several studies indicated that DNA synthesis continues to occur in cells that fail to divide following treatment with cisplatin [121], suggesting that a cellular mechanism for bypassing Pt-DNA lesions may be operative. This mechanism is now known to be translesion synthesis (TLS), and the efficiency and fidelity with which cells are able to bypass DNA-platinum lesions is linked with drug sensitivity, resistance, and mutagenicity.

Specialized TLS polymerases [122] have evolved in mammalian cells to incorporate nucleotides opposite damaged nucleotides on the template strand, including members of the Y family of polymerases (η, ι, κ, and Rev1) and DNA polymerase ζ [123, 124]. For TLS, polymerase switching must occur, where the replication polymerase is displaced by the TLS polymerase. This process is signaled by mono-ubiquitination of proliferating cell nuclear antigen (PCNA) at Lys-164. Once ubiquitinated, PCNA has a higher affinity for the Y-family polymerases, all of which share a novel ubiquitin binding motif that localizes TLS polymerases at the site of the blocked replication machinery. Following recruitment of the TLS polymerase three distinct steps occur to bypass a bifunctional lesion such as a 1,2-d(GpG) adduct, namely, (i) insertion of a nucleotide opposite the 3′-G, (ii) insertion of a nucleotide opposite the 5′-G, and (iii) extension onward from the 5′-G. Each of these steps has distinct kinetics that depend on the specific polymerase and the nature of the lesion [123, 125]. Differences in the solution geometry of cisplatin- and oxaliplatin-DNA adducts account for the differential TLS efficiency and fidelity observed for these two species, with polymerase η and β bypassing oxaliplatin lesions more readily than cisplatin lesions [99, 126]. In vivo studies subsequently revealed that Pol η or κ incorporate the correct or incorrect nucleotide, respectively, opposite a 1,2-d(GpG) cisplatin intrastrand cross-link, while the extension step is performed by Pol ζ. Pol ζ is an error-prone polymerase that, in the absence of other polymerases, performs TLS past cisplatin adducts with low efficiency [99, 127].

Recent studies have also discovered a role for TLS polymerases in the repair of ICLs. In general, ICL bypass consists of three steps. The first is an unhooking step where incisions are made at either side of the damaged nucleotide on one strand of the DNA. This process is followed by TLS past the unhooked ICL, which restores one of two strands and provides an intact template to complete the repair process [128]. Difficulties studying this process at a mechanistic level persist, however, owing to the combined effects of multiple ICL repair pathways, potential redundancy between polymerases, and the limited options currently available to study these pathways [129].

4.2.Transcription Inhibition

As for DNA polymerases, RNA polymerases that transcribe mRNA encoded on DNA are efficiently blocked by bifunctional platinum adducts. Early studies exploring the mechanism of action of cisplatin revealed that cell death was proceeded by G2/M arrest [121], indicating that, under physiologically relevant conditions, sufficient DNA synthesis occurred to satisfy progression of the cell cycle through the S phase. More detailed studies correlated the cellular sensitivity of Chinese hamster ovary cells both proficient and deficient in DNA repair with G2 arrest following cisplatin treatment. These experiments confirmed that cisplatin affected the extent of DNA synthesis but importantly was not linked to the sensitivity of the cell line [130].

Taken together, the results are consistent with the hypothesis that transcription inhibition is the main mechanism by which cisplatin exerts its action, arresting the cell cycle at G2/M, resulting in cellular failure to transcribe the genes necessary to enter mitosis. More detailed experiments performed with site-specifically modified Pt-DNA adducts indicate transcription by RNA

polymerase II (Pol II) and E. coli polymerase (RNAP) to be blocked by 1,2-d(GpG) and 1,2-d(ApG) adducts on the template strand but only slightly inhibited when the adduct is on the non-template strand [131]. To date a vast body of research into transcription inhibition has been reported with Pol II, the mammalian polymerase involved in transcription of the majority of eukaryotic genes. Inhibition of RNA polymerase I (Pol I), which is involved in transcription of rRNA, has received considerably less attention but is speculated to be operative via a comparable mechanism [24, 132]. Pol I transcribes far more DNA overall than Pol II and its inhibition may be responsible for ribosome biogenesis stress (see below, Section 5.5), the mechanism to which the anticancer properties of oxaliplatin were recently attributed [133].

In addition to failing to transcribe the requisite genes, once stalled, the polymerases act as damage recognition sentinals that may either instigate repair pathways or mediate cell death, as discussed in the following sections. Normal cells have multiple different repair pathways, and often more than one pathway is operative in the removal of a lesion. 4.3.Repair of Platinum Lesions 4.3.1.Nucleotide Excision Repair

Nucleotide excision repair (NER) is a programmed cellular repair mechanism for removal of DNA lesions and the primary mechanism by which cisplatin-DNA 1,2-intrastrand cross-links are removed [24]. In vitro experiments indicate that intrastrand adducts of oxaliplatin are repaired in a similar manner [134], revealing that the carrier ligand does not influence the repair mechanism. However, inter-strand cross-links generated by cisplatin are not substrates for NER [135].

Figure 6. Schematic representation of the NER pathway and the multiple proteins involved in recognition and excision of the platinum lesion followed by regeneration of the double-stranded DNA.

NER is a complex, multistep process [136] (Figure 6) that requires six repair factors: RPA, XPA, XPC, TFIIH, XPG, and XPF•ERCC1. The initial step is an ATP-independent recognition process that is believed to involve indirect readout of the damage through sensing abnormal DNA backbone conformations. In this step RPA, XPA, and XPC-TFIIH bind cooperatively in a random order at the site of the damage-generating pre-incision complex 1 (PIC1). The DNA is then unwound by up to 20 base pairs and a kinetic proofreading step takes place, ensuring that only damaged DNA undergoes NER. Once DNA is unwound, XPG binds the damaged site with high affinity displacing XPC to generate PIC2. Finally, XPF•ERCC1 is recruited to the damage site and generates dual incisions around the damaged nucleotide (PIC3) liberating a 24–32 nucleotide oligomer containing the damaged site. The excised platinum oligomer is expected to undergo degradation in the nucleus, like other small oligomers, but the fate of the platinum remains unknown [24]. DNA polymerases δ and ε fill the gapped region left following excision of the damaged strand before ligation of the ends regenerates double-stranded DNA.

Xeroderma pigmentosum (XP) cells lacking one of more components of the NER pathway are 5- to 10-fold more sensitive to cisplatin treatment than normal cells [24, 137], confirming the role of NER in processing platinum lesions. Complementary data indicate a correlation between increased expression of NER genes and cisplatin resistance.

As discussed in Section 3.3, platinum lesions on nuclear DNA effect the positioning of genomic DNA in nucleosomes, with lesions being preferentially oriented toward the histone core proteins, thus shielding damage recognition and repair by NER proteins [119]. In vivo, NER is further modulated by post-translational modification of the histones [119]. 4.3.2.Recombination Repair

Recombination repair (RR) operates by two pathways, homologous recombination (HR) and non-homologous end joining (NHEJ) [24, 136]. Like NER-deficient cells, cells deficient in RR are sensitive to cisplatin treatment. Furthermore, cells deficient in both NER and RR are more sensitive

to cisplatin than either deficient cell line alone [138]. DNA recombination is most commonly implicated in the repair of interstrand cross-links [139].

As with all repair mechanisms the first stage is damage recognition. Following double-stranded break recognition, nucleolytic processing generates single-stranded DNA with free 3′-ends facilitating Rad51-mediated strand invasion forming a Holliday junction. Subsequent DNA synthesis, ligation, and resolution of the Holliday junction regenerates double-stranded DNA with high fidelity, with information lost from the broken double-stranded DNA being regenerated from the homologous duplex. In contrast, NHEJ is an error-prone repair pathway that ligates the two duplex termini regardless of whether they come from the same or a different chromosome [136, 139]. Experiments support HR as an important repair mechanism for cisplatin lesions, whereas knockout of the NHEJ pathway does not significantly affect the cisplatin sensitivity of cells [140].

4.3.3.Fanconi's Anemia

Fanconi's Anemia (FA) is a rare autosomal disease arising from deregulation of replication-dependent removal of interstrand cross-links [141]. It is generally believed that the FA pathway has evolved in eukaryotes to deal with the difficulties associated with repairing ICLs where both strands of a DNA duplex are damaged. Patients with Fanconi's Anemia are thus unable to efficiently remove ICLs and are therefore hypersensitive to cisplatin and other anticancer agents operative through formation of ICLs [142].

The FA pathway utilizes elements of the HR, NER, and TLS pathways in the repair processes and its activity is closely regulated. The pathway is only operative in the S phase of the cell cycle and is turned off following repair of DNA damage [141, 143]. The current mechanism for DNA repair by FA proteins is incomplete but is believed to be instigated by ATR activation and ubiquitination of FANCD2, which then co-localizes with repair proteins at the site of DNA damage. Subsequently, endonucleases generate double-strand breaks that uncouple the two sister chromatids. The structure-specific nucleases ERCC1-XPF and MUS81-EME1 have been linked with ICL repair, and cells lacking these proteins are sensitive to cross-linking agents. Following formation of the double-strand break it is speculated that the cross-link is unhooked to generate a single nucleotide lesion that can be processed like a monofunctional adduct by NER and TLS proteins. Finally, following replication through the site of DNA damage, HR proteins are implicated in regeneration of the replication fork [141].

4.3.4.Mismatch Repair

The mismatch repair (MMR) pathway is involved in recognition and repair of base-base mismatches and insertion and deletion loops generated where one partnerless nucleotide in double-stranded DNA is partially extrahelical [144]. MMR has been linked with repair of both intra- and interstrand platinum cross-links, but mismatch repair proteins do not recognize oxaliplatin cross-links [145]. Furthermore, several studies have demonstrated correlations between cisplatinresistant cell lines and MMR defects, having both intrinsic and acquired resistance [24, 144, 146, 147].

Current research indicates that hMSH2, which forms heterodimers hMutSα and -β with MSH6 and MSH3, respectively, binds with high affinity (Kd ~67 nM [148]) to 1,2-d(G pG) intrastrand cross-links but poorly with 1,3-d(GpTpG) cisplatin adducts [149]. MutSβ also binds ICLs generated with cisplatin [150]. Preferential recognition of cisplatin over oxaliplatin, trans-diamminedichloroplatinum(II), and [Pt(dien)Cl]+ adducts has also been reported [148, 149]. Following damage recognition, MutLα, a heterodimer of MSH1 and postmeiotic segregation increased 2 (PMS2), is recruited to the site of action and a relatively stable ATP-dependent ternary complex is generated with the damaged DNA. Additionally, the PCNA sliding clamp, DNA polymerase δ, exonuclease 1, and DNA ligase are required to complete the MMR process [24]. MMR proteins also play an important role in signaling DNA-damage induced apoptosis [151], effecting phosphorylation of p53, and activating the stress-activated kinase, JNK, as discussed in

Section 5 in greater detail. 4.3.5.Base Excision Repair

Base excision repair (BER) has evolved to repair damaged bases that result in minimal distortion of the DNA duplex [152], and recent evidence supports an additional role for BER in cross-link repair [153]. Data supporting dysregulation of BER proteins in cisplatin-resistant cancers [154] has led researchers to investigate its role in the development of cisplatin resistance. Results [153] obtained with BER-defective MEFs in the presence and absence of a small molecule inhibitor of APE1, a critical enzyme in the BER pathway, support the involvement of BER in ICL repair while showing that BER does not affect intrastrand cross-link processing. In contrast, minimal changes in sensitivity of the BER-deficient/inhibited cells compared with wild-type cells occurs when they were treated with oxaliplatin, indicating a specific role for cisplatin.

Two BER pathways have been reported – short and long patch pathways [136]. Damage recognition and excision are initiated in each pathway by DNA glycosylases that bind the damaged DNA, compressing it and flipping the damaged base out of the helix into the active site of the enzyme. Cleavage of the damaged base generates an abasic site on the DNA, and subsequent removal of the abasic sugar generates a one-nucleotide gap, which is filled by DNA Pol β, APE1, and DNA ligase III-XRCC1 in mammalian cells. Alternatively, the long patch pathway requires APE1 to make an incision on the 5′-side of the abasic site, followed by a 3′-incision made by FEN1 endonuclease. This cut liberates a 2–10 nucleotide long excision product and DNA Pol δ/ε and PCNA then synthesize a new patch that is ligated by DNA ligase I to regenerate the double-stranded DNA. 4.4.Protein Binding to Platinated DNA

In addition to specific repair proteins highlighted in the preceding sections several other mammalian proteins recognize and bind to platinated DNA [24, 100, 155]. The best studied of these are the high-mobility group (HMG) domain proteins (Figure 7). High mobility group box (HMGB) proteins 1–4 share considerable structural homology, and all contain two tandem HMG domains, capable of recognizing and binding with high affinity to bent and distorted duplex DNA.

HMGB1 is a 30 kDa protein comprising two HMG domains appended with an acidic tail that is not essential for DNA binding. Owing to its high intercellular concentration, short residence time on DNA, and affinity for bent DNA, HMGB1 has a high probability of encountering platinum adducts; it has therefore long been postulated to be involved in cisplatin sensitization [24, 156, 157]. However, experimental results aiming to correlate HMGB1 expression levels with cisplatin sensitivity [158], or to introduce foreign HMGB1 to modulate cisplatin sensitivity, have proved inconclusive [24]. Recent results from our laboratory [159, 160] support the initial hypothesis that HMGB binding to cisplatin-damaged DNA prevents NER via a repair shielding mechanism, thereby sensitizing cells to cisplatin treatment. Critically, however, formation of a disulfide bond between Cys22 and Cys44 in the second HMG domain must be prevented if cisplatin sensitization is to be achieved. Experiments performed with HMGB4, a variant of the HMG box protein that contains a tyrosine residue in place of Cys22 and therefore is not affected by the intracellular redox potential, unambiguously allowed correlation of HMGB4 expression with cisplatin sensitivity. Supporting these results are clinical observations that link the exceptionally high cure rates of testicular germ cell tumors (TGCTs) with the preferential expression of HMGB4 in testes. Finally, a two-fold increase in cisplatin sensitivity was demonstrated for cisplatin-resistant breast cancer cells MDA-MB-231 following transfection of HMGB4 cDNA and subsequent HMGB4 expression in the cell line. Figure 7. X-ray structure of HMGB1 bound to duplex DNA containing a cisplatin 1,2-d(GpG) intrastrand cross-link (1CKT [224]; see also [159]). Cisplatin is shown as a space-filling model with platinum in gray and nitrogen in blue, and HMGB1 is represented as cyan ribbon.

Several non-HMGB proteins contain one or more HMG domains, including the structure-specific

recognition protein SSRP1 and the ribosomal RNA transcription factor hUBF (human upstream binding factor). SSRP1 is an 81 kDa protein containing one HMG domain that forms a heterodimer with Spt16/Cdc68 [24]. The heterodimer FACT (facilitates chromatin transcription) is a chromatin modulator that binds cisplatin DNA adducts. The isolated HMG domain of SSRP1 also binds damaged DNA, whereas the full SSRP1 complex alone does not [161]. hUBF contains six HMG domains [100] and binds cisplatin-damaged DNA with high specificity and the strongest reported affinity (Kd = 60 pM [162]). The binding affinity of hUBF for its natural substrate, the rRNA promotor, and cisplatin-damaged DNA are comparable, leading the authors to propose transcription hijacking [100, 162] as an alternative means by which protein binding to cisplatin-damaged DNA may sensitize cells to treatment. At saturated levels of hUBF and platinum concentrations below those reported in cancer patients, complexation of hUBF with the rRNA promotor was completely inhibited. Such dysregulation of rRNA synthesis is likely to have a negative effect on a cell's welfare.

The TATA-binding protein (TBP) also binds cisplatin-damaged DNA with a preference for 1,2-d(GpG) intrastrand over 1,3-d(GpG) cross-links [163]. The binding affinity and kinetics of TBP to the TATA box are similar to that observed for TBP binding to cisplatin-damaged DNA and increased by 20-fold in the presence of HMGB1, leading to speculation that a TBP-HMGB1 complex interacts with platinated DNA. TBP binding to damaged DNA results in transcription inhibition through reduced interaction of the TBP with the TATA box and to reduced transcription factor recruitment [24].

YB-1 is a transcription factor that binds to an inverted CCAAT box sequence called the Y-box [164]. It also binds preferentially to platinated DNA sequences including 1,2-d(GpG), 1,2-d(ApG), and 1,3-d(GpTpG). YB-1 also physically interacts with many cellular proteins including PCNA, MSH2, and DNA polymerase δ, many of which are elements of various repair pathways suggesting a possible role for YB-1 in modulation of DNA damage repair [24].

The 104 amino acid protein poly(ADP-ribose)polymerase 1 (PARP-1) is a platinum damage response protein that binds to cisplatin 1,2-d(GpG) and 1,3-d(GpTpG) modified DNA in both its activated and unactivated forms [165]. PARP-1 also binds DNA adducts generated with oxaliplatin and the monofunctional platinum agent pyriplatin ([Pt(NH3)2(pyridine)Cl]+) indicating that the protein binding event occurs in response to the presence of a foreign substance on DNA rather than a specific structural distortion [165]. PARP-1 has been associated with BER [166] and induces apoptosis through formation of poly(ADP-ribose) polymers that signal release of apoptosis-inducing factors from mitochondria [167]. In response to DNA damage PARP is heavily upregulated [165], resulting in NAD+ depletion and ultimately cell death via necrosis as a result of glycolysis shutdown. Recently, the concept of synthetic lethality has given rise to the development of PARP inhibitors for treatment of patients with defective BRCA genes [166, 168].

Tumor suppressor protein p53 contains two DNA-binding domains, both of which are required for binding to platinated DNA [169]. However, the C-terminal domain is more critical for preferential binding of damaged over undamaged DNA [24, 170]. Purified, active p53 binds 1,2-d(GpG) intrastrand cross-links (Kd = 150 nM) but has no affinity for 1,3-d(GpG) cross-links, ICLs, or monofunctional adducts [171]. Like PARP, p53 interacts with damage recognition elements in DNA repair pathways and also enhances HMGB1 binding [172], thereby modulating repair of adducts.

5.SIGNAL TRANSDUCTION PATHWAYS ACTIVATED BY PLATINUM DNA DAMAGE

5.1.Overview of Signal Transduction

Before DNA damage can be repaired it must first be identified and the information communicated to damage response proteins within the cell. Once the DNA damage response (DDR) has been initiated the cell cycle check points, Chk1 and Chk2, become activated, halting cell cycle

progression and providing the opportunity for the cell to repair the damage before cell cycle re-entry (checkpoint recovery) [136, 173, 174]. Cell cycle check points are thus implicated in control and activation of DNA repair pathways, in addition to composition of telomeric chromatin, localization of DNA repair proteins, and in some cells induction of apoptosis [173]. Cell cycle arrest may occur at the G1, intra-S, or G2 phase of the cell cycle [136]. If the cell is unable to efficiently repair the damage it will remain arrested or undergo apoptosis, preventing genetically unstable cells from progressing through replication. The cytotoxicity of classical platinum agents thus relies not only on their ability to inhibit DNA and RNA synthesis but also on the inability of cells to sense and signal repair of platinum lesions.

5.1.1.DNA Damage Sensors

Until recently little was known about proteins involved in detecting DNA damage [173], but a growing body of evidence supports the role of the so-called 9-1-1 complex [175] in combination with Rad17 and the proximal kinases ataxiatelangiectasia mutated (ATM) and ATM- and Rad3-related kinases (ATR) [176]. The 9-1-1 complex is a heterotrimeric, toroidal clamp, comprising Rad9, Hus1, and Rad1 proteins, that shares structural and mechanistic features with the better known PCNA [177]. Following genotoxic stress induced through replication inhibition as well as other mechanisms the 9-1-1 complex is loaded onto the chromatin by the clamp loader Rad17-replication factor C (RFC) [178, 179]. Independently but simultaneously, ATM-Rad3 related kinases-ATR-interacting protein (ATR-ATRIP) binds to the damaged DNA. Finally recruitment of TopBP1, which bridges between the 9-1-1 complex and ATRIP-ATR, facilitates ATR-mediated Chk1 phosphorylation and activation [180]. Whereas ATR can phosphorylate some substrates in the absence of Rad9, TopBP1 localization depends on the Rad9 tail and is therefore essential for Chk1 phosphorylation.

5.1.2.Signal Transducers

Following recognition of damage by the cell, information is transferred via a series of signal transducers to effectors that instigate repair of damaged DNA or halt cell cycle progression [173]. Several sequential steps are therefore required to execute the function of the DNA damage response pathway, and these steps must occur within a timeframe fast enough to prevent transition of damaged cells into the next phase of the cell cycle [181]. Additionally, the damage signal must be durable enough to persist as long as the damage. Distinct mechanisms are therefore implicated in the induction and maintenance of checkpoint responses.

5.2.Checkpoint Kinases

In addition to regulating cell cycle, checkpoint kinases Chk1 and Chk2 are DNA damage kinases activated by ATR and ATM, respectively [182]. ATR and ATM are protein kinases structurally related to the phosphatidylinositol-3-OH kinases (PI(3)K) family. Broadly, ATM is activated in response to double-strand breaks whereas ATR responds to breaks created by a variety of agents including stalled replication forks caused by bulky base adducts. Secondary activation of ATR is also observed during the processing of double-strand breaks, which generates single-stranded lesions [181].

The role of Chk1, which is expressed in the S and G2 phases of the cell cycle, is conserved from yeast to humans [181] and involves ATR-mediated phosphorylation of claspin complexed Chk1 at Ser317 and Ser345 [182, 183]. Chk1 is encoded on the CHEK1 gene and is essential for genome integrity, with early studies confirming the embryonic lethality of Chk1 knockout mice [184, 185]. Following the initial ATR-promoted phosphorylation steps, Chk1 dissociates from chromatin and autophosphorylation of Chk1 Ser296 generates a docking site for 14-3-3γ that in turn promotes Chk1 phosphorylation of Cdc25A at Ser76, which in turn signals proteasomal degradation [181–183]. Transitions between different phases of the cell cycle are governed by the cyclin-dependent kinases (Cdks) in combination with a variety of cyclins. Negative regulation of Cdks is achieved through phosphorylation at Thr14 and Tyr15 by the Wee1 and Myt1 kinases, while dephosphorylation by Cdc25 kinases activates Cdks. Cell cycle arrest thus occurs as a result of unregulated phosphorylation of Cdk2 or Cdk1 [181], bringing about G1 or G2 checkpoint arrest, respectively.

In contrast to Chk1, studies with Chk2-knockout mice have confirmed that Chk2 is redundant in higher eukaryotic systems [183], leading to the hypothesis that Chk1 is the main checkpoint inhibitor and Chk2 may be a supportive kinase. Chk2 functions in a similar manner to Chk1, inhibiting Cdc25 phosphatases following ATM-mediated Chk2 Thr68 phosphorylation and subsequent autophosphorylation events [181].

5.3.MAPK Proteins (ERK/JNK/p38)

The mitogen-activated protein kinase (MAPK) cascade has also been implicated in signal transduction following recognition of cisplatin-induced DNA lesions [186]. The major MAPK family members [187] include the extracellular signal-regulating kinases (ERK), the c-Jun N-terminal kinases (JNKs, also known as the stress-activated protein kinases), and the p38 kinases [28, 174]. In healthy cells MAPKs are responsible for signal transduction from the cell surface to the nucleus, thereby modulating gene expression and controlling cell proliferation, differentiation, and death [186]. Activation of MAPK in response to cisplatin is cell-dependent and may induce, suppress, or have no role in apoptosis [186]. MAPK cascades will be activated not only in response to platinum-DNA lesions but also to platinum adducts generated with other biological nucleophiles [186]. MAPKs have thus been implicated in both induction of apoptosis and the development of resistance to platinum agents, but the exact role of each of these kinases is controversial owing to complex, cell-specific responses.

In addition to activation by endogenous growth factors and mitogens, ERK is activated in response to cisplatin treatment [188, 189]. Following dual phosphorylation by MAPK kinase (MAPKK) and MAPKK kinase (MAPKKK), ERK phosphorylates p53, thereby upregulating p21, GADD45, and Mdm2 and effecting cell cycle regulation [28]. Data supporting ERK activation both contributing to and preventing cisplatin-induced apoptosis [190] have been reported for a variety of cell lines.

Like ERK, p38 proteins respond to a wide variety of stimuli including inflammatory cytokines and environmental stress, and they have been implicated in cisplatin-induced apoptosis [191]. Activation of p38 sensitizes cells to cisplatin whereas inactivation of p38 makes cells cisplatin-resistant [28, 191]. As with Chk1, ATM, and ATR may under certain circumstances activate p38. Once activated, p38 can then go on to phosphorylate the downstream MAP kinase-activated protein kinase 2 (MAPKAP kinase 2; MK2), which can induce a checkpoint response through phosphorylation of Cdc25 in an analogous manner to Chk1. Checkpoint maintenance is regulated through stabilization of Gadd45a mRNA that further potentiates MK2 activation [182]. Additionally, p38 kinases and the downstream kinase, mitogen- and stress-activated protein kinase 1 (MSK1), are involved in cisplatin-induced phosphorylation of histones [192].

JNKs have been implicated in both cell proliferation and apoptosis, as determined by the duration of JNK activation [174, 193]. Sustained activation of JNK correlates with apoptosis induction, whereas acute and transient activation of JNK signals cell survival. Most of the factors that activate p38 also activate JNK and, like p38, JNK is activated through phosphorylation of Thr and Tyr residues by MKK. Apoptosis is then controlled by modulating the activity of pro-apoptotic proteins via phosphorylation and increased expression of pro-apoptotic genes such as TNF-alpha, Bak, and Fas-L [193]. Initial observations for the role of JNK in cisplatin induced apoptosis found that JNK-defective cells are cisplatinresistant[194]. Since then others have provided evidence to support the hypothesis that the JNK pathway is involved in cisplatin-induced apoptosis [174].

A considerable amount of research has thus been performed to determine the role of the MAP kinases, but much remains to be done if their complex roles in cisplatin-induced apoptosis and resistance are to be fully elucidated. As noted above, in many instances activation of the MAPKs may be triggered by different events and cause multiple different cellular responses, sometimes in parallel. More recently the role of ras oncogenes, which are upstream regulators of ERK and JNK, has received attention [195]. The ras superfamily consists of H-, K-, and N-ras G proteins that function as molecular switches [196]. When bound to GTP ras activates ERK, and the upstream

kinases MEK and raf, which ultimately signal p53 phosphorylation following signal transduction. However, when in the GDP-bound state, ras is unable to induce signal transduction [196]. Ras over-expression and mutation has been implicated in cisplatin resistance. 5.4.Tumor Suppressor Protein p53

The status of p53 is closely linked with the ability of a cell to tolerate DNA damage and hence a patient's prognosis and likelihood of developing resistance [197, 198]. Roughly half of all human cancers exhibit a mutation in the TP53 gene [195], approximately 75 % of which are missense mutations that prevent p53-induced apoptosis and often result in aggressive tumor growth [199].

In normal cells, p53 is regulated by the E3 ubiquitin ligase Mdm2, which both tags p53 for ubiquitin proteasomal degradation and binds directly to p53 trans-activation domains 1 and 2 (TAD1 and 2) [199]. Thus the concentration of p53 is maintained at a low steady state around 103–104 molecules per cell [200]. Following activation in response to diverse stress signals including DNA damage, p53 undergoes ATM and/or ATR phosphorylation, leading to its stabilization [28]. A growing body of evidence supports p53 having both a potent transcriptional activation domain and the ability to indirectly modify gene transcription [199]. Wild-type and several mutants of p53 can directly bind cisplatin-modified DNA [169, 170, 201].

Cisplatin-induced cell death is regulated by p53 via several pathways including degradation of the FLICE-like inhibitory protein (FLIP), overexpression of the phosphatase and tension homolog (PTEN), and inhibition of AMP-kinase. Additionally, binding of p53 to Bcl-xL counteracts the antiapoptotic function of this protein [28]. 5.5.Oxaliplatin Does Not Induce a DNA Damage Response

Recent experimental evidence has identified key differences in the way cisplatin and oxaliplatin induce cell death [133]. Oxaliplatin, unlike cisplatin, does not induce a DNA damage response but instead kills cells by ribosomal biogenesis stress, providing a fundamental explanation for the differential side-effects and spectrum of activity reported for these classical platinum anticancer agents. Initial observations, made using an RNAi platform [202] indicated that, unlike cisplatin and carboplatin that classify as DNA cross-linkers, oxaliplatin exhibits a distinctive mechanism of action consistent with that of the monofunctional agent, phenanthriplatin. Oxaliplatin and phenanthriplatin are mechanistically closer to the transcription/translation inhibitors rapamycin and actinomycin D. Further studies indicated that oxaliplatin treatment results in fewer double strand breaks on DNA than formed by other platinum agents and confirmed a relative lack of sensitivity to the silencing of genes involved in HR and those implicated in repair of ICLs within DT40 cells, by comparison to the properties of cisplatin. In support of the conclusion that oxaliplatin induces cell death through ribosome bio-genesis stress is the fact that pre-rRNA was upregulated following treatment with this agent when measured at time points greater than thirty minutes, while RNA Pol II transcripts remained stable. Moreover, knock down of RplII, an essential component of the ribosome, rendered Eu-Myc p19Arf-/- lymphoma and A549 human lung adenocarcinoma cells oxaliplatin-resistant. Ribosome biogenesis stress induces overexpression of RplII subunits that bind to Mdm2 and prevent it from interacting with p53. Western blot analysis of p53 expression supported diminution of p53 upon oxaliplatin treatment.

Finally, using The Cancer Genome Atlas the authors compared gene expression for colorectal cancer, which responds well to oxaliplatin treatment, to that for ovarian cancer, for which cisplatin and carboplatin are the preferred treatment. The greatest differences in expression levels between colorectal and ovarian cancers were observed for ribosomal genes. Notably, expression of ribosomal genes in colorectal cancer was significantly upregulated by comparison to equivalent ovarian cancer genes, thus establishing clinical relevance for the different mechanisms of cisplatin and oxaliplatin cell killing [133]. 6.UNDESIRED CONSEQUENCES OF

6.1.Toxicity

One of the limitations associated with platinum based-chemotherapy is the development of dose-limiting toxicities that prevent continuation of treatment. Several systematic toxicities are commonly encountered including gastrointestinal, oto-, nephro-, hepato-, and neurotoxicities as well as myelosuppression [203, 204]. Broadly, toxicity occurs as a result of drug accumulation at non-cancerous sites. Typically, the sites involve rapidly growing cells such as those found in the lining of the gasterointestinal tract, bone marrow, and hair cells [203], although it is unclear whether this classification applies to platinum drugs. Research is ongoing into the precise origin of each of these toxicities in the hope that a better understanding of the mechanism by which each drug becomes toxic will allow development of treatment regimens or next generation drugs specifically designed to minimize toxic side effects. Our laboratory has made significant contributions to this effort, in recent years elucidating the role and thus potential clinical implications for HMGB4 in improving the efficacy of cisplatin [159], and initiating development of novel platinum drug delivery constructs, one of which, BTP-114, is currently undergoing clinical trials. Preclinical trials with BTP-114, a cisplatin derivative, have demonstrated reduced toxicity in addition to a 13-fold increase in platinum loading in lung and ovarian cancer tumor models compared to cisplatin.

Neurotoxicity commonly referred to as peripheral neuropathy is dose-limiting in both cisplatin and oxaliplatin treatment, but there is evidence for dissimilar mechanisms of action for the two platinum-induced neuropathies [205]. Peripheral neuropathy is reported to occur in around 85 % of patients [206] receiving cisplatin at a cumulative dose greater than 300 mg/m2 and, whereas oxaliplatin is generally less toxic than cisplatin, it still generates a high incidence of peripheral neuropathy that is further classified as either acute or chronic in nature depending on its presentation immediately following treatment or after high cumulative doses, respectively. Clinically, peripheral neuropathy is characterized by the initial development of paraesthesia (tingling) and dysaesthesias of the toes and fingers, which extends with time to a ‘glove and stocking’ distribution [26, 207]. The pain induced is severe and may affect a patient’s functional abilities as well as lowering the quality of life. Factors that affect the onset of peripheral neuropathy in response to chemotherapy include a patient’s age and pre-existing medical conditions as well as the drug-dose intensity, cumulative dose, and therapy duration [207]. Strategies to limit neurotoxicity include the co-administration of thiols, particular glutathione (GSH) [207], or vitamin E together with the platinum agent [207]. Contradictory reports of the success of glutathione treatment for peripheral neuropathy have been reported, and there are concerns at the observation that GSH expression correlates with platinum resistance [195] that have diminished the interest in this approach in recent years. In contrast, clinical data support alleviation of peripheral neuropathy for patients treated with a calcium and magnesium infusion on the day of oxaliplatin treatment, without loss of oxaliplatin anticancer activity [204]. The non-pharmacological approach of ‘stop and go’ treatment has also demonstrated similar response rates and progression-free survival compared to the classical oxaliplatin continuous treatment model. In ’stop and go’ treatment a patient is treated with oxaliplatin up to the point where they exhibit peripheral neuropathy. The treatment is then discontinued, and only when the effects of peripheral neuropathy have worn off is the patient again treated with oxaliplatin. In this manner the long term and accumulating effects of oxaliplatin are managed [204]. Recently, research has linked acute oxaliplatin-induced neuropathy with impairment of voltage-gated sodium channels [205, 208]. In particular, increased sodium influx due to prolonged opening of the sodium channels is implicated in the presentation of unwanted neuropathological side effects.

Nephrotoxicity is commonly encountered in cisplatin treatment and it has been estimated that 28–36 % of patients who receive an initial dose of 50–100 mg/m2 develop acute renal failure [203], from which most patients fail to fully recover. Similarly, ototoxicity, which includes hearing loss, ear pain, and tinnitus is a common dose-dependent side effect of cisplatin [26, 209]. In contrast,

reports of nephrotoxicity and ototoxocity are rare following oxaliplatin treatment [210]. The origins of oto- and nephrotoxicity have been briefly highlighted in Section 2.3 and involve the differential transport of platinum agents by membrane transporters [26, 72]. Early studies indicated that hydration with saline or saline infused with mannitol- or furosemide induces diuresis effectively reducing the nephrotoxicity of cisplatin to the point where it is no longer dose-limiting [203, 211]. More recently exogenous thiol treatment, particularly with the pro-drug amifostine (S-2(3-amino-propylamino)ethylphosphorothioic acid), has been proposed as a means to further reduce nephro- and ototoxicity in addition to previously mentioned neurotoxicity [203, 206].

Gastrointestinal symptoms including vomiting and hepatotoxicity [212] are also observed with both cisplatin and oxaliplatin treatment. Neither is considered limiting as gastrointestinal conditions are effectively treated with 5HT3 antagonists [13] and hepatotoxicity remains a secondary concern to nephrotoxicity.

6.2.Multifactorial Resistance

In addition to dose-limiting toxicities, acquired and intrinsic cellular resistance to platinum agents limits their efficacy as anticancer agents. The multistep mechanism of action required for platinum anticancer agents to bring about a desired therapeutic response is matched at each stage by multiple resistance mechanisms. Intracellular accumulation of platinum is modulated by membrane transporters that alter both influx and efflux, and the number of platinum-DNA lesions is minimized by deactivation of platinum agents in the cytoplasm and increased repair of the adducts [25]. In general, cellular resistance arises when several mechanisms are operative simultaneously, a phenomenon termed multifactorial resistance [195].

Reduced cisplatin accumulation has been reported for several cisplatin resistant cell lines in comparison to the parental line [213]. However, resistance is often mediated by more than one mechanism, and a direct correlation between reduced cisplatin accumulation and resistance is rarely observed [195]. Moreover, continuing controversy surrounding the mechanism of transport for platinum agents further confounds attempts to determine whether reduced accumulation is due to reduced cellular uptake, increased efflux, or both [195, 213].

In contrast, more consistent data are available for identifying the role of thiols in resistance, with the concentration of several biological thiols being correlated with resistance both in vitro and in the clinic [195, 213]. In particular, several cisplatin-resistant cell lines have elevated concentrations of GSH, including a testicular tumor cell line that is normally cisplatin-sensitive but which acquired cisplatin resistance in vitro [214]. Similarly, increased concentrations of cysteine-containing metallothioneins have been identified in cisplatin-resistant tumor models [25, 215].

Another factor operative in platinum resistance is the ability of cells to tolerate or repair platinum lesions on DNA. Downregulation or mutation of the MMR proteins hMLH1 or hMSH6 increases replicative bypass by 3- to 6-fold past cisplatin lesions, but the same defects have little effect on the extent of bypass across oxaliplatin lesions [25], indicating that cisplatin lesions are better tolerated by MMR-deficient cell lines. Moreover, cisplatin-resistant cells deficient in MMR often have abrogated p53 function, which is implicated in the downregulation of hMSH2 [195]. When operative, the contribution of increased repair is low, but nonetheless clinically significant, and typically gives rise to 1.5- to 2-fold resistance [25]. Increased repair of platinum-DNA lesions is linked with increased NER protein expression, specifically, increased mRNA levels of ERCC1 and XPA have been reported for samples taken from patients exhibiting acquired resistance to cisplatin [195]. This result correlates with data supporting low levels of these proteins in testicular tumor cells known to be sensitive to cisplatin [195].

6.3.Cancer Stem Cell Enrichment

Some researchers now believe that cancer stem cells (CSC), which make up as little as 1 % of the tumor population [216], may be responsible for the development of resistance and tumor

recurrence following chemotherapy [217–219]. It is postulated that conventional chemotherapeutic agents, which are targeted to bulk tumor cells, spare CSCs, thereby enriching the population of resistant CSCs. Research into CSC treatment is still in its infancy, and much work has yet to be done to fully understand the properties of CSCs, develop tools required to study this distinct subpopulation of cancer cells [218], and ultimately discover new anticancer agents capable of effectively targeting CSC, specifically without affecting normal stem cells.

6.4.Mutagenicity

In addition, to concerns related to resistance, toxicity, and cancer stem cell enrichment, the mutagenic potential of platinum anticancer agents must also be noted. Early mouse models indicated that, at therapeutic doses, cisplatin was carcinogenic and that treatment may induce secondary tumor formation [220]. Subsequent research has investigated the mutagenicity of cisplatin and other platinum agents in a variety of cell types, and assessed the relative mutagenicity of different cisplatin lesions [221]. It is known [222, 223] that the major 1,2-d(GpG) cisplatin intrastrand cross-link was the most lethal among 1,2-d(GpG), 1,2-d(ApG), and 1,3-d(GpTpG) adducts investigated and therefore could account for most of the cytotoxicity displayed by cisplatin. The 1,2-d(GpG) link is also considerably less mutagenic than the 1,2-d(ApG) link, with relative mutation frequencies of 1.4 % for 1,2-d(GpG) and 6 % for 1,2-d(ApG). No specific mutations were reported for the 1,3-d(GpTpG) cross-links.

7.CONCLUDING REMARKS AND FUTURE DIRECTIONS

The value of metallo-drugs as anticancer agents has been firmly established in the half century that has passed since the seminal discovery of cisplatin as a potent anticancer agent. The mechanism of action that governs the activity of the classical bifunctional platinum agents, including cisplatin and oxaliplatin, has been extensively studied during this time, allowing scientists to appreciate the many factors that dictate the efficacy of new drug candidates.

More recently, non-classical anticancer agents that differ from the traditional classical agents in their metal identity or coordination preference have been developed in a bid to overcome some of the remaining limitations with the currently approved platinum drugs. In particular, non-classical anticancer agents, including pro-drugs such as BTP-114 discussed above, polynuclear metal complexes, and drugs based on non-platinum metals including gold and ruthenium, discussed in detail in later chapters of this book, may have improved efficacy and cellular uptake over classical platinum(II) agents, while at the same time showing reduced incidence of drug resistance, tumor recurrence, and toxic side-effects.

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ABBREVIATIONS AND DEFINITIONS

9-1-1	Rad9, Hus1, and Rad1
ATM	ataxia-telangiectasia mutated
ATOX1	copper chaperone
ATP	adenosine 5′-triphosphate
ATR(ATRIP)	ATM-Rad3 related kinases-ATR-interacting protein
BER	base excision repair
bp	base pair
Cdks	cyclin-dependent kinases
chk1/2	cell cycle checkpoint 1/2
cisplatin/CDDP	cis-dichlorodiammineplatinum(II)
CSC	cancer stem cell
Ctr1/2	copper transporter 1/2
DACH	trans-R,R-1,2-diaminocyclohexane
DDR	DNA damage response
dien	diethylenetriamine = 1.4.7-triazaheptane

E. coli Escherichia coli
enloplatin 1,1-cyclobutane dicarboxylato-O′,O′ tetrahydro-4H pyran-4,4-dimethylamine-N′,N′ platinum(II)
ERCC excision repair cross complementing
ERK extracellular signal regulating kinases
FA Fanconi's Anemia
FACT facilitates chromatin transcription
FDA US Food and Drug Administration
FLIP FLICE-like inhibitory protein
GSH glutathione
HMG(B) high mobility group (box)
HPLC high performance liquid chromatography
HR homologous recombination
ICL interstrand cross-link
JNK c-Jun N-terminal kinases
lobaplatin [2-hydroxypropanoato-O1,O2][1,2-cyclobutanedimethanamine-N,N#]platinum(II)
MAP(K) mitogen activated protein (kinase)
MAPKAP kinase 2 MAP kinase-activated protein kinase 2
(h)MATE (human) multidrug and toxin extrusion antiporters
MMR mismatch repair
heptaplatin [propanedioato-O,O#][2-(1-methylethyl)-1,3-dioxolane-4,5-dimethanamine-N,N#]platinum(II)
miboplatin R-2-amino methyl pyrrolidine 1,1-cyclobutane dicarboxylate platinum(II)
MEF mouse embryonic fibroblasts
MSK1 mitogen- and stress-activated protein kinase 1
NCI National Cancer Institute
nedaplatin diammine[hydroxyacetato-O,O#]platinum(II)
NER nucleotide excision repair
NHEJ non-homologous end joining
NK-121/CI-973 cis-1,1-cyclobutane dicarboxylato(2R)-2-methyl-1,4-butanediamine platinum(II)
OCT organic cation transporter
(h/y)OCT1/2/3 (human/yeast) organic cation transporter 1/2/3
oxaliplatin trans-L-diaminocyclohexane oxalate platinum(II)
PARP poly(ADP-ribose)polymerase 1
PCNA proliferating cell nuclear antigen
PIC1/2/3 preincision complex 1/2/3
PI(3)K phosphatidylinositol-3-OH kinases
PMS2 postmeiotic segregation increased 2
Pol polymerase
PTEN phosphatase and tension homolog
RR recombination repair
(r/m)RNA (ribosomal/messenger) ribonucleic acid
siRNA small interfering RNA
SSRP1 structure specific recognition protein
t1/2 half-life
TAD1/2 transactivation domains 1/2
TBP TATA-binding protein
TLS translesion synthesis
(h)UBF (human)upstream binding factor
XP xeroderma pigmentosum

zeniplatin 2,2-bis aminomethyl-1,3-propandiol-N-N′ 1,1-cyclo-butane dicarboxylate-O′,O#platinum(II)

REFERENCES

1.R. A. Alderden, M. D. Hall, T. W. Hambley, J. Chem. Edu. 2006, 83, 728–734.

2.F. M. Muggia, A. Bonetti, J. D. Hoeschele, M. Rozencweig, S. B. Howell, J. Clin. Oncol. 2015, 33, 4219–4226.

3.B. Rosenberg, L. Van Camp, T. Krigas, Nature 1965, 205, 698–699.

4.B. Rosenberg, L. Van Camp, E. B. Grimley, A. J. Thomson, J. Biol. Chem. 1967, 242, 1347–1352.

5.B. Rosenberg, Platin. Met. Rev. 1971, 15, 42–51.

6.B. Rosenberg, L. Van Camp, J. E. Trosko, V. H. Mansour, Nature 1969, 222, 385–386.

7.D. J. Higby, H. J. Wallace, D. J. Albert, J. F. Holland, Cancer 1974, 33, 1219–1225.

8.N. Hanna, L. H. Einhorn, J. Clin. Oncol. 2014, 32, 3085–3092.

9.M. Rozencweig, D. D. Von Hoff, M. Slavik, F. M. Muggia, Ann. Intern. Med. 1977, 86, 803–812.

10.E. Cvitkovic, J. Spaulding, V. Bethune, J. Martin, W. F. Whitmore, Cancer 1977, 39, 1357–1361.

11.C. F. J. Barnard, Platin. Met. Rev. 1989, 33, 162–167.

12.D. Lebwohl, R. Canetta, Eur. J. Cancer 1998, 34, 1522–1534.

13.L. X. Cubeddu, I. S. Hoffmann, N. T. Fuenmayor, A. L. Finn, New Eng. J. Med. 1990, 322, 810–816.

14.L. Kelland, Nat. Rev. Cancer 2007, 7, 573–584.

15.R. J. Knox, F. Friedlos, D. A. Lydall, J. J. Roberts, Cancer Res. 1986, 46, 1972–1979.

16.L. R. Kelland, S. J. Clarke, M. J. McKeage, Platin. Met. Rev. 1992, 36, 178–189.

17.Y. Kidani, K. Inagaki, M. Iigo, A. Hoshi, K. Kuretani, J. Med. Chem. 1978, 21, 1315–1318.

18.N. J. Wheate, S. Walker, G. E. Craig, R. Oun, Dalton Trans. 2010, 39, 8113–8127.

19.O. Rixe, W. Ortuzar, M. Alvarez, R. Parker, E. Reed, K. Paull, T. Fojo, Biochem. Pharmacol. 1996, 52, 1855–1865.

20.J. L. Grem, N. McAtee, F. Balis, R. Murphy, D. Venzon, B. Kramer, B. Goldspiel, M. Begley, C. J. Allegra, Cancer 1993, 72, 663–668.

21.P. J. Loehrer, S. Turner, P. Kubilis, S. Hui, J. Correa, R. Ansari, D. Stephens, R. Woodburn, S. Meyer, J. Clin. Oncol. 1988, 6, 642–648.

22.A. de Gramont, A. Figer, M. Seymour, M. Homerin, A. Hmissi, J. Cassidy, C. Boni, H. Cortes-Funes, A. Cervantes, G. Freyer, D. Papamichael, N. Le Bail, C. Louvet, D. Hendler, F. de Braud, C. Wilson, F. Morvan, A. Bonetti, J. Clin. Oncol. 2000, 18, 2938–2947.

23.R. L. Siegel, K. D. Miller, A. Jemal, Cancer J. Clin. 2016, 66, 7–30.

24.Y. Jung, S. J. Lippard, Chem. Rev. 2007, 107, 1387–1407.

25.M. Kartalou, J. M. Essigmann, Mutat. Res. 2001, 478, 23–43.

26.G. Ciarimboli, Scientifica 2012, 2012, 18, 473829.

27.M. D. Hall, M. Okabe, D.-W. Shen, X.-J. Liang, M. M. Gottesman, Annu. Rev. Pharmacol. Toxicol. 2008, 48, 495–535.

28.A. Basu, S. Krishnamurthy, J. Nucleic Acids 2010, 2010, 16; doi: 10.4061/2010/201367.

29.S. P. Binks, M. Dobrota, Biochem. Pharmacol. 1990, 40, 1329–1336.

30.N. D. Eljack, H.-Y. M. Ma, J. Drucker, C. Shen, T. W. Hambley, E. J. New, T. Friedrich, R. J. Clarke, Metallomics 2014, 6, 2126–2133.

31.R. Safaei, Cancer Lett. 2006, 234, 34–39.

32.P. Abada, S. B. Howell, Met. Based Drugs 2010, 9; doi: 10.1155/2010/317581.

33.K. D. Ivy, J. H. Kaplan, Mol. Pharmacol. 2013, 83, 1237–1246.

34.S. Ishida, F. McCormick, K. Smith-McCune, D. Hanahan, Cancer Cell 2010, 17, 574–583.

35.S. Ishida, J. Lee, D. J. Thiele, I. Herskowitz, Proc. Natl. Acad. Sci. USA 2002, 99, 14298–14302.

36.X. Lin, T. Okuda, A. Holzer, S. B. Howell, Mol. Pharmacol. 2002, 62, 1154–1159.

37.I.-S. Song, N. Savaraj, Z. H. Siddik, P. Liu, Y. Wei, C. J. Wu, M. T. Kuo, Mol. Cancer Ther. 2004, 3, 1543–1549.

38.J. Zisowsky, S. Koegel, S. Leyers, K. Devarakonda, M. U. Kassack, M. Osmak, U. Jaehde, Biochem. Pharmacol. 2007, 73, 298–307.

39.Z. D. Liang, Y. Long, W.-B. Tsai, S. Fu, R. Kurzrock, M. Gagea-Iurascu, F. Zhang, H. H. W. Chen, B. T. Hennessy, G. B. Mills, N. Savaraj, M. T. Kuo, Mol. Cancer Ther. 2012, 11, 2483–2494.

40.A. K. Holzer, K. Katano, L. W. J. Klomp, S. B. Howell, Clin. Cancer Res. 2004, 10, 6744–6749.

41.A. K. Holzer, S. B. Howell, Cancer Res. 2006, 66, 10944–10952.

42.X. Xu, L. Duan, B. Zhou, R. Ma, H. Zhou, Z. Liu, Clin. Exp. Pharmacol. Physiol. 2012, 39, 786–792.

43.K. M. Bompiani, C.-Y. Tsai, F. P. Achatz, J. K. Liebig, S. B. Howell, Metallomics 2016, 8, 951–962.

44.C. A. Larson, P. L. Adams, D. D. Jandial, B. G. Blair, R. Safaei, S. B. Howell, Biochem. Pharmacol. 2010, 80, 448–454.

45.C. A. Larson, P. L. Adams, B. G. Blair, R. Safaei, S. B. Howell, Mol. Pharmacol. 2010, 78, 333–359.

46.B. G. Blair, C. A. Larson, R. Safaei, S. B. Howell, Clin Cancer Res. 2009, 15, 4312–4321.

47.C. P. Huang, M. Fofana, J. Chan, C. J. Chang, S. B. Howell, Metallomics 2014, 6, 654–661.

48.B. G. Blair, C. A. Larson, P. L. Adams, P. B. Abada, C. E. Pesce, R. Safaei, S. B. Howell, Mol. Pharmacol. 2011, 79, 157–166.

49.V. Calandrini, F. Arnesano, A. Galliani, T. H. Nguyen, E. Ippoliti, P. Carloni, G. Natile, Dalton Trans. 2014, 43, 12085–12094.

50.K. Katano, R. Safaei, G. Samimi, A. Holzer, M. Tomioka, M. Goodman, S. B. Howell, Clin. Cancer Res. 2004, 10, 4578–4588.

51.M. Komatsu, T. Sumizawa, M. Mutoh, Z.-S. Chen, K. Terada, T. Furukawa, X.-L. Yang, H. Gao, N. Miura, T. Sugiyama, S. Akiyama, Cancer Res. 2000, 60, 1312–1316.

52.K. Yoshizawa, S. Nozaki, H. Kitahara, T. Ohara, K. Kato, S. Kawashiri, E. Yamamoto, Oncol. Rep. 2007, 18, 987–991.

53.E. Martinez-Balibrea, A. Martínez-Cardús, E. Musulén, A. Ginés, J. L. Manzano, E. Aranda, C. Plasencia, N. Neamati, A. Abad, Int. J. Cancer 2009, 124, 2905–2910.

54.S. Harrach, G. Ciarimboli, Front. Pharmacol. 2015, 6, 85; doi: 10.3389/ fphar.2015.00085.

55.J. W. Jonker, A. H. Schinkel, J. Pharm. Exp. Ther. 2004, 308, 2–9.

56.H. Koepsell, K. Lips, C. Volk, Pharmaceut. Res. 2007, 24, 1227–1251.

57.E. Schlatter, P. Klassen, V. Massmann, S. K. Holle, D. Guckel, B. Edemir, H. Pavenstädt, G. Ciarimboli, Pflügers Arch., EJP 2014, 466, 1581–1589.

58.D. Gründemann, V. Gorboulev, S. Gambaryan, M. Veyhl, H. Koepsell, Nature 1994, 372, 549–552.

59.J. W. Jonker, E. Wagenaar, S. van Eijl, A. H. Schinkel, Mol. Cell. Biol. 2003, 23, 7902–7908.

60.A. Yonezawa, S. Masuda, K. Nishihara, I. Yano, T. Katsura, K.-i. Inui, Biochem. Pharmacol. 2005, 70, 1823–1831.

61.G. Ciarimboli, T. Ludwig, D. Lang, H. Pavenstädt, H. Koepsell, H.-J. Piechota, J. Haier, U. Jaehde, J. Zisowsky, E. Schlatter, Am. J. Path., 167, 1477–1484.

62.H. Katsuda, M. Yamashita, H. Katsura, J. Yu, Y. Waki, N. Nagata, Y. Sai, K. Miyamoto, Biol. Pharm. Bull. 2010, 33, 1867–1871.

63.K. K. Filipski, R. H. Mathijssen, T. S. Mikkelsen, A. H. Schinkel, A. Sparreboom, Clin. Pharmacol. Ther. 2009, 86, 396–402.

64.D. Th. Sleijfer, J. J. G. Offerman, N. H. Mulder, M. Verweij, G. K. van der Hem, H. S. Koops, S. Meijer, Cancer 1987, 60, 2823–2828.

65.J. Zhang, W. Zhou, Food Chem.Toxicol. 2012, 50, 2289–2293.

66.G. Ciarimboli, D. Deuster, A. Knief, M. Sperling, M. Holtkamp, B. Edemir, H. Pavenstädt, C. Lanvers-Kaminsky, A. am Zehnhoff-Dinnesen, A. H. Schinkel, H. Koepsell, H. Jürgens, E. Schlatter, Am. J. Path. 2010, 176, 1169–1180.

67.A. Yonezawa, S. Masuda, S. Yokoo, T. Katsura, K.-i. Inui, J. Pharm. Exp. Ther. 2006, 319, 879–886.

68.S. Zhang, K. S. Lovejoy, J. E. Shima, L. L. Lagpacan, Y. Shu, A. Lapuk, Y. Chen, T. Komori, J. W. Gray, X. Chen, S. J. Lippard, K. M. Giacomini, Cancer Res. 2006, 66, 8847–8857.

69.T. Kanou, J. Uozumi, K. Soejima, Y. Tokuda, Z. Masaki, Clin. Exp. Nephrol. 2004, 8, 310–315.

70.S. Yokoo, S. Masuda, A. Yonezawa, T. Terada, T. Katsura, K.-i. Inui, Drug Metab. Dispos. 2008, 36, 2299–2306.

71.T. Nakamura, A. Yonezawa, S. Hashimoto, T. Katsura, K.-i. Inui, Biochem. Pharmacol. 2010, 80, 1762–1767.

72.A. Yonezawa, K.-i. Inui, Biochem. Pharmacol. 2011, 81, 563–568.

73.M. Jennerwein, P. A. Andrews, Drug Metab. Dispos. 1995, 23, 178–184

74.S. J. Berners-Price, T. G. Appleton, in Platinum-Based Drugs in Cancer Therapy, Eds L. R. Kelland, N. P. Farrell, Humana Press, Totowa, NJ, 2000, pp. 3–35.

75.O. Pinato, C. Musetti, C. Sissi, Metallomics 2014, 6, 380–395.

76.P. C. Dedon, R. F. Borch, Biochem. Pharmacol. 1987, 36, 1955–1964.

77.D. P. Bancroft, C. A. Lepre, S. J. Lippard, J. Am. Chem. Soc. 1990, 112, 6860–6871.

78.S. J. Berners-Price, T. A. Frenkiel, U. Frey, J. D. Ranford, P. J. Sadler, J. Chem. Soc., Chem. Commun. 1992, 789–791.

79.R. Faggiani, B. Lippert, C. J. L. Lock, B. Rosenberg, Inorg. Chem. 1977, 16, 1192–1196.

80.R. Faggiani, B. Lippert, C. J. L. Lock, B. Rosenberg, J. Am. Chem. Soc. 1977, 99, 777–781.

81.B. Rosenberg, Biochimie 1978, 60, 859–867.

82.D. S. Gill, B. Rosenberg, J. Am. Chem. Soc. 1982, 104, 4598–4604.

83.T. G. Appleton, R. D. Berry, C. A. Davis, J. R. Hall, H. A. Kimlin, Inorg. Chem. 1984, 23, 3514–3521.

84.M. C. Lim, R. B. Martin, J. Inorg. Nucl. Chem. 1976, 38, 1911–1914.

85.R. C. Todd, K. S. Lovejoy, S. J. Lippard, J. Am. Chem. Soc. 2007, 129, 6370–6371.

86.K. Zhang, M. Chew, E. B. Yang, K. P. Wong, P. Mack, Mol. Pharmacol. 2001, 59, 837–843.

87.D. M. Townsend, K. D. Tew, Oncogene 2003, 22, 7369–7375.

88.O. Heudi, A. Cailleux, P. Allain, J. Inorg. Biochem. 1998, 71, 61–69.

89.K. J. Barnham, M. I. Djuran, P. del Socorro Murdoch, J. D. Ranford, P. J. Sadler, Dalton Trans. 1995, 3721–3726.

90.M. A. Graham, G. F. Lockwood, D. Greenslade, S. Brienza, M. Bayssas, E. Gamelin, Clin. Cancer Res. 2000, 6, 1205–1218.

91.E. R. Jamieson, S. J. Lippard, Chem. Rev. 1999, 99, 2467–2498.

92.S. P. LeDoux, G. L. Wilson, E. J. Beecham, T. Stevnsner, K. Wassermann, V. A. Bohr, Carcinogenesis 1992, 13, 1967–1973.

93.X. Shu, X. Xiong, J. Song, C. He, C. Yi, Angew. Chem. Int. Ed. 2016, 55, 14246–14249.

94.O. A. Olivero, C. Semino, A. Kassim, D. M. Lopez-Larraza, M. C. Poirier, Mutat. Res. Lett. 1995, 346, 221–230.

95.O. A. Olivero, P. K. Chang, D. M. Lopez-Larraza, M. Cristina Semino-Mora, M. C. Poirier, Mutat. Res. 1997, 391, 79–86.

96.H. C. Harder, B. Rosenberg, Int. J. Cancer 1970, 6, 207–216.

97.M. Kartalou, J. M. Essigmann, Mutat. Res. 2001, 478, 1–21.

98.S. E. Sherman, S. J. Lippard, Chem. Rev. 1987, 87, 1153–1181.

99.S. G. Chaney, S. L. Campbell, E. Bassett, Y. Wu, Crit. Rev. Oncol. Hematol. 2005, 53, 3–11.

100.R. C. Todd, S. J.

Lippard, Metallomics 2009, 1, 280–291.

101.P. M. Takahara, A. C. Rosenzweig, C. A. Frederick, S. J. Lippard, Nature 1995, 377, 649–652.

102.D. Yang, S. S. G. E. van Boom, J. Reedijk, J. H. van Boom, A. H.–J. Wang, Biochem. 1995, 34, 12912–12920.

103.A. Gelasco, S. J. Lippard, Biochem. 1998, 37, 9230–9239.

104.C. J. Van Garderen, L. P. A. Van Houte, Eur. J. Biochem. 1994, 225, 1169–1179.

105.F. Coste, J.-M. Malinge, L. Serre, W. Shepard, M. Roth, M. Leng, C. Zelwer, Nucleic Acids Res. 1999, 27, 1837–1846.

106.B. Spingler, D. A. Whittington, S. J. Lippard, Inorg. Chem. 2001, 40, 5596–5602.

107.Y. Wu, P. Pradhan, J. Havener, G. Boysen, J. A. Swenberg, S. L. Campbell, S. G. Chaney, J. Mol. Biol. 2004, 341, 1251–1269.

108.B. Wu, C. A. Davey, Chem. Biol. 2008, 15, 1023–1028.

109.I. Ioshikhes, A. Bolshoy, K. Derenshteyn, M. Borodovsky, E. N. Trifonov, J. Mol. Biol. 1996, 262, 129–139.

110.A. J. Danford, D. Wang, Q. Wang, T. D. Tullius, S. J. Lippard, Proc. Natl. Acad. Sci. USA 2005, 102, 12311–12316.

111.S. J. Lippard, J. D. Hoeschele, Proc, Natl Acad. Sci. USA 1979, 76, 6091–6095.

112.M. Foka, J. Paoletti, Biochem. Pharmacol. 1986, 35, 3283–3291.

113.J. J. Hayes, W. M. Scovell, Biochim. Biophysi. Acta 1991, 1088, 413–418.

114.A. M. Galea, V. Murray, Biochim. Biophys. Acta 2002, 1579, 142–152.

115.N. P. Davies, L. C. Hardman, V. Murray, Nucleic Acids Res. 2000, 28, 2954–2958.

116.E. Segal, Y. Fondufe-Mittendorf, L. Chen, A. Thåström, Y. Field, I. K. Moore, J.-P. Z. Wang, J. Widom, Nature 2006, 442, 772–778.

117.M. Ober, S. J. Lippard, J. Am. Chem. Soc. 2007, 129, 6278–6286.

118.M. Ober, S. J. Lippard, J. Am. Chem. Soc. 2008, 130, 2851–2861.

119.D. Wang, R. Hara, G. Singh, A. Sancar, S. J. Lippard, Biochem. 2003, 42, 6747–6753.

120.K. M. Comess, J. N. Burstyn, J. M. Essigmann, S. J. Lippard, Biochem. 1992, 31, 3975–3990.

121.C. M. Sorenson, A. Eastman, Cancer Res. 1988, 48, 4484–4488.

122.S. Prakash, R. E. Johnson, L. Prakash, Annu. Rev. Biochem. 2005, 74, 317–353.

123.J. K. Hicks, C. L. Chute, M. T. Paulsen, R. L. Ragland, N. G. Howlett, Q. Guéranger, T. W. Glover, C. E. Canman, Mol. Cell. Biol. 2010, 30, 1217–1230.

124.A. Vaisman, S. E. Lim, S. M. Patrick, W. C. Copeland, D. C. Hinkle, J. J. Turchi, S. G. Chaney, Biochem. 1999, 38, 11026–11039.

125.A. R. Lehmann, A. Niimi, T. Ogi, S. Brown, S. Sabbioneda, J. F. Wing, P. L. Kannouche, C. M. Green, DNA Repair 2007, 6, 891–899.

126.S. G. Chaney, S. L. Campbell, B. Temple, E. Bassett, Y. Wu, M. Faldu, J. Inorg. Biochem. 2004, 98, 1551–1559.

127.P. A. Knobel, T. M. Marti, Cancer Cell Int. 2011, 11, 39–39.

128.T. V. Ho, A. Guainazzi, S. B. Derkunt, M. Enoiu, O. D. Schärer, Nucleic Acids Res. 2011, 39, 7455–7464.

129.U. Roy, O. D. Schärer, DNA Repair 2016, 44, 33–41.

130.C. M. Sorenson, A. Eastman, Cancer Res. 1988, 48, 6703–6707.

131.Y. Corda, C. Job, M.-F. Anin, M. Leng, D. Job, Biochem. 1991, 30, 222–230.

132.R. Hara, C. P. Selby, M. Liu, D. H. Price, A. Sancar, J. Biol. Chem. 1999, 274, 24779–24786.

133.P. M. Bruno, Y. Liu, G. Y. Park, J. Murai, C. E. Koch, T. J. Eisen, J. R. Pritchard, Y. Pommier, S. J. Lippard, M. T. Hemann, Nat. Med. 2017, 23, 461–471.

134.J. T. Reardon, A. Vaisman, S. G. Chaney, A. Sancar, Cancer Res. 1999, 59, 3968–3971.

135.D. B. Zamble, D. Mu, J. T. Reardon, A. Sancar, S. J. Lippard, Biochem. 1996, 35, 10004–10013.

136.A. Sancar, L. A. Lindsey-Boltz, K. Ünsal-Kaçmaz, S. Linn, Annu. Rev. Biochem. 2004, 73, 39–85.

137.T. Furuta, T. Ueda, G. Aune, A. Sarasin, K. H. Kraemer, Y. Pommier, Cancer Res. 2002, 62, 4899–4902.

138.Z. Z. Zdraveski, J. A. Mello, M. G. Marinus, J. M. Essigmann, Chem. Biol. 2000, 7, 39–50.

139.D. M. Noll, T. M. Mason, P. S. Miller, Chem. Rev. 2006, 106, 277–301.

140.G. P. Raaphorst, J.-M. Leblanc, L. F. Li, Anticancer Res. 2005, 25, 53–58.

141.G.-L. Moldovan, A. D. D'Andrea, Annu. Rev. Genet. 2009, 43, 223–249.

142.A. D. D'Andrea, M. Grompe, Nat. Rev. Cancer 2003, 3, 23–34.

143.B. Haynes, N. Saadat, B. Myung, M. P. V. Shekhar, Mutat. Res. 2015, 763, 258–266.

144.J. Jiricny, Nat. Rev. Mol. Cell. Biol. 2006, 7, 335–346.

145.A. Sawant, A. Kothandapani, A. Zhitkovich, R. W. Sobol, S. M. Patrick, DNA Repair 2015, 35, 126–136.

146.D. Fink, S. Nebel, S. Aebi, H. Zheng, B. Cenni, A. Nehmé, R. D. Christen, S. B. Howell, Cancer Res. 1996, 56, 4881–4886.

147.D. Fink, S. Aebi, S. B. Howell, Clin. Cancer Res. 1998, 4, 1–6.

148.J. A. Mello, S. Acharya, R. Fishel, J. M. Essigmann, Chem. Biol. 1996, 3, 579–589.

149.D. R. Duckett, J. T. Drummond, A. I. H. Murchie, J. T. Reardon, A. Sancar, D. M. J. Lilley, P. Modrich, Proc. Natl. Acad. Sci. USA 1996, 93, 6443–6447.

150.G. Zhu, S. J. Lippard, Biochem. 2009, 48, 4916–4925.

151.Z. Li, A. H. Pearlman, P. Hsieh, DNA Repair 2016, 38, 94–101.

152.H. E. Krokan, M. Bjørås, Cold Spring Harb. Perspect. Biol. 2013, 5, a012583; doi: 10.1101/cshperspect.a012583.

153.A. Kothandapani, V. S. M. N. Dangeti, A. R. Brown, L. A. Banze, X.-H. Wang, R. W. Sobol, S. M. Patrick, J. Biol. Chem. 2011, 286, 14564–14574.

154.D. Wang, D.-B. Xiang, X.-q. Yang, L.-S. Chen, M.-X. Li, Z.-Y. Zhong, Y.-S. Zhang, Lung Cancer 2009, 66, 298–304.

155.J. Zlatanova, J. Yaneva, S. H. Leuba, FASEB J. 1998, 12, 791–799.

156.W. M. Scovell, N. Muirhead, L. R. Kroos, Biochem. Biophys. Res. Commun. 1987, 142, 826–835.

157.S. L. Bruhn, P. M. Pil, J. M. Essigmann, D. E. Housman, S. J. Lippard, Proc. Natl. Acad. Sci. USA 1992, 89, 2307–2311.

158.M. Wei, O. Burenkova, S. J. Lippard, J. Biol. Chem. 2003, 278, 1769–1773.

159.S. G. Awuah, I. A. Riddell, S. J. Lippard, Proc. Natl. Acad. Sci. USA 2017, 114, 950–955.

160.S. Park, S. J. Lippard, Biochemistry 2012, 51, 6728–6737.

161.A. T. Yarnell, S. Oh, D. Reinberg, S. J. Lippard, J. Biol. Chem. 2001, 276, 25736–25741.

162.D. K. Treiber, X. Zhai, H. M. Jantzen, J. M. Essigmann, Proc. Natl Acad. Sci. USA 1994, 91, 5672–5676.

163.F. Coin, P. Frit, B. Viollet, B. Salles, J.-M. Egly, Mol. Cell. Biol. 1998, 18, 3907–3914.

164.T. Ohga, K. Koike, M. Ono, Y. Makino, Y. Itagaki, M. Tanimoto, M. Kuwano, K. Kohno, Cancer Res. 1996, 56, 4224–4228.

165.G. Zhu, P. Chang, S. J. Lippard, Biochem. 2010, 49, 6177–6183.

166.H. Farmer, N. McCabe, C. J. Lord, A. N. J. Tutt, D. A. Johnson, T. B. Richardson, M. Santarosa, K. J. Dillon, I. Hickson, C. Knights, N. M. B. Martin, S. P. Jackson, G. C. M. Smith, A. Ashworth, Nature 2005, 434, 917–921.

167.V. Schreiber, F. Dantzer, J.-C. Amé, G. de Murcia, Nat. Rev. Mol. Cell Biol. 2006, 7, 517–528.

168.J. Michels, I. Vitale, L. Galluzzi, J. Adam, K. A. Olaussen, O. Kepp, L. Senovilla, I. Talhaoui, J. Guegan, D. P. Enot, M. Talbot, A. Robin, P. Girard, C. Oréar, D. Lissa, A. Q. Sukkurwala, P. Garcia, P. Behnam-Motlagh, K. Kohno, G. S. Wu, C. Brenner, P. Dessen, M. Saparbaev, J.-C. Soria, M. Castedo, G. Kroemer, Cancer Res. 2013, 73, 2271–2280.

169.C. C. Wetzel, S. J. Berberich, Biochim. Biophys. Acta 2001, 1517, 392–397.

170.H. Pivoňková, M. Brázdová, J. Kašpárková, V. Brabec, M. Fojta, Biochem. Biophys. Res. Commun. 2006, 339, 477–484.

171.J. Kasparkova, S. Pospisilova, V. Brabec, J. Biol. Chem. 2001, 276, 16064–16069.

172.T. Imamura, H. Izumi, G. Nagatani, T. Ise, M. Nomoto, Y. Iwamoto, K. Kohno, J. Biol. Chem. 2001, 276, 7534–7540.

173.B.-B. S. Zhou, S. J. Elledge, Nature 2000, 408, 433–439.

174.D. Wang, S. J. Lippard, Nat. Rev. Drug Discov. 2005, 4, 307–320.

175.P. Roos-Mattjus, B. T. Vroman, M. A. Burtelow, M. Rauen, A. K. Eapen, L. M. Karnitz, J. Biol. Chem. 2002, 277, 43809–43812.

176.A. Maréchal, L. Zou, Cold Spring Harb. Perspect. Biol. 2013, 5, a012716; doi: 10.1101/cshperspect.a012716.

177.M. Xu, L. Bai, Y. Gong, W. Xie, H. Hang, T. Jiang, J. Biol. Chem. 2009, 284, 20457–20461.

178.E. R. Parrilla-Castellar, S. J. H. Arlander, L. Karnitz, DNA Repair 2004, 3, 1009–1014.

179.M. Toueille, N. El-Andaloussi, I. Frouin, R. Freire, D. Funk, I. Shevelev, E. Friedrich-Heineken, G. Villani, M. O. Hottiger, U. Hübscher, Nucleic Acids Res. 2004, 32, 3316–3324.

180.S. Delacroix, J. M. Wagner, M. Kobayashi, K.-i. Yamamoto, L. M. Karnitz, Genes Dev. 2007, 21, 1472–1477.

181.R. H. Medema, L. Macurek, Oncogene 2012, 31, 2601–2613.

182.J. Benada, L. Macurek, Biomolecules 2015, 5, 1912–1937.

183.B.-B. S. Zhou, J. Bartek, Nat. Rev. Cancer 2004, 4, 216–225.

184.H. Takai, K. Tominaga, N. Motoyama, Y. A. Minamishima, H. Nagahama, T. Tsukiyama, K. Ikeda, K. Nakayama, M. Nakanishi, K.-i. Nakayama, Genes Dev. 2000, 14, 1439–1447.

185.C. S. Sørensen, R. G. Syljuåsen, Nucleic Acids Res. 2012, 40, 477–486.

186.A. Brozovic, M. Osmak, Cancer Lett. 2007, 251, 1–16.

187.J. M. Kyriakis, J. Avruch, Physiol. Rev. 2001, 81, 807–869.

188.X. Wang, J. L. Martindale, N. J. Holbrook, J. Biol. Chem. 2000, 275, 39435–39443.

189.Y. K. Kim, H. J. Kim, C. H. Kwon, J. H. Kim, J. S. Woo, J. S. Jung, J. M. Kim, J. App. Toxicol. 2005, 25, 374–382.

190.S. Lee, S. Yoon, D.-H. Kim, Gynecol. Oncol. 2007, 104, 338–344.

191.J. H. Losa, C. P. Cobo, J. G. Viniegra, V. J. S.-A. Lobo, S. Ramon y Cajal, R. Sánchez-Prieto, Oncogene 2003, 22, 3998–4006.

192.D. Wang, S. J. Lippard, J. Biol. Chem. 2004, 279, 20622–20625.

193.D. N. Dhanasekaran, E. P. Reddy, Oncogene 2008, 27, 6245–6251.

194.B. W. Zanke, K. Boudreau, E. Rubie, E. Winnett, L. A. Tibbles, L. Zon, J. Kyriakis, F.-F. Liu, J. R. Woodgett, Curr. Biol. 1996, 6, 606–613.

195.Z. H. Siddik, Oncogene 2003, 22, 7265–7279.

196.W. Dempke, W. Voigt, A. Grothey, B. T. Hill, H.-J. Schmoll, Anticancer Drugs 2000, 11, 225–236.

197.J. M. Nigro, S. J. Baker, A. C. Preisinger, J. M. Jessup, R. Hosteller, K. Cleary, S. H. Bigner, N. Davidson, S. Baylin, P. Devilee, T. Glover, F. S. Collins, A. Weston, R. Modali, C. C. Harris, B. Vogelstein, Nature 1989, 342, 705–708.

198.M. Hollstein, D. Sidransky, B. Vogelstein, C. C. Harris, Science 1991, 253, 49–53.

199.G. L. Y. Zhang, Annu. Rev. Cancer Biol. 2017, 1, null.

200.C. C. Harris, J. Natl. Cancer Inst. 1996, 88, 1442–1455.

201.T. Saha, R. K. Kar, G. Sa, Prog. Biophys. Mol. Bio. 2015, 117, 250–263.

202.H. Jiang, J. R. Pritchard, R. T. Williams, D. A. Lauffenburger, M. T. Hemann, Nat. Chem. Biol. 2011, 7, 92–100.

203.K. Barabas, R. Milner, D. Lurie, C. Adin, Vet. Comp. Oncol. 2008, 6, 1–18.

204.P. M. Hoff, E. D. Saad, F. Costa, A. K. Coutinho, R. Caponero, G. Prolla, R. C. Gansl, Clin. Colorectal Cancer 2012, 11, 93–100.

205.H. Adelsberger, S. Quasthoff, J. Grosskreutz, A. Lepier, F. Eckel, C. Lersch, Eur. J. Pharmacol. 2000, 406, 25–32.

206.R. S. Go, A. A. Adjei, J. Clin. Oncol. 1999, 17, 409–409.

207.S. Wolf, D. Barton, L. Kottschade, A. Grothey, C. Loprinzi, Eur. J. Cancer 2008, 44, 1507–1515.

208.S. B. Park, C. S.-Y. Lin, A. V. Krishnan, D. Goldstein, M. L. Friedlander, M. C. Kiernan, PLOS ONE 2011, 6, e18469; doi: doi:10.1371/journal.pone.0018469.

209.K. U. Wensing, G. Ciarimboli, Anticancer Res. 2013, 33, 4183–4188.

210.E. Raymond, S. G. Chaney, A. Taamma, E. Cvitkovic, Ann. Oncol. 1998, 9, 1053–1071.

211.V. Pinzani, F. Bressolle, I. J. Haug, M. Galtier, J. P. Blayac, P. Balmès, Cancer Chemother. Pharmacol. 1994, 35, 1–9.

212.D. McWhirter, N. Kitteringham, R. P. Jones, H. Malik, K. Park, D. Palmer, Crit. Rev. Oncol. Hematol. 2013, 88, 404–415.

213.L. R. Kelland, Crit. Rev. Oncol. Hematol. 1993, 15, 191–219.

214.J. R. W. Masters, R. Thomas, A. G. Hall, L. Hogarth, E. C. Matheson, A. R. Cattan, H. Lohrer, Eur. J. Cancer 1996, 32, 1248–1253.

215.K. Kasahara, Y. Fujiwara, K. Nishio, T. Ohmori, Y. Sugimoto, K. Komiya, T. Matsuda, N. Saijo, Cancer Res. 1991, 51, 3237–3243.

216.F.-S. Liu, Taiwan. J. Obstet. Gynecol. 2009, 48, 239–244.

217.J. E. Visvader, G. J. Lindeman, Nat. Rev. Cancer 2008, 8, 755–768.

218.A. Wiechert, C. Saygin, P. S. Thiagarajan, V. S. Rao, J. S. Hale, N. Gupta, M. Hitomi, A. B. Nagaraj, A. DiFeo, J. D. Lathia, O. Reizes, Oncotarget 2016, 7, 30511–30522.

219.A. Vassilopoulos, C. Xiao, C. Chisholm, W. Chen, X. Xu, T. J. Lahusen, C. Bewley, C.-X. Deng, J. Biol. Chem. 2014, 289, 24202–24214.

220.W. R. Leopold, E. C. Miller, J. A. Miller, Cancer Res. 1979, 39, 913–918.

221.B. J. S. Sanderson, L. R. Ferguson, W. A. Denny, Mutat. Res. 1996, 355, 59–70.

222.L. J. N. Bradley, K. J. Yarema, S. J. Lippard, J. M. Essigmann, Biochem. 1993, 32, 982–988.

223.K. J. Yarema, S. J. Lippard, J. M. Essigmann, Nucleic Acids Res. 1995, 23, 4066–4072.

224.U. M. Ohndorf, M. A. Rould, Q. He, C. O. Pabo, S. J. Lippard, Nature 1999, 399, 708–712.

Viktor Brabec,1 Jana Kasparkova,1

Vijay Menon,2 and Nicholas P. Farrell2

1 Institute of Biophysics, Czech Academy of Sciences,

Kralovopolska 135, CZ-612 65 Brno, Czech Republic

<brabec@ibp.cz>

2 Department of Chemistry, Virginia Commonwealth University,

Richmond, VA 23284–2006, USA

<npfarrell@vcu.edu>

Abstract: Polynuclear platinum complexes (PPCs) represent a discrete structural class of DNA-binding agents with excellent antitumor properties. The use of at least two platinum coordinating units automatically means that multifunctional DNA binding modes are possible. The

structural variability inherent in a polynuclear platinum structure can be harnessed to produce discrete modes of DNA binding, with conformational changes distinct from and indeed inaccessible to, the mononuclear agents such as cisplatin. Since our original contributions in this field a wide variety of dinuclear complexes especially have been prepared, their DNA binding studied, and potential relevance to cytotoxicity examined. This chapter focuses on how DNA structure and reactivity is modulated through interactions with PPCs with emphasis on novel aspects of such structure and reactivity. How these major changes are further reflected in damaged DNA-protein binding and cellular effects are reviewed. We further review, for the first time, the great structural diversity achieved in PPC complex design and summarize their major DNA binding effects.

Keywords: DNA conformations • DNA-protein crosslinking • polynuclear platinums • structural diversity 1.INTRODUCTION

The acceptance of DNA as cellular target of cisplatin (cis-[PtCl2(NH3)2]; cis-DDP) and its congeners has led to significant understanding on the factors affecting platinum complex-DNA adduct structure and how these can be modulated by suitable complex design. DNA as a cellular target for platinum metal-based anticancer drugs remains an active area of research including the search for (i) new, more specific or more potent analogs of existing drugs; (ii) agents to induce, interfere with, or preferentially interact with, unusual or “non-B DNA” structures such as Z-DNA, Holliday junctions, and G-quadruplexes; and (iii) agents capable of interacting at the level of DNA-protein complexes, such as telomerase and topoisomerase. Figure 1. Structures of principal alkanediamine-linked polynuclear platinum complexes (PPCs). Triplatin is BBR3464; 1,1/t,t is BBR3005; 1,1/t,t refers to two monofunctional Pt units where the Pt-Cl is trans to the diamine bridge, etc.

Polynuclear platinum complexes (PPCs) represent a discrete class of platinum-based anticancer agents whose development was based on the concepts that alteration of DNA adduct structure in comparison to those formed by cisplatin and congeners would induce differential downstream effects with respect to protein recognition and cellular signaling pathways (Figure 1). Tolerance of DNA adducts, a property linked in part to the development of cellular resistance to cisplatin, may be considered to be altered through formation of different adduct structures by circumventing the cellular processes associated with cisplatin-DNA adduct recognition and repair [1–3]. In this way logical approaches to design drugs effective against cisplatin-resistant cancers can be envisaged. Proof of principle for this hypothesis was achieved by the advance to the clinic of Triplatin (BBR3464, [{trans-PtCl(NH3)2}2μ-{trans-Pt(NH3)2(H2N(CH2)6NH2)2}]4+), a charged trinuclear bifunctional DNA-binding agent (Figure 1). The pharmacology and antitumor activity of Triplatin and dinuclear analogs have been reviewed [4–6].

The polynuclear platinum structure as shown in Figure 1 inherently leads to a very diverse array of complexes by varying the nature and geometry of the coordination sphere as well as the nature of the linker. The major studies have been on alkanediamine-linked dinuclear and trinuclear complexes but many researchers have now modified this basic structure. DNA represents a rich template for coordination chemistry and, in this review, we will emphasise how research in the polynuclear field has delineated specific DNA modifications, structures and reactivity patterns not readily available to mononuclear complexes. The majority of complexes have good cytotoxicity and in general are collaterally sensitive to cisplatin – these aspects will not be covered in detail, but References are provided. 2.DINUCLEAR BIFUNCTIONAL PLATINUM(II) COMPLEXES WITH ALKANEDIAMINE LINKERS

The predominant DNA adducts in all cases of dinuclear bifunctional Pt(II) complexes are long-range {Pt,Pt} inter- and intrastrand crosslinks (CLs) and DNA-protein crosslinks may also be formed (Figure 2). In [{PtCl(NH3)2}2(H2N(CH2)nNH2)]2+ the leaving chloride ligands are either cis

(1,1/c,c) or trans (1,1/t,t) to the diamine bridge (Figure 1). Both geometries display in vivo antitumor activity comparable with that of cisplatin but importantly they retain activity in acquired cisplatin-resistant cell lines [1, 4, 5]. This situation represents a fundamental difference between mononuclear and dinuclear platinum chemistry and biology – in the mononuclear case cisplatin is antitumoractive, while transplatin is not. 2.1.{Pt,Pt} Interstrand Crosslinks

{Pt,Pt} interstrand CLs are preferentially formed between N7-platinated G residues and are oriented in the 5′/5′ direction. Besides 1,2 interstrand CLs (between G residues in neighboring base pairs), 1,3 or 1,4 CLs are also possible where the platination sites are separated by one or 2 base pairs, respectively. Geometry affects the relative proportion of interstrand CLs with the efficiency of formation being much higher for the 1,1/c,c over the 1,1/t,t isomer [7–9]. The 1,1/t,t complex preferentially forms interstrand CLs even when the specific sequence contains the possibility to form a 1,2-intrastrand adduct [10]. The 1,2 CLs are formed with a pronouncedly slower rate than the longer-range 1,3 or 1,4 CLs [11]. The conformational distortions induced in DNA by the 1,3- or 1,4-interstrand CLs of 1,1/t,t show that these lesions result only in a very small directional bending of the helix axis (~10°) and duplex unwinding (9°) and are thus conformationally flexible [11]. Figure 2. Schematic of major DNA and DNA-protein adducts accessible to PPCs. The {Pt,Pt} crosslinks may be long-range where the platinating sites are separated by up to 4 intervening base pairs (see text).

The structure of the alkanediamine linker in the dinuclear Pt(II) complexes can control the substitution process with small nucleophiles [12]. DNA reactions proceed primarily via formation of the monoaqua monochloro species in the rate-limiting step [13]. Aquation and subsequent formation of the monofunctional adducts of 1,1/t,t is preceded by preassociation with the polyanionic DNA surface through electrostatic interactions and hydrogen bonding [9, 13]. Transformation of monofunctional to bifunctional adducts proceeds via the aquated intermediate and this closure is markedly faster than that found for the major 1,2-intrastrand CL formed from the diaqua form of cisplatin [9]. The rate of aquation of 1,1/t,t is enhanced in the presence of single-stranded over double-stranded DNA showing that the nature of template DNA may affect substrate specificity [14]. Notably, 1,1/c,c is hydrolyzed less readily than 1,1/t,t and there is no evidence for the preassociation of 1,1/c,c [15]. 2.2.{Pt,Pt} Intrastrand Crosslinks

The 1,1/t,t isomer forms minor 1,2-GG intrastrand CLs producing a flexible, non-directional bend in DNA [16] which noticeably reduces thermal and thermodynamic stability of the duplex more than the equivalent mononuclear cisplatin adduct [17]. {Pt,Pt} intrastrand CLs have not been observed in DNA modified by 1,1/c,c [7, 8]. NMR studies have shown restricted rotation around the Pt-3′-G bond in single-stranded r(GpG), d(GpG), and d(TGGT) adducts and this steric hindrance may be responsible for the inability to form the 1,2-GG intrastrand CLs with sterically more demanding double-helical DNA [18]. 2.3.Conformational Changes in DNA

On a global level, both dinuclear platinums induce the B/Z transition in poly(dG-dC) % poly(dG-dC) and Pt-DNA bond formation is not an absolute necessity for the Z-DNA induction, but the {Pt,Pt} interstrand crosslink may be important in ‘locking’ the Z-conformation [8, 19]. This is a general property and the polyamine-linked dinuclear complexes (Section 3) are also very effective in inducing irreversible conformational changes including the B/A transition [20].

The structural changes on site-specific oligonucleotides have been summarized [21]. The {Pt,Pt} 1,4-interstrand crosslink of 1,1/t,t-modified (5′-ATGTACAT)2 shows that both A and G pur ine residues adopt a syn conformation of the nucleoside unit – a pre-requisite for induction of the left-handed conformation [22]. The structure of this adduct closely resembles that formed by the trinuclear compound Triplatin (BBR3464) (see below, Section 7). The combined biophysical features – bifunctional DNA binding through two monofunctional Pt units and changes to the sugar residues in an extended sequence – may explain the conformational flexibility noted,

significantly different to the rigid bending of cisplatin [11, 21]. 2.4.Protein Recognition

DNA interstrand crosslinks pose a special challenge to repair enzymes because they involve both strands of DNA and therefore cannot be repaired using the information in the complementary strand for resynthesis [23]. High-mobilitygroup (HMG)-domain proteins play a role in sensitizing cells to cisplatin [23, 24]. An important structural motif recognized by HMG-domain proteins on DNA modified by cisplatin is a stable, directional bend of the helix axis. One possible consequence of binding of HMG-domain proteins to cisplatin-modified DNA is the shielding of damaged DNA from intracellular nucleotide excision repair (NER) [25]. The conformational flexibility of the major {Pt,Pt} crosslinks results in very weak or no recognition of their DNA adducts by HMGB1 proteins [11]. The {Pt,Pt} interstrand adducts may, however, present a block to DNA or RNA polymerase [9, 26].

With respect to intrastrand crosslinks, the affinity of HMG-domain proteins to the duplex containing 1,2-GG intrastrand CL of cisplatin is sequence-dependent and is reduced with increasing thermodynamic destabilization of the duplex [27]. The weak affinity of the minor 1,2-GG intrastrand CL of 1,1/t,t to HMG-domain proteins is consistent with the observation that this lesion reduces the thermal and thermodynamic stability of DNA markedly more than the same lesion of cisplatin [16, 17]. Consistent with the weak recognition by HMGB1 proteins, effective removal of {Pt,Pt} intrastrand adducts by NER has been observed [28].

The major {Pt,Pt} interstrand CLs are repaired much less easily than the {Pt,Pt} intrastrand CLs and are not removed in an in vitro assay using mammalian and rodent cell-free extracts capable of removing the intrastrand CLs [11, 29]. Hence, the {Pt,Pt} interstrand adducts do not have to be shielded by damaged DNA recognition proteins, such as those containing HMG domains, to prevent their repair. Clearly, the mechanism of antitumor activity of bifunctional dinuclear Pt(II) complexes does not involve recognition by HMG-domain proteins as a crucial step, in contrast to the proposals for cisplatin and its direct analogs. This critical ability to dictate a biological effect is reasonably attributed to the design and formation of a structurally unique set of Pt-DNA adducts accessible only to the dinuclear structure. 3.POLYAMINE-LINKED BIFUNCTIONAL DINUCLEAR PLATINUM(II) COMPLEXES

An important subset of bifunctional dinuclear platinum complexes are those where the platinum units are linked through natural and synthetic polyamines, adding extra charge to the overall structure (Figure 1) [30]. Their promising preclinical activity has been summarized [5, 6, 31, 32]. The design of BBR3610 mimics the charge and the distances between platinating centers in the trinuclear Triplatin (BBR3464). The BBR3610-DACH (DACH = 1,2-diaminocyclohexane) compound is the first dinuclear analog of oxaliplatin. The DNA binding mode of these dinuclear Pt(II) complexes, including sequence preference, type of the major adducts, and resulting conformational alterations, is not very different from that of the alkanediamine-linked analogs. The kinetics of binding of the spermine and spermidine compounds corresponds to their relatively high charge (2C to 4C). The preference for the formation of {Pt,Pt} interstrand CLs, however, does not follow a charge-based pattern nor the length of the polyamine chain (Table 1) [33, 34]. The presence of the central positively-charged moiety reduces the interstrand crosslinking efficiency – synthesis of the central N-blocked spermidine derivative (BBR3571) such as in [{trans-PtCl(NH3)2}2{μ-BOC-spermidine}]2+ (BOC = t-BuOCO), [35], results in markedly less crosslinking than the parent protonated compound [33]. Both the spermidine and spermine-linked compounds are very effective inducers of irreversible B/Z and B/A transitions in DNA [20].

Table 1.Summary of the DNA-binding characteristics of selected polynuclear platinum compounds. [a]

	unwinding angle/adduct (°)	interstrand CL/adduct (%)

1,1/t,t n = 6	10–14	70–90
BBR3571	12.3	40
BBR3535	15.4	57
Triplatin (BBR3464)	14	20
BBR3610	14	23
BBR3610-DACH	13	26
Cisplatin	13	6

a See [33, 34]. All compounds have trans-oriented platinating groups (Figure 1).

3.1.Consequences of DNA Binding of Dinuclear Polyamine-Linked Complexes

The major adducts of BBR3610 and BBR3610-DACH are removed from DNA by DNA repair systems with a markedly lower efficiency than the adducts of cisplatin [34]. The increased length of the linker allows for formation of longer-range {Pt,Pt} intrastrand CLs in comparison to the 1,2-adducts formed by 1,1/ t,t. The ability of {Pt,Pt} intrastrand CLs of BBR3610 and BBR3610-DACH to thermodynamically destabilize DNA depends on the number of base pairs separating the platinated bases and the greatest destabilization is observed for the long-range CL in which the platinated sites are separated by four base pairs

[36]. The extent of destabilization correlates with the extent of conformational distortions induced. The efficiency of excinucleases to remove these CLs from DNA also depends on their length; the trend is identical to that observed for the ability to thermodynamically destabilize the duplex.

A second example of how DNA downstream effects may be “fine-controlled” by the nature of the linking diamine/polyamine chain is seen in the inhibition of DNA replication by the site-specific {Pt,Pt} 1,2-intrastrand crosslinks of BBR3571 [37]. The interaction of DNA polymerases with a Pt-DNA adduct is an important determinant of the propensity of a given adduct to be cytotoxic, mutagenic, or ultimately, of no long-term consequence. The 1,2-GG intrastrand CL of BBR3571 inhibits DNA translesion synthesis markedly more efficiently than the equivalent adduct of cisplatin [37]. This result has been explained by the bulkier adduct of the dinuclear complex and by the flexibility induced in DNA which can make the productive binding of this adduct at the polymerase site more difficult.

3.2.Interduplex Crosslinking

The properties of DNA globally modified by polynuclear complexes are highlighted by markedly enhanced intraduplex {Pt,Pt} interstrand crosslinking (Figure 2). In general, DNA interstrand crosslinking requires close proximity of binding sites in the two DNA strands. This requirement may be easily fulfilled in the case of formation of the intraduplex DNA interstrand CLs by bifunctional Pt(II) complexes because binding via one leaving group inevitably leaves the other close to other binding sites in the same duplex. However, if the reactive sites of the bifunctional crosslinking agents are sufficiently distant, then binding to adjacent duplexes may occur. Thus, bifunctional Pt(II) compounds might also be effective interduplex crosslinkers in cases when two fragments of double-helical DNA molecules are forced to lie together, for instance, during recombination, at replication forks or sites of topoisomerase action, or more generally in cellular environmental conditions. Under molecular crowding conditions mimicking environmental conditions in the cellular nucleus the spermine-linked BBR3535 fulfills the requirements placed on interduplex DNA crosslinkers considerably better than mononuclear cisplatin or transplatin [38]. Platinating sites in 1,1/t,t-spermine are markedly more distant (2.7 nm) than those in cisplatin (0.28 nm) and moreover, the trans geometry of leaving ligands in the dinuclear complex may allow the spermine linker to direct the reactive sites in opposite directions, which may facilitate binding to adjacent duplexes.

The structural features promoting interduplex CLs may, in fact, be quite varied when we consider polynuclear platinum complexes. The trinuclear tridentate [Pt3Cl3(hptab)]3+ (hptab =

N,N,N′,N′,N″,N″-hexakis(2-pyridylmethyl)-1,3,5-tris-(aminomethyl)benzene) forms mainly trifunctional {Pt,Pt,Pt} intrastrand CLs in the absence of proteins and molecular crowding agents, where all Pt(II) centers are coordinated to G residues [39–41]. In the presence of a molecular crowding agent two DNA duplexes are bound in high yield [41]. The increased functionality of the complex also allows for observation of DNA-protein cross-links in high yield (see Section 4). These examples suggest concepts for the systematic design of polynuclear agents capable of forming interduplex DNADNA crosslinks. 3.3.Susceptibility to Metabolic Decomposition and Structural Variations

Decomposition of the PPC structure occurs when the substitution-labile ligand is trans to the linker because replacement of the Pt–Cl bond by a trans-labilizing sulfur donor results in breaking the Pt-amine (linker) bond [42, 43]. The kinetic data for these reactions indicate that aquation is not a rate-limiting step for reactions with sulfur nucleophiles. This metabolic effect is deactivating and also diminishes the capacity to form long-range CLs. On the other hand, the cis geometry as in [{cis-PtCl(NH3)2}2-μ-Y]n+ should preserve the main features of antitumor polynuclear Pt(II) complexes but with enhanced stability to metabolic deactivation [44, 45].

Figure 3. Structures of various dinuclear platinum(II) complexes discussed in the text for their DNA binding properties.

A unique 11-member chelate ring contains a glutathione-bridged Pt-μ-GS-Pt structure [44]. The BBR3610-DACH compound was synthesized for this reason and shows enhanced metabolic stability over BBR3610 [46]. A second example of enhanced stability to metabolic deactivation is afforded by use of a semi-rigid linker in the compound [{cis-Pt(NH3)2Cl}]2(μ-4,4′-methylenedianiline)]2+ (I, Figure 3), [47–49]. The DNA adducts of I demonstrated for the first time for PPCs a strong specific recognition and binding of HMG-domain proteins to modified DNA [49]. Dinuclear trans-oriented Pt(II) complexes may remain stable in the presence of sulfur-containing compounds as observed for long chain [{trans-PtCl(dien)}2-μ-(CH2)n]2+ (n = 7,10,12, dien = diethylenetriamine) (II, Figure 3) [50]. DNA conformational changes are also dependent on the linking chain length [50].

3.4.Macrocyclic Polyamine-Linked Complexes

The compound [Pt2(DTBPA)Cl2] (DTBPA = 2,2′-(4,11-dimethyl-1,4,8,11-tetraaza-cyclotetradecane-1,8-diyl)bis-(N-(2-(pyridin-2-yl)ethyl)acetamide)), which combines a modified macrocyclic polyamine (cyclam) and two pyridine moieties, shows only moderate affinity to DNA possibly due to the steric hindrance of the cyclam ring which affects the DNA-binding of the two Pt(II) centers but it does unwind the DNA double helix [51, 52]. The analogue [Pt2(TPXA)Cl2]Cl2 (TPXA = N,N, N′,N′-tetra(2-pyridylmethyl)-m-xylylene diamine), in which the Pt(II) centers are now bridged by a bulky aromatic linker, forms 1,4-{Pt,Pt} intrastrand rather than 1,3-intra- and interstrand CLs which exert more perturbation on the tertiary structure of negatively supercoiled DNA than cisplatin [52, 53]. 4.DNA-PROTEIN CROSSLINKING

The presence of two or more Pt centers automatically leads to the possibility of “higher-order“ tri- and tetrafunctional DNA binding when cis-[PtCl2(amine)2] units are used as in the canonical 2,2/c,c (Figure 1). Dinuclear tri- and tetrafunctional platinum complexes form very efficient coordinative ternary DNA-protein CLs to a range of proteins including components of the UVrABC repair system and the Klenow fragment [54–56]. The protein binding is effected from a first formed {Pt,Pt} interstrand CL, thus significantly differentiating the structures from mononuclear DNA-protein adducts, where by definition only monofunctional Pt-DNA binding is possible. The bulky DNA–protein CLs represent a more distinct and persisting structural motif recognized by the components of downstream cellular systems processing DNA damage in a considerably different manner than the DNA adducts of mononuclear platinum drugs.

An interesting implication is that the formation of DNA-protein crosslinks by tri- or tetrafunctional

dinuclear Pt(II) complexes is related to their different cytotoxicity profile in comparison with the dinuclear bifunctional analogues [4, 5]. Use of a rigid linker in the tetrafunctional [{trans-PtCl2(NH3)}2(μ-piperazine)], (III, Figure 3), also allows for observation of DNA-protein CLs while {Pt,Pt} interstrand crosslinking is diminished relative to the alkanediaminelinked 1,1/c,c or 1,1/t,t [57].

5.DINUCLEAR PLATINUM(II) COMPLEXES STABILIZING G-DNA QUADRUPLEXES

Certain guanine (G)-rich nucleic acid sequences can form four-stranded structures which can adopt a wide diversity of structures and topologies (Figure 4) [58]. These structures have many interesting biological roles including roles in telomeres, DNA replication, gene regulation, transcription, and translation. These properties make them appealing therapeutic targets so that the identification of small molecules that demonstrate selectivity for biologically relevant G-quadruplexes is an active area in drug discovery.

G-quadruplexes have been shown to be a target for several dinuclear Pt(II) complexes as exemplified by [{trans-PtCl(NH3)2}2-μ-H2N(CH2)nNH2]2+ (n = 2 or 6) [59]. The folding of AG3(T2AG3)3 in either Na+ (antiparallel) or K+ (parallel) forms and the complexes showed that the parallel structure exists whatever the cation and confirmed the existence of the antiparallel structure in the presence of both cations. The unique {Pt,Pt} CLs are formed between Gs belonging to the same quartet (Figure 4). Molecular dynamics rationalized these findings, where it was shown that the guanines were flexible allowing reversible migration to form the top G-quartet, thereby making the N7 atoms accessible to platination [59]. Many planar ligands have been used to stack with the G-quartets. Figure 4. The parallel and antiparallel structures of the G-quadruplex AG 3(T 2AG 3) 3. Note the bifunctional platination sites in the same quadruplex. Adapted with permission from [59]; copyright 2005 American Chemical Society.

The dinuclear terpyridine-based Pt(II) complex (IV, Figure 3) interacts via π-π stacking and with the DNA phosphate backbone via direct coordination or electrostatic interactions [60]. The complex induces the formation of quadruplex DNA (largely the antiparallel conformation) even in the absence of potassium ions and with good selectivity (up to 100-fold) over duplex DNA. Another type of Pt(II) complex stabilizing DNA quadruplexes is represented by symmetric dinuclear terpyridine-Pt(II) units – in general [{Pt(terpy}2μ-Y]4+ where Y is a flexible thiol bridging ligand or 4,4′-trimethylene-dipyridine ligand [61]. These substitution-inert complexes markedly increase the melting temperature of various G-DNA quadruplex motifs and maintain this binding in up to a 600-fold excess of double-helical DNA. The isomeric dinuclear cations, [{Pt(2,2′-bpy)}2(tppz)]4+ (tppz = tetrakis(pyridine-2-yl)pyrazine), differ in their overall shape and display different affinities toward duplex DNA and human telomeric quadruplex DNA [62].

Trinuclear Pt(II) complexes are also effective and selective G-quadruplex binders and good telomerase inhibitors. Two propeller-shaped, trigeminal-ligand-containing, flexible trinuclear Pt(II) cations, [{Pt(dien)}3(ptp)]6+ and [{Pt(dpa)}3(ptp)]6+ (dpa = bis-(2-pyridylmethyl)amine; ptp = 6′-(pyridin-3-yl)-3,2′: 4′,3″-terpyridine), exhibit higher affinity for human telomeric and c-myc promoter G4 sequences than duplex DNA [63]. Both complexes are good telomerase inhibitors, with IC50 values in the micromolar range.

6.STRUCTURAL VARIATION IN DINUCLEAR PLATINUM(II) COMPLEXES

6.1.Azole- and Azine-Bridged Dinuclear Bifunctional Pt(II) Complexes

A consistent theme in platinum complex drug development is the design of agents capable of pharmacological inhibition of DNA repair [1, 2, 24, 64]. An interesting series in this respect is the set of dinuclear Pt(II) complexes where two cis-{Pt(NH3)2} units are bridged by various azole-based bridging ligands such as [{cis-Pt(NH3)2}2(μ-OH)(μ-pyrazolato)](NO3)2 (1,1/c,c-prz), [{cis -Pt(NH3)2}2(μ-OH)(μtri- or tetraazolato)](NO3)2 (V and VI, Figure 3) [65–68]. The μ-hydroxo

acts as a leaving group in these complexes, and the rigid bridging azolates keep the appropriate distance between the two Pt atoms to enable binding of two neighboring G residues in double-helical DNA. Complex V forms in DNA duplexes major 1,2-GG intrastrand CLs but the distortion induced is significantly less pronounced than that induced by similar CLs from cisplatin and concomitantly, the thermodynamic stability of the modified DNA duplex is lessened considerably [69, 70]. As a corollary, the dinuclear adducts, although formally similar to those formed by cisplatin, are weak substrates for HMGB1 protein recognition and represent poor substrates for DNA repair through a “cisplatin-like” mechanism [70]. Notably, V and VI cause irreversible compaction of DNA through an intermediate state in which coil and compact parts coexist in a single DNA molecule, a feature different from that of typical condensing agents [71, 72].

In contrast to the azole-bridged dinuclear bifunctional Pt(II) complexes, the benzotriazolate (Btaz)-bridged one, [{cis-PtCl(NH3)2}2(l-Btaz-H)]Cl, utilizes the rigid aromatic ring as a linker with non-bridging chloride ions as leaving groups [73]. Monofunctional DNA adducts of this dinuclear complex are converted to more toxic bifunctional CLs considerably more slowly in comparison with cisplatin, or the 1,1/t,t or 1,1/c,c [73]. The compound is weakly antitumor-active but susceptible to metabolic deactivation. Use of the rigid aromatic rings of azines as bridging ligands affords compounds such as ([{cis-PtCl(NH3)2}2(μ-Y)]2+ (Y = pyrazine, pyrimidine or pyridazine) [74, 75]. The complexes with the least steric hindrance and those with more delocalized ligands capable of additional DNA intercalations such as base stacking are the most cytotoxic. Finally, a series of pyrazine-bridged dinuclear Pt(II) complexes with general formulas [{PtCl(L)}2(μ-prz)]2+ (L = chelating diamines such as ethylenediamine, en; (G)-1,2-propylenediamine; isobutylenediamine; trans-(G)-1,2-diaminocyclohexane; 1,3-propylenediamine; 2,2-dimethyl-1,3-propylenediamine) and one pyridazine (pydz)-bridged complex, [{PtCl(en)}]2(μ-pydz)]2+, effectively interact with DNA in cell-free media [76].

6.2.Miscellaneous Dinuclear Platinum(II) Complexes

Additional DNA-binding modes may be incorporated into dinuclear Pt(II) complexes by use of intercalating moieties such as acridines and anthraquinones in the linker [77, 78]. Another group of antitumor dinuclear Pt(II) compounds that bind DNA and also interact via intercalation comprise the complexes [{PtCl(bpy)}2(μ-L-H)2] and [{PtCl(phen)}2(μ-L-H)2] (bpy = 2,2′-bipyridine, phen = 1,10-phenanthroline, and L = 2,2′-azanediyldibenzoic dianion or 1,3-benzothiazol-2-amine) dibridged by H2L ligands [79, 80]. The results performed in cell-free media have shown that the DNA binding mode of the complexes involve their intercalative DNA interaction and that these complexes can cleave DNA.

A series of organometallic dinuclear Pt(II) complexes was synthesized with the aim to tune the electronic and steric properties of the Pt centers so that the bifunctional dinuclear Pt(II) compounds could act by different mechanistic pathways in comparison with classical 1,1/c,c [81, 82]. Modifications of DNA by [{Pt(CH3)Cl((CH3)2SO)}2(μ-H2N(CH2)6NH2)] (VII, Figure 3), show a DNA binding mode different from that of the formally equivalent 1,1/c,c, with mostly monofunctional adducts. The minor {Pt,Pt} interstrand CLs (2 %) are capable of terminating RNA synthesis in vitro while the major monofunctional adducts are not.

The dinuclear [{trans-PtCl(NH3)2}2μ-dpzm]2+ (dzpm = 4,4′-dipyrazolylmethane) (VIII, Figure 3) forms {Pt,Pt} intrastrand and interstrand crosslinks in double-stranded DNA, but with a distinct preference for AA or AG sites [83, 84]. Use of modified pyridine groups such as isonicotinamide or substituted isonicotinamide as linker results in dinuclear compounds formally similar to picoplatin (cis-[PtCl2(NH3)(2-mepyridine)] [85, 86].

Finally, dinuclear boron-containing bifunctional and tetrafunctional Pt(II)-amine complexes, in which two Pt(II) moieties are bridged by 1,7-carborane [carborane = dicarba-closo-dodecaborane(12)], [{trans-PtCl(NH3)2}2μ-1,7-NH2(CH2)3CB10H10-C(CH2)3NH2]2+

(1,1/t,t-carborane) and [{cis-PtCl2(NH3)}2μ-1,7-NH2(CH2)3-CB10H10C(CH2)3NH2] (2,2/c,c-carborane), were synthesized as potential DNA targeting agents in boron neutron capture therapy (BNCT) [87]. 7.TRINUCLEAR PLATINUM(II) COMPLEXES 7.1.The Trinuclear Bifunctional Platinum(II) Complex Triplatin (BBR3464)

The synthetic pathways developed for alkanediamine-linked dinuclear compounds automatically lead the way to trinuclear compounds and a cisplatin synthon can be developed using three sequential cis-[PtCl2(amine)2 units [88]. The most studied compound is Triplatin (BBR3464) [{trans-PtCl(NH3)2}2μ- {trans-Pt(NH3)2(H2N(CH2)6NH2)2}]4+, a trinuclear, bifunctional DNA binding agent with an overall 4C charge where the bridging between the two platinating units is formally made by the [{trans-Pt(NH3)2}μ-{H2N(CH2)6NH2}2] unit (Figure 1). The drug advanced to Phase II clinical trials where durable responses in cisplatin-resistant ovarian cancer were noted. The drug did not advance further due to a combination of pharmacokinetics involving loss of the trinuclear structure (albeit with overall reactivity similar to cisplatin if we consider that <5 % of administered cisplatin is considered to get to DNA) and pharmaceutical company takeovers.

The trinuclear compound and the dinuclear polyamine-linked species are formally equivalent with both linking units containing a charged moiety capable of hydrogen-bonding and electrostatic interactions with DNA, and both series can be seen as logical extensions of the “original” alkanediamine-linked series. It may be noted that the polyamine-linked dinuclear compounds are as similarly potent as Triplatin. Several reviews on various aspects of the pre-clinical and clinical studies on Triplatin, including DNA binding studies, have been published [1, 2, 5, 6, 21, 89]. It is the purpose in this chapter to highlight the contributions that Triplatin-DNA studies have made to delineation of novel DNA structure and reactivity.

The high charge on Triplatin facilitates rapid binding to DNA with a t1/2 of ~40 min, significantly faster than the neutral cisplatin [90]. Triplatin forms {Pt,Pt} long-range interstrand CLs in natural DNA in a considerably higher amount (~20 %) than cisplatin (Table 1) [90]. Changing the geometry of the central unit from trans to cis, as in [{trans-PtCl(NH3)2}2μ-cis-Pt(NH3)2{H2N(CH2)6NH2}2]4+ (BBR3499, 1,0,1/t,c,t) results in enhanced {Pt,Pt} interstrand crosslinks with reduced sequence specificity and slower binding to DNA [91]. The BBR3499-DNA adducts distort DNA conformation and are repaired by cell-free extracts considerably better than the adducts of BBR3464 [91]. DNA molecules aggregate and compact upon treatment with Triplatin, as revealed by high-resolution atomic force microscopy, reasonably attributed the combination of charge and formation of the long-range CLs [92]. 7.2.Directional Isomers

The {Pt,Pt} interstrand crosslinks of Triplatin occur in both the 5′-5′ and 3′-3′ sense, forming “directional isomers” (Figure 5) [93, 94]. The directionality is dependent on the nature of the crosslink. The 1,2-interstrand CL forms preferentially the 3′-3′ direction (in an antiparallel manner), the 1,4-interstrand CL forms in both directions in approximately equal proportions, whereas the 1,6-interstrand CL forms in preferentially the 5′-5′ direction [89, 93]. The kinetics of formation of 1,2- and 1,4-interstrand CLs were found to be similar and faster than that for the analogous 1,6-interstrand CL [93, 94]. The kinetics of binding follows the trend seen for the dinuclear alkanediamine-bridged 1,1/t,t compound with aquation followed by monofunctional binding and then closure to the bifunctional crosslink [95]. Figure 5. A. A scheme for the formation of novel 5′-5′ and 3′-3′ directional isomers on DNA. B. The “walking” of {Pt,Pt} DNA adducts is induced by different thermodynamic destabilization of the double helix by structurally different adducts.

In examining the factors affecting the formation of directional isomers by {1H,15N} HSQC NMR spectroscopy, differences occur at the monofunctional binding step. In the 5′-5′ case, pre-association with initial hydrogen-bonding and electrostatic interactions with DNA are observed in the minor grove [96]. Two distinct pathways for the terminal {PtN3Cl} groups to approach and

bind the guanine N7 in the major groove with the central linker anchored in the minor groove were inferred. To achieve platination of the guanine residues the central linker remains in the minor groove but triplatin must diffuse off the DNA for covalent binding to occur. Unlike the 5′-5′ case a number of 3′-3′ crosslinked adducts are observed [97].

Molecular dynamics simulations showed a highly distorted structure with considerable base fraying and widening of the minor groove [96]. In contrast to the 1,4-situation the 3′-3′ 1,2-interstrand crosslink is formed preferentially in the sequence d(ACGTATACGT)2 where two simultaneous adducts are formed bet ween the adjacent guanines, the first examples of a structurally characterized 3′-3′ adduct [98]. The structure is quite distinct from the analogous cisplatin 1,2-interstrand (GC)2 adduct and from the 5′-5′ 1,2-Cl formed by [{trans-(PtCl(NH3)2}2μ-H2(CH2)4NH2]2+ [89, 98, 99]. The ability to form directional isomers is a property shared with BBR3571, but not 1,1/t,t, suggesting that the presence of charge in the central moiety, implying pre-association, is a key factor in dictating this property.

Both directional {Pt,Pt} 1,4-interstrand CLs formed by Triplatin exist as two distinct non-interconvertible conformers [100]. Analysis of the conformers by differential scanning calorimetry, chemical probes of DNA conformation, and minor groove binder Hoechst 33258 have demonstrated that each of the four conformers affects DNA in a distinctly different way and adopts a different conformation and are distinct from those of the short-range adducts of cisplatin [100]. The properties of these site-specific adducts of Triplatin, such as conformational distortions, are also distinctly different from those of the short-range adducts of mononuclear cisplatin [93, 101]. The structural distortions on site-specific {Pt,Pt} CLs of Triplatin have been summarized [21].

7.3.Walking on Double-Helical DNA and Linkage Isomerization

In studying the properties of site-specific long-range CLs of Triplatin, some CLs of this platinum compound were found unstable [102]. The inherent steric effects around the Pt center of the mononuclear bifunctional adduct are replaced by the steric constraints of the conformational change as a whole. Since specific Triplatin adducts distort DNA conformation differently, it is reasonable to expect that the energetic signatures of these dissimilar adducts are different. Under physiological conditions the Pt-G(N7) bonds are reactive leading to linkage isomerization reactions on the double-helical DNA substrate. Upon incubation of DNA duplexes containing a single, site-specific intrastrand CL between G residues the coordination bonds between Pt and the N7 of one of the G residues within the intrastrand adduct are cleaved leading to the formation of interstrand CLs (linking both strands of DNA). These interstrand CLs react further to form intrastrand CL in the strand complementary to that in which original intrastrand CL was formed. This successive rearrangement may proceed in the way that the molecule of Triplatin originally coordinated to one strand of DNA can spontaneously translocate from this strand to its complementary counterpart via intermediate interstrand CL (Figure 5), which may evoke walking of this platinum complex on DNA molecules.

Differential scanning calorimetry of duplexes containing single, site-specific CLs of Triplatin revealed that one of the driving forces that leads to the lability of DNA CLs of Triplatin is a difference between the thermodynamic destabilization induced by the CL and by the adduct into which it could isomerize [102]. Thus, one of the driving forces that leads to the lability of DNA CLs of Triplatin is a difference between the thermodynamic destabilization induced by the CL and by the adduct into which it could isomerize.

7.4.Interactions of DNA Modified by Triplatin by Damaged-DNA Binding Proteins

Binding of cellular damaged DNA-binding proteins to DNA modified by platinum complexes plays an important role in initial phases of the mechanism of cytotoxic action of platinum drugs [23–25]. Thus, due to its ability to modify DNA in a unique manner, Triplatin could distinctly evoke different pathways of cellular response to DNA damage such as triggering of the apoptotic pathway.

7.4.1.Recognition by HMG-Domain Proteins and DNA Repair

In contrast to distortions induced by major CLs of cisplatin, the CLs of Triplatin do not extensively unwind and rigidly bend DNA so that they are not substrates for damaged DNA-binding proteins, such as HMG-domain proteins [93, 94]. Thus, the antitumor effects of BBR3464 do not involve a shielding or hijacking mechanism as the effects of cisplatin (vide supra). On the other hand, while intrastrand adducts of Triplatin are readily removed from DNA by the NER systems, the interstrand CLs are not.

7.4.2.Recognition by the Tumor Suppressor Protein p53

The DNA binding activity of the p53 protein is crucial for its tumor suppressor function. The active protein p53 is a nuclear phosphoprotein that consists of 393 amino acids and contains four major functional domains [102]. Active p53 binds as a tetramer to ~50 different response elements that occur naturally in the human genome and shows functionality [103]. Free DNA in the segments corresponding to the consensus sequence is already intrinsically bent toward the major groove [104, 105]. The interactions of active and latent p53 proteins with DNA fragments and oligodeoxyribonucleotide duplexes modified by Triplatin in a cell-free medium has been examined and the results have been compared with those describing interactions of these proteins with DNA modified by cisplatin [106].

The results indicate that structurally different DNA adducts of Triplatin and cisplatin exhibit a different efficiency to affect the binding affinity of the modified DNA to p53 protein. It has been suggested that different structural perturbations induced in DNA by the adducts of Triplatin and cisplatin produce differential response to p53 protein activation and recognition. Triplatin retains significant activity in human tumor cell lines and xenografts refractory or poorly responsive to cisplatin and displays high activity in human tumor cell lines characterized by both wild-type and mutant p53 gene. In contrast, on average, cells with mutant p53 are more resistant to the effect of cisplatin. The results support the hypothesis that the mechanism of antitumor activity of Triplatin may also be associated with its efficiency to affect the binding affinity of platinated DNA to active p53 protein. Thus, a “molecular approach” to control downstream effects such as protein recognition and pathways of apoptosis induction may consist in design of structurally unique DNA adducts as cell signals.

7.4.3.Recognition by the Nuclear Transcription Factor κB

Multiple signaling pathways have been linked to tumor resistance to mononuclear cisplatin, among them also activation of nuclear transcription factor kappaB (NF-κB) [107]. Interestingly, suppression of apoptosis or necrosis is an important NF-κB function [108, 109]. Binding of NF-κB proteins to their consensus sequences in DNA (κB sites) is the key biochemical activity responsible for the biological functions of NF-κB [110, 111].

Structurally different DNA adducts of Triplatin, cisplatin, and transplatin exhibit a different efficiency to affect the affinity of the platinated DNA (κB sites) to NF-κB proteins [112]. Triplatin-DNA adducts exhibited the highest efficiency to inhibit binding of NF-κB protein to its κB very likely connected with the enhanced extent of the conformational perturbations induced in DNA.

7.4.4.DNA Structural Conformational Changes, Protein Recognition, and Cell Cycle Effects

A major question for all this work is to ask how are the structural modifications and modulations of DNA adduct-protein recognition reflected in changes in cell cycle and signaling pathways. Real changes could affect signaling pathways and thus be truly complementary to other clinically used anticancer drugs, beyond the cisplatin class. An interesting example comes from the PPC work (Figure 6).

Mismatch repair is an important determinant in the efficacy of cisplatin treatment [113]. HCT116

cells deficient in the mismatch repair protein, MLH1, which was shown earlier to be resistant to cisplatin, were not resistant to Triplatin, indicating that Triplatin overrides one of the main factors contributing toward cisplatin resistance, in this case the mismatch repair status of the cells [114]. In both melphalan-sensitive and resistant OAW42 ovarian cancer cells, Triplatin induced a persistent G2/M phase cell cycle arrest as compared to cisplatin, which caused an initial S phase accumulation followed by a G2/M arrest that was later resolved [115].

In another study, A431 cells (human cervix squamous carcinoma) and its cisplatin-resistant counterpart, A431/Pt on treatment with Triplatin showed a varying degree of cellular effects which mainly included upregulation of genes like E2F1 (A431/Pt), antimetastatic factors (Nm23-H2 in A431 cells, Nm23-H1 and SAP102 in A431/Pt cells, and CD9 in both A431 and A431/Pt cells) and downregulation of pro-metastatic factors (e.g., Axl and VEGF in A431 cells and IL0–1b in A431/Pt cells) [116]. Further, Triplatin treatment showed a G2/M phase arrest in both A431 and A431/Pt cells although it was a much stronger effect in the former. Cytoflow analysis indicated a high proportion of sub-G1 cells in Triplatin-treated A431/Pt cells. In this context, a comparative study of U2OS cells showed a persistent increase in S phase cells following cisplatin treatment whereas Triplatin treatment showed a persistent accumulation of cells in the G2/M phase with some cells still retained in the G1 phase [117].

BBR3610 exhibits low-dose toxicity in colon cancer cells that harbored either wild-type p53 (HCT116) or mutant p53 (DLD1) suggesting that the cellular effects of BBR3610 were p53-independent [32]. BBR3610 induces G2/M arrest, early autophagy, and late apoptosis in glioma cells [118]. One of the hallmarks of cancer development is a deregulated cell cycle progression. Cells exposed to DNA-damaging agents trigger various cell cycle checkpoints, subsequently leading to G1/S or G2/M cell cycle arrest. Similar to cisplatin, BBR3464 and BBR3610 block DNA synthesis, cause S phase accumulation, and eventually this leads to G2/M arrest. However, BBR3610-DACH showed a paradigm shift, causing both a G1/S and G2/M arrest with complete depletion of S phase [119]. Both BBR3610 and BBR3610-DACH formed approximately the same number of interstrand CLs but their downstream cell cycle effects are different. This aspect of Pt-DNA conformational changes, by structurally distinct complexes, in general, requires further exploration. Figure 6. Differential cell-cycle effects caused by Triplatin, BBr3610, and BBR3610-DACH showing different downstream consequences of {Pt,Pt} interstrand crosslinking. 8.CONCLUSIONS AND OUTLOOK

The major structural alterations in DNA such as irreversible conformational changes, stabilization of G-quadruplexes, high efficiency of DNA-protein adduct formation, the observation of directional isomers and the ‘walking’ of adducts on DNA are all effected by the design and structure of polynuclear platinum complexes. Many of these features are not accessible to mononuclear complexes.

The high charge of many of the PPCs suggests that pre-association plays important roles in dictating many of these properties. Substitution-inert or “non-covalent” complexes, which have also a wide variety of effects on DNA, are beyond the scope of this chapter but have been reviewed recently [89, 120]. Since the early publications on the straight chain alkanediamine-linked dinuclear complexes, various researchers have adapted the basic structure to obtain a wide variety of complexes and DNA binding modes and, eventually, antitumor activity equivalent to the original series. Triplatin remains the only “non-classical” platinum complex to enter human clinical trials. This chapter shows the rich diversity of PPCs and the potential for further clinical development, based on the strategy to produce DNA adducts structurally dissimilar to those of cisplatin and oxaliplatin. ACKNOWLEDGMENTS

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ABBREVIATIONS AND DEFINITIONS

BOC t-BuOCO
bpy 2,2′-bipyridine
Btaz benzotriazolate
cisplatin cis-PtCl2(NH3)2, cis-DDP
CLs crosslinks
DACH 1,2-diaminocyclohexane
dien diethylenetriamine = bis-(2-aminoethyl)amine
dpa bis-(2-pyridylmethyl)amine
DTBPA 2,2′-(4,11-dimethyl-1,4,8,11-tetraaza-cyclotetradecane-1,8-diyl)bis-(N-(2-(pyridin-2-yl)ethyl)acetamide
dzpm 4,4′-dipyrazolylmethane
GS glutathione
HMG high-mobility-group
hptab N,N,N′,N′,N$,N$-hexakis(2-pyridylmethyl)-1,3,5-tris(aminomethyl)benzene)
HSQC heteronuclear single quantum coherence
IC50 half maximal inhibitory concentration
L 2,2′-azanediyldibenzoic dianion or 1,3-benzothiazol-2-amine
NER nucleotide excision repair
phen 1,10-phenanthroline
PPCs polynuclear platinum complexes
prz pyrazine
ptp 6′-(pyridin-3-yl)-3,2#:4′,3$-terpyridine
pydz pyridazine
tppz tetrakis(pyridine-2-yl)pyrazine
TPXA N,N,N′,N′-tetra(2-pyridylmethyl)-m-xylylene diamine
Triplatin BBR3464, [{trans-PtCl(NH3)2}2μ-{trans- Pt(NH3)2(H2N(CH2)6NH2)2}]4+

REFERENCES

1.N. Farrell, Metal Ions in Biological Systems, Eds. A. Sigel, H. Sigel, Marcel Dekker, Inc., New York, Basel, 2004, Vol. 42, pp 251–296.

2.V. Brabec, Prog. Nuc. Acid Res. Mol. Biol. 2002, 71, 1–68.

3.V. Brabec, J. Kasparkova, Drug Resistance Updates 2005, 8, 131–146.

4.N. Farrell, Y. Qu, U. Bierbach, M. Valsecchi, E. Menta, in Cisplatin. Chemistry and Biochemistry of a Leading Anticancer Drug, Ed. B. Lippert, Wiley-VCH, Zurich, Weinheim, 1999, pp 479–496.

5.N. P. Farrell, in Platinum-based Drugs in Cancer Therapy, Eds. L. R. Kelland, N. P. Farrell, Humana Press Inc., Totowa/NJ, 2000, pp 321–338.

6.N. Farrell, Drugs of The Future 2012, 37, 795–806.

7.J. Kasparkova, O. Novakova, O. Vrana, N. Farrell, V. Brabec, Biochemistry 1999, 38, 10997–11005.

8.N. Farrell, T. G. Appleton, Y. Qu, J. D. Roberts, A. P. S. Fontes, K. A Skov, P. Wu, Y. Zou, Biochemistry 1995, 34, 15480–15486.

9.R. Zaludova, A. Zakovska, J. Kasparkova, Z. Balcarova, V. Kleinwächter, O. Vrana, N. Farrell, V.

Brabec, Eur. J. Biochem. 1997, 246, 508–517.

10.S. J. Berners-Price, M. S. Davies, J. W. Cox, D. S. Thomas, N. Farrell, Chem. Eur. J. 2003, 9, 713–725.

11.J. Kasparkova, N. Farrell, V. Brabec, J. Biol. Chem. 2000, 275, 15789–15798.

12.E. Selimović, J. Bogojeski, Int. J. Chem. Kinetics 2015, 47, 327–333.

13.J. W. Cox, S. Berners-Price, M. S. Davies, Y. Qu, N. Farrell, J. Am. Chem. Soc. 2001, 123, 1316–1326.

14.M. S. Davies, S. J. Berners-Price, J. W. Cox, N. P. Farrell, Chem. Commun. 2003, 122–123.

15.J. Zhang, D. Thomas, M. Davies, S. Berners-Price, N. Farrell, J. Biol. Inorg. Chem. 2005, 10, 1–15.

16.J. Kasparkova, K. J. Mellish, Y. Qu, V. Brabec, N. Farrell, Biochemistry 1996, 35, 16705–16713.

17.C. Hofr, N. Farrell, V. Brabec, Nuc. Acids Res. 2001, 29, 2034–2040.

18.K. J. Mellish, Y. Qu, N. Scarsdale, N. Farrell, Nuc. Acids Res. 1997, 25, 1265–1271.

19.A. Johnson, Y. Qu, B. Vanhouten, N. Farrell, Nuc. Acids Res. 1992, 20, 1697–1703.

20.T. D. McGregor, W. Bousfield, Y. Qu, N. Farrell, J. Inorg. Biochem. 2002, 91, 212–219.

21.J. B. Mangrum, N. P. Farrell, Chem. Commun. 2010, 46, 6640–6650.

22.Y. Qu, N. J. Scarsdale, M. C. Tran, N. Farrell, J. Inorg. Biochem. 2004, 98, 1585–1590.

23.M. Kartalou, J. M. Essigmann, Mutation Res. 2001, 478, 1–21.

24.T. C. Johnstone, K. Suntharalingam, S. J. Lippard, Chem. Rev. 2016, 116, 3436–3486.

25.Y. Jung, S. J. Lippard, Chem. Rev. 2007, 107, 1387–1407.

26.Y. Zou, B. Van Houten, N. Farrell, Biochemistry 1994, 33, 5404–5410.

27.N. Poklar, D. S. Pilch, S. J. Lippard, E. A. Redding, S. U. Dunham, K. J. Breslauer, Proc. Natl. Acad. Sci. USA 1996, 93, 7606–7611.

28.M. Missura, T. Buterin, R. Hindges, U. Hubscher, J. Kasparkova, V. Brabec, H. Naegeli, EMBO J. 2001, 20, 3554–3564.

29.V. Brabec, in Platinum-based Drugs in Cancer Therapy, Eds. L. R. Kelland, N. P. Farrell, Humana Press Inc., Totowa/NJ, 2000; pp 37–61.

30.H. Rauter, R. Di Domenico, E. Menta, A. Oliva, Y. Qu, N. Farrell, Inorg. Chem. 1997, 36, 3919–3927.

31.C. Billecke, S. Finniss, L. Tahash, C. Miller, T. Mikkelsen, N. P. Farrell, O. Boegler,

Neuro-Oncology 2006, 8, 215–226.

32.C. Mitchell, P. Kabolizadeh, J. Ryan, J. D. Roberts, A. Yacoub, D. T. Curiel, P. B. Fisher, M. P. Hagan, N. P. Farrell, S. Grant, P. Dent, Mol. Pharmacol. 2007, 72, 704–714.

33.T. D. McGregor, A. Hegmans, J. Kasparkova, K. Neplechova, O. Novakova, H. Penazova, O. Vrana, V. Brabec, N. Farrell, J. Biol. Inorg. Chem. 2002, 7, 397–404.

34.L. Zerzankova, T. Suchankova, O. Vrana, N. P. Farrell, V. Brabec, J. Kasparkova, Biochem. Pharmacol. 2010, 79, 112–121.

35.A. Hegmans, J. Kasparkova, O. Vrana, V. Brabec, N. P. Farrell, J. Med. Chem. 2008, 51, 2254–2260.

36.J. Florian, J. Kasparkova, N. Farrell, V. Brabec, J. Biol. Inorg. Chem. 2012, 17, 187–196.

37.B. Moriarity, O. Novakova, N. Farrell, V. Brabec, J. Kasparkova, Arch. Biochem.Biophys. 2007, 459, 264–272.

38.T. Muchova, S. Quintal, N. Farrell, V. Brabec, J. Kasparkova, J. Biol. Inorg. Chem. 2012, 17, 239–245.

39.Y. Zhao, W. He, P. Shi, J. Zhu, L. Qiu, L. Linb, Z. Guo, Dalton Trans. 2006, 2617–2619.

40.Y. Zhu, Y. Wang, G. Chen, Nucl. Acids. Res. 2009, 37, 5930–5942.

41.R. Olivova, J. Kasparkova, O. Vrana, M. Vojtiskova, T. Suchankova, O. Novakova, W. He, Z. Guo, V. Brabec, Mol. Pharmaceutics 2011, 8, 2368–2378.

42.M. E. Oehlsen, Y. Qu, N. Farrell, Inorg. Chem. 2003, 42, 5498–5506.

43.N. Summa, J. Maigut, R. Puchta, R. van Eldik, Inorg. Chem. 2007, 46, 2094–2104.

44.M. E. Oehlsen, A. Hegmans, Y. Qu, N. Farrell, Inorg. Chem. 2005, 44, 3004–3006.

45.M. E. Oehlsen, A. Hegmans, Y. Qu, N. Farrell, J. Biol. Inorg. Chem. 2005, 10, 433–442.

46.J. W. Williams, Y. Qu, G. H. Bulluss, E. Alvorado, N. P. Farrell, Inorg. Chem. 2007, 46, 5820–5822.

47.D. Fan, X. Yang, X. Wang, S. Zhang, J. Mao, J. Ding, L. Lin, Z. Guo, J. Biol. Inorg. Chem. 2007, 12, 655–665.

48.L. Zerzankova, T. Suchankova, O. Vrana, N. P. Farrell, V. Brabec, J. Kasparkova, Biochem. Pharmacol. 2010, 79, 112–121.

49.L. Zerzankova, H. Kostrhunova, M. Vojtiskova, O. Novakova, T. Suchankova, M. Lin, Z. Guo, J. Kasparkova, V. Brabec, Biochem. Pharmacol. 2010, 80, 344–351.

50.J. Pracharova, O. Novakova, J. Kasparkova, D. Gibson, V. Brabec, Pure Appl. Chem. 2013, 85, 343–354.

51.Z. H. Xu, Y. M. Zhang, Z. Q. Xue, X. L. Yang, Z. Y. Wu, Z. J. Guo, Inorg. Chim. Acta 2009, 362,

2347–2352.

52.H. W. Yue, B. Yang, Y. Wang, G. J. Chen, Int. J. Mol. Sci. 2013, 14, 19556–19586.

53.J. Zhu, Y. Zhao, Y. Zhu, Z. Wu, M. Lin, W. He, Y. Wang, G. Chen, L. Dong, J. Zhang, Y. Lu, Z. Guo, Chem. Eur. J. 2009, 15, 5245–5253.

54.Y. Qu, H. Rauter, A. P. S. Fontes, R. Bandarage, L. R. Kelland, N. Farrell, J. Med. Chem. 2000, 43, 3189–3192.

55.M. Kloster, H. Kostrhunova, R. Zaludova, J. Malina, J. Kasparkova, V. Brabec, N. Farrell, Biochemistry 2004, 43, 7776–7786.

56.B. van Houten, S. Illenye, Y. Qu, N. Farrell, Biochemistry 1993, 32, 11794–11801.

57.V. Brabec, P. Christofis, M. Slamova, H. Kostrhunova, O. Novakova, Y. Najajreh, D. Gibson, J. Kasparkova, Biochem. Pharmacol. 2007, 73, 1887–1900.

58.S. Neidle, J. Med. Chem. 2016, 59, 5987–6011.

59.I. Ourliac-Garnier, M. A. Elizondo-Riojas, S. Redon, N. Farrell, S. Bombard, Biochemistry 2005, 44, 10620–10634.

60.K. Suntharalingam, A. J. P. White, R. Vilar, Inorg. Chem. 2010, 49, 8371–8380.

61.D. L. Ang, B. W. J. Harper, L. Cubo, O. Mendoza, R. Vilar, J. Aldrich-Wright, Chem. Eur. J. 2016, 22, 2317–2325.

62.P. von Grebe, K. Suntharalingam, R. Vilar, P. J. Sanz Miguel, S. Herres-Pawlis. B. Lippert, Chem. Eur. J. 2013, 19, 11429–11438.

63.C.-X. Xu, Y. Shen, Q. Hu, Y. X. Zheng, Q. Cao,P. Z. Qin, Y. Zhao, L. N. Ji, Z. W. Mao Chem. Asian J. 2014, 9, 2519–2526.

64.C. A. Rabikz, M. E. Dolan, Cancer Treatment Rev. 2007, 33, 9–23.

65.S. Komeda, Metallomics 2011, 3, 650–655.

66.S. Teletchéa, S. Komeda, J.-M. Teuben, M. A. Elizondo-Riojas, J. Reedijk, J. Kozelka, Chem. Eur. J. 2006, 12, 3741–3753.

67.K. Spiegel, A. Magistrato, P. Carloni, J. Reedijk, M. L. Klein, J. Phys. Chem. B 2007, 111, 11873–11876.

68.S. Komeda, Y.-L. Lin, M. Chikuma, ChemMedChem 2011, 6, 987 – 990.

69.J. Mlcouskova, J. Malina, V. Novohradsky, J. Kasparkova, S. Komeda, V. Brabec, Biochim. Biophys. Acta 2012, 1820, 1502–1511.

70.J. Mlcouskova, J. Kasparkova, T. Suchankova, S. Komeda, V. Brabec, J. Inorg. Biochem. 2012, 114, 15–23.

71.N. Kida, Y. Katsuda, Y. Yoshikawa, S. Komeda, T. Sato, Y. Saito, M. Chikuma, M. Suzuki, T.

Imanaka, K. Yoshikawa, J. Biol. Inorg. Chem 2010, 15, 701–707.

72.Y. Yoshikawa, S. Komeda, M. Uemura, T. Kanbe, M. Chikuma, K. Yoshikawa, T. Imanaka, Inorg. Chem. 2011, 50, 11729–11735.

73.R. Olivova, J. Stepankova, T. Muchova, V. Novohradsky, O. Novakova, O. Vrana, J. Kasparkova, V. Brabec, Inorg. Chim. Acta 2012, 393, 204–211.

74.S. Komeda, G. V. Kalayda, M. Lutz, A. L. Spek, Y. Yamanaka, T. Sato, M. Chikuma, J. Reedijk, J. Med. Chem. 2003, 46, 1210–1219.

75.G. V. Kalayda, S. Komeda, K. Ikeda, T. Sato, M. Chikuma, J. Reedijk, Eur. J. Inorg. Chem. 2003, 24, 4347–4355.

76.L. Senerovic, M. D. Zivkovic, A. Veselinovic, A. Pavic, M. I. Djuran, S. Rajkovic, J. Nikodinovic-Runic, J. Med. Chem. 2015, 58, 1442–1451.

77.B. A. J. Jansen, P. Wielaard, G. V. Kalayda, M. Ferrari, C. Molenaar, H. J. Tanke, J. Brouwer, J. Reedijk, J. Biol. Inorg. Chem 2004, 9, 403–413.

78.H. -L. Chan, D.-M. Ma, M. Yang, C.-M Che, J. Biol. Inorg. Chem. 2003, 8, 761–769.

79.E. J. Gao, M. C. Zhu, H. X. Yin, L. Liu, Q. Wu, Y. G. Sun, J. Inorg. Biochem. 2008, 102, 1958–1964.

80.E. Gao, L. Liu, M. Zhu, Y. Huang, F. Guan, X. Gao, M. Zhang, L. Wang, W. Zhang, Y. Sun, Inorg. Chem. 2011, 50, 4732–4741.

81.G. Cafeo, C. Lo Passo, L. Monsu Scolaro, I. Pernice, R. Romeo, Inorg. Chim. Acta 1998, 275–276, 141–149.

82.V. Marini, J. Kasparkova, O. Novakova, L. M. Scolaro, R. Romeo, V. Brabec, J. Biol. Inorg. Chem. 2002, 7, 725–734.

83.N. J. Wheate, B. J. Evison, A. J. Herlt, D. R. Phillips, J. G. Collins, Dalton Trans. 2003, 3486–3492.

84.N. J. Wheate, G. M. Abbott, R. J. Tate, C. J. Clements, R. Edrada-Ebel, B. F. Johnston, J. Inorg. Biochem. 2009, 103, 448–454.

85.B. W. Johnson, V. Murray, M. D. Temple, BMC Cancer 2016, 16, 333–349.

86.S. D. Brown, K. D. Trotter, O. B. Sutcliffe, J. A. Plumb, B. Waddell, N. E. B. Briggs, N. J. Wheate, Dalton Trans. 2012, 41, 11330–11339.

87.S. L. Woodhouse, L. M. Rendina, Chem. Commun. 2001, 2464–2465.

88.Y. Qu, T. G. Appleton, J. D. Hoeschele, N. P. Farrell, Inorg. Chem. 1993, 32, 2591–2593.

89.N. P. Farrell, Chem. Soc. Rev. 2015, 44, 8773–8785.

90.V. Brabec, J. Kasparkova, O. Vrana, O. Novakova, J. W. Cox, Y. Qu, N. Farrell, Biochemistry 1999, 38, 6781–6790.

91.J. Kasparkova, O. Vrana, N. Farrell, V. Brabec, J. Inorg. Biochem. 2004, 98, 1560–1569.

92.T. Banerjee, P. Dubey, R. Mukhopadhyay, Biochimie 2010, 92, 846–851.

93.J. Kasparkova, J. Zehnulova, N. Farrell, V. Brabec, J. Biol. Chem. 2002, 277, 48076–48086.

94.J. Zehnulova, J. Kasparkova, N. Farrell, V. Brabec, J. Biol. Chem. 2001, 276, 22191–22199.

95.M. S. Davies, D. S. Thomas, A. Hegmans, S. J. Berners-Price, N. Farrell, Inorg. Chem. 2002, 41, 1101–1109.

96.A. Hegmans, S. J. Berners-Price, M. S. Davies, D. Thomas, A. Humphreys, N. Farrell, J. Am. Chem. Soc. 2004, 126, 2166–2180.

97.R. Ruhayel, J. Moniodis, X. Yang, J. Kasparkova, V. Brabec, S. J. Berners-Price, N. Farrell, Chem. Eur. J. 2009, 15, 9365–9374.

98.Y. Qu, M.-C. Tran, N. P. Farrell, J. Biol Inorg. Chem. 2009, 14, 969–977.

99.D., Yang, S. van Boom, J. Reedijk, J. van Boom, N. Farrell, A. H.-J. Wang, Nature Struct. Biol., 1995, 2, 577–581.

100.J. Malina, N. P. Farrell, V. Brabec, Chem. Asian J. 2011, 6, 1566–1574.

101.J. Malina, J. Kasparkova, N. P. Farrell, V. Brabec, Nuc. Acids Res. 2011, 39, 720–728.

102.P. May, E. May, Oncogene 1999, 18, 7621–7636.

103.T. Tokino, S. Thiagalingam, W. S. Eldeiry, T. Waldman, K. W. Kinzler, B. Vogelstein, Hum. Mol. Genet. 1994, 3, 1537–1542.

104.A. K. Nagaich, V. B. Zhurkin, S. R. Durell, R. L. Jernigan, E. Appella, R. E. Harrington, Proc. Natl. Acad. Sci. USA 1999, 96, 1875–1880.

105.A. Lebrun, R. Lavery, H. Weinstein, Protein Eng. 2001, 14, 233–243.

106.J. Kasparkova, M. Fojta, N. Farrell, V. Brabec, Nucleic Acids Res. 2004, 32, 5546–5552.

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