PLATINUM COMPLEXES AND THEIR ANTI-TUMOUR ACTIVITY AGAINST CHRONIC LYMPHOCYTIC LEUKAEMIA CELLS

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ANTITUMORSKO DEJSTVO KOMPLEKSA PLATINE NA ĆELIJE HRONIČNE LIMFOCITNE LEUKEMIJE

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ABSTRACT

Since the discovery of the antitumor activity of cisplatin by Rosenberg and co-workers, the use of metal complexes in cancer treatment has caused a huge interest. Today, platinum-based drugs are part of standard chemotherapy in the management of a variety of cancers, germ cell tumours, sarcomas, and lymphomas. Unfortunately, toxicity and drug resistance are major obstacles to wider clinical application of these drugs. Their use is greatly limited by severe side effects such as nephrotoxicity, ototoxicity, and neurotoxicity. Although cisplatin is one of the most successful anticancer drugs to date, its biochemical mechanism of action is still unclear. Cisplatin is generally accepted as having the ability to interact with the purine bases on the DNA, causing DNA damage, interfering with DNA repair mechanisms, and subsequently inducing apoptosis in cancer cells.

Chronic lymphocytic leukaemia is a neoplastic B cell lymphoproliferative disease characterized by a highly variable clinical course. Clinical stage at the diagnosis and biological prognostic factors are the important predictors for survival. The Rai and Binet staging systems describe three major prognostic subgroups. Commonly used prognostic biomarkers in chronic lymphocytic leukaemia can be divided into genotypic, DNA-level changes and phenotypic, expression-level changes. For chronic lymphocytic leukaemia, substantial progress in therapy has not been made over the past 40 years. The main goal of future scientific research is to find new platinum complexes that have better efficacy in cancer treatment, the ability to be administered orally, without developing a cancer-drug resistance, and reduced toxic side effects.

Keywords: *Platinum-based anticancer drugs, Chronic lymphocytic leukaemia, Cisplatin mechanisms of action, Cancer treatment*

SAŽETAK

Otkad su Rosenberg i saradnici otkrili antitumorsko delovanje cisplatine, upotreba metalnih kompleksa u lečenju raka izazvala je veliki interes. Danas su lekovi bazirani na platini deo standardne hemoterapije u lečenju raznih karcinoma, tumora matičnih ćelija, sarkoma i limfoma. Nažalost, toksičnost i rezistencija glavne su prepreke za širu kliničku primenu tih lekova. Uprkos konzistentnoj brzini početnog odgovora, terapija cisplatinom često rezultira razvojem rezistencije, što dovodi do neuspeha u lečenju. Upotreba cisplatine znatno je ograničena jakim nuspojavama, kao što su nefrotoksičnost, ototoksičnosti i neurotoksičnost. Iako je cisplatina jedan od najuspešnijih lekova protiv raka do danas, njen biohemijski mehanizam delovanja još uvek je nejasan. Opšte je prihvaćeno da cisplatina ima sposobnost interakcije s purinskim bazama DNA, uzrokuje oštećenja DNA, utiče na mehanizme popravka DNA, a nakon toga indukuje apoptozu u ćelijama raka. Hronična limfocitna leukemija je neoplastična limfoproliferativna bolest B limfocita koja se karakteriše vrlo promenjivim kliničkim tokom. Klinički stadijum bolesti i biološki prognostički faktori su važni prediktori za preživljenje bolesnika. Rai i Binet klasifikacija bolesti opisuje tri glavne prognostičke podskupine. Često korišćeni prognostički biomarkeri u hroničnoj limfocitnoj leukemiji mogu se podeliti u genotipske i fenotipske promene. Za hroničnu limfocitnu leukemiju značajnog napretka u terapiji nije zabeleženo zadnjih 40 godina. Buduća naučna istraživanja imaju za cilj da pronađu nove komplekse platine efikasnije u lečenju malignih tumora, koji se mogu oralno primenjivati, ne razvijaju rezistenciju na lek i imaju manje toksične nuspojave.

Ključne reči: *Antitumorski lekovi, derivati platine, Hronična limfocitna leukemija, Cisplatina mehanizmi delovanja, Lecenje tumora*

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INTRODUCTION

Cancer is one of the most deadly diseases worldwide, and chemotherapy is a main strategy for the systemic treatment of cancer. Cancer patients are treated by repeated cycles of chemotherapy, but the clinical course in some patients is characterized by a series of relapses. Cisplatin was the first metal-based agent used in the clinics for the treatment of cancer. Platinum-based anticancer drugs are among the most potent chemotherapeutic agents for the treatment of various malignant tumours. At present, cisplatin, carboplatin, and oxaliplatin are the only metal-based anticancer agents in worldwide clinical use (1). Platinum complexes are clinically used as adjuvant therapy of cancers aiming to induce tumour cell death (2). Platinum-based drugs are used for the treatment of human and animal tumours and are currently indicated for ovarian, testicular, bladder, colorectal, non-small cell and small cell lung cancers, as well as melanomas, lymphomas, and myelomas (3). There are currently six platinum drugs with marketing approval in various regions throughout the world: cisplatin, carboplatin, oxaliplatin, nedaplatin, lobaplatin, and heptaplatin (3). Severe side effects, as well as drug resistance, have limited their clinical applications (4, 5).

The World Health Organization classification of hematopoietic malignancies describes chronic lymphocytic leukaemia/small lymphocytic lymphoma (CLL/SLL) as a neoplasm composed of monomorphic small round to slightly irregular B lymphocytes in peripheral blood, bone marrow, spleen and lymph nodes, admixed with prolymphocytes and paraimmunoblasts forming proliferation centres in tissue infiltrates (6). These two disorders are morphologically, phenotypically and genotypically indistinguishable, different only in the degree of peripheral blood lymphocytosis (7). The term SLL is used for non-leukemic cases with the tissue morphology and immunophenotype of CLL, and SLL requires lymphadenopathy, no cytopenias due to bone marrow infiltration by CLL/SLL and less than 5 x 10^9/L B lymphocytes in peripheral blood (6). Chronic lymphocytic leukaemia is the most common type of leukaemia in the Western world, accounting for approximately 30% of all leukaemias (8). Individuals over 65 years of age account for 40% of cases, but CLL is extremely rare below the age of 30 years. The overall incidence is approximately three per 100,000 per year. Studies on the racial and geographic distribution show that CLL is 20-30 times more common in white and black populations of Europe, Australasia and North America than in populations of India, China and Japan. The male/female ratio in all populations is approximately 1.5-2:1 (6).

In 70-80% cases, CLL is diagnosed as an incidental finding on a routine full blood count. A definitive diagnosis of CLL is based on the combination of a lymphocytosis and characteristic lymphocyte morphology and immunophenotype. The predominant cell is a small lymphocyte with a narrow border of scanty cytoplasm and a dense nucleus with partially aggregated chromatin and without recognizable nucleoli (perspectives). Gumprecht nuclear shadows, or smudge cells, found as cell debris, are characteristic findings in CLL (9). CLL/SLL has a distinctive immunophenotype. Using flow cytometry, the tumour cells express dim surface IgM/IgD, CD5, CD19, CD20, CD22, CD23, CD43,CD79a and CD11c (weak); however, CD10 is negative, and FMC-7 and CD79b are usually negative or weakly expressed in typical CLL (6). Unlike most other lymphoid malignancies, chromosomal translocations are rare in CLL/SLL but approximately 80% of the cases have cytogenetic abnormalities detected by FISH (10). Approximately 50% of CLL show del 13q14.3, approximately 20% trisomy 12, deletions in 10-20% of 11q22-23, and less commonly, deletions in 17p13 and 6q21 (6, 10). Somatic mutations of the Immunoglobulin Heavy Chain Variable (IGHV) genes are present in at least half of CLL patients, indicating that antigenic exposure may be relevant in the pathogenesis of CLL (11).

The clinical course of B-CLL shows a marked heterogeneity from an indolent type without need for treatment for a long period, to a rapidly progressing disease that requires immediate therapy. The decision to treat a patient is mainly based on clinical and laboratory features indicating active and advanced disease. The Rai and Binet clinical staging systems are used to define disease extent and prognosis, and this definition is based on the extent of lymphadenopathy, splenomegaly, and hepatomegaly, measured by palpation, and anaemia and thrombocytopenia measured by blood cell counts (12, 13). New biological prognostic factors have become important especially in the early stage (9). The expression of ZAP-70 and CD38 are both associated with an adverse prognosis. Deletions of 11q22-23, 17p13 and 6q21 are associated with worse outcome, and isolated del 13q14.3 is associated with a more favourable clinical course (6). Additional adverse predictive factors include a rapid lymphocyte doubling time in peripheral blood (<12 months) and serum markers of rapid cell turnover, including elevated thymidin kinase, sCD23 and β-2 microglobulin (9). Two subsets of CLL can be determined based on the presence or absence of somatic hypermutation by molecular sequencing methods (10). Patients with unmutated IGVH tend to have more advanced disease stages and a more aggressive clinical course and tend to acquire high-risk cytogenetic alterations associated with CD38 and ZAP-70 expression (10).

The standard first-line therapy includes a purine analogue-based combination (fludarabin, cladribine, and pentostatin) e.g., fludarabine, cyclophosphamide with or without rituximab or alkylating agent (chlorambucil in comorbid patients). The other protocol for CLL treatment includes steroids, vincristine, doxorubicine mitoxantrone and monoclonal antibodies (alemtuzumab) (14). Investigations on the intrinsic ability of B-CLL cells to avoid apoptosis have been mainly centred on the Bcl-2 gene product, which is the overexpressed prototype antidote to apoptosis. Targeting the apoptotic pathways may provide a new therapeutic approach. Identifying a new agent with novel

mechanism of action that complement cytotoxicity and fight the resistance will be necessary for the future therapy protocols.

Platinum - physical and chemical properties

Platinum is a transition metal located in the centre of the Periodic Table along with the other members of the group. These metals are less reactive than the typical metals (15). Because of the small energy differences between the valence shells, a number of oxidation states occur: 0, +2, +4, and +5. Cisplatin is a simple neutral inorganic compound having square planar geometry and containing a Pt (II) centre bonded to two non-labile ammine ligands and two labile chloride ligands (4).

When cisplatin enters the cell, it becomes aquated, meaning that the chloride ligands are exchanged for water. This is due to the lower concentration of NaCl in the cytoplasm compared to the blood plasma. The aquated molecule can cross-link DNA through a covalent coordinate bond to the nitrogen atom on guanine and also to a lesser extent, to adenine. This DNA damage prevents replication and transcription, which leads to apoptosis and cell death (16).

History of platinum-based therapy

Cisplatin was first synthesized in the late nineteenth century, after being described in 1845 by Michele Peyrone but its anticancer properties were accidentally discovered by Barnett Rosenberg in the 1960s while he was studying the effects of electric fields on the growth of *Escherichia coli* bacteria (3). Rosenberg and his group found that the electric current itself had no effect on cell division, but the current was causing a chemical reaction dissolving platinum from the electrodes into the medium. Cisplatin was approved for use as an anticancer drug in the 1970s. At that time, researchers focused their attention on identifying organic molecules as new anticancer agents, so cisplatin expanded the drug testing with metal-based molecules.

Carboplatin is the second-generation platinum anticancer drug. This drug was developed as a less-toxic derivative of cisplatin; it is equally effective and is used as the platinum drug of choice in the treatment of ovarian cancers (15). The main difference between carboplatin and cisplatin is the six-membered chelate ring that makes the drug less reactive and prone to hydrolase and thereby reduces some of the unwanted side reactions (15). Carboplatin was approved for clinical use in treating tumours in the 1980s.

Oxaliplatin is the third-generation platinum-based anticancer drug and it was approved in the 1990s for the treatment of colorectal carcinoma. Oxaliplatin has a more complex structure than previous platinum-based anticancer drugs and is also effective against some tumours that have become resistant to cisplatin and carboplatin (15).

Currently, there are many platinum-based drugs in various stages of clinical trials (3). Platinum (IV) complexes have greater inertness than the corresponding platinum (II) complexes. They have some advantages such as oral administration, reduced toxicity and a decrease in the amount of the complex that is lost or deactivated in the path to the target cell (17, 18).

Platinum-based drugs - mechanism of action

The target of platinum-based drugs is nuclear DNA. Once cisplatin has been intravenously administered to the patient, it rapidly diffuses into tissues and is highly bound to plasma proteins (19). The biochemical mechanism by which cisplatin crosses the cell membrane still remains unclear. Passive diffusion across the cellular membrane and active transport can both play a role in the cellular uptake of platinum-based antitumor drugs (4). Early studies noted that cellular uptake of cisplatin was linear, concentration dependent, and nonsaturable, and passively transported across the cellular membrane (20), whereas in higher concentration, endocytosis may contribute to cisplatin uptake (4, 20). In the cytoplasm, many cellular components that have soft nucleophilic sites, such as cytoskeletal microfilaments, thiol-containing peptides and proteins and RNA, may react with cisplatin (19). The most important non-DNA targets of cisplatin probably are the tripeptide glutathione and metallothioneins, and their binding has been associated with negative pharmacological properties including the development of resistance and toxicity (19). The first step in activation of cisplatin is aquation, where a chloride leaving group is replaced by water. Subsequent displacement of this water allows the platinum to coordinate to a nitrogen atom in DNA. The N7 atoms of guanine and adenine that are located in the major groove of the double helix are the most accessible and reactive nucleophilic sites for platinum coordination to DNA (19). Each platinum drug will bind two bases either through nucleosides on the same strand (intra-strand binding) or through individual bases on different strands (inter-strand binding) (19, 21). Intrastrand binding causes the DNA helix to unwind and bend, preventing DNA transcription and replication. This DNA damage initiates apoptosis.

Recently, active/facilitated transport pathways, such as copper transporter (Ctr1), have been identified. A connection between copper and platinum trafficking is bidirectional cross-residence. Cells selected for resistance to high levels of copper were found to be resistant to platinum and vice versa. Human Ctr1 is a member of a highly conserved family of copper transporters that subdivide into three regions: 1) an extracellular N-terminal domain, 2) a membrane embedded domain composed of three transmembrane helices and 3) an intracellular C-terminal domain (4). If the mechanism of cisplatin uptake involves endocytosis of the drug bound to Ctr1 and degradation of the

cisplatin-Ctr1 complex, then additional steps are needed to complete the process; that is, cisplatin needs to be released from the endocytic vesicles into the cytosol or delivered to the nucleus, mitochondria, and microsomal compartments which are known to accumulate platinum after drug exposure. Endosome and lysosome compartments of mammalian contain a Ctr2 for the release of intracellular copper stores. Ctr2 has a large effect on the accumulation of cisplatin and carboplatin (4, 22).

Platinum complexes, protein interaction and toxicity

Cisplatin and related compounds are known to bind to several classes of proteins, affecting aspects as diverse as structural, antioxidant, electron transfer, small molecule or iron transport, or DNA processing (23). These interactions should be considered among the mechanism whereby platinum-containing drugs induce toxic side effects such as nausea, vomiting, fatigue, alopecia, haematological suppression, peripheral sensory neuropathy, renal damage, and others.

More than 95% of the cisplatin that enters a cell is estimated to bind to proteins and peptides, rather than to DNA (23). Platinum-based anticancer drugs can bind to a range of proteins, especially at sulphur atoms, affecting their conformation and functions. One possible mechanism of resistance and toxicity induced by these drugs may be explained according to this interaction (23). Cytotoxicity in cancer cells should always be viewed in relation to general toxicity and not be mistaken for anticancer activity.

Clinical resistance to platinum-based therapy

The major problem with platinum-based anticancer therapy is that cancer cell exposure to the drugs causes resistance, and tumours stop responding. Increasing the dose will increase the toxicity but not gain any antitumour effect (16). The resistance mechanism is multifactorial, and several causes have been proposed. From the molecular point of view, the resistance can be caused mainly in three ways (16). First, the cell can block the influx of the drug. Second, if the drug does enter, the cells can efflux it back outside or block it from reaching the target. Third, if the drug reaches the DNA, it can be removed by a repair system (2).

The pathway controlling the copper homeostasis in the cell becomes interesting when the cisplatin-resistant cancer cells show an over-expression of the copper transporting protein (Ctr1). Ctr1 is the main copper uptake transporter in human cells. It is positioned in the cell membrane where a central pore that functions as a canal has been formed. Studies of crystallized Ctr1 with electron microscopy showed a series of rings of methionines, histidines, and cysteines lingering on the inside of the pore to facilitate copper transport (16). Later studies have discovered a second potential copper transporter, Ctr2 (24). Ctr2 is not located in the plasma membrane like Ctr1 but is located mostly in the membranes of the intracellular compartments (16). Atox 1 is a copper chaperone that transports copper from Ctr1 to ATP7A/B (16). ATP7A and ATP7B are two homologous copper ATPases in human cells located at the trans-Golgi network. They use the energy of the ATP hydrolysis to transport Cu from the cytosol across the cellular membranes. More than likely, this is an indirect process. Several studies have shown the importance of the Ctr1 in cisplatin drug uptake, regulation of the cellular accumulation, and cytotoxicity. The overexpression of Ctr1 is found to sensitize cells to the toxic effects of platinum agents (16). After Ctr1 and ATP7A/B were found to mediate cisplatin resistance, Atox 1 has gained interest in case of platinum anticancer drugs resistance. The cells resistant to cisplatin had elevated levels of ATP7B, which suggest that copper ATPases are involved in resistance to platinum anticancer drugs.

Cisplatin and carboplatin generate mutual cross-resistant cells, but oxaliplatin-resistant cells are often not cross-resistant to cisplatin, pointing to a different mechanism of action. Cisplatin resistance occurs intrinsically (e.g., colon carcinomas) or is acquired (e.g., ovarian carcinomas), and some cancers show no tendency to acquire resistance at all (e.g., testicular cancer) (25). Platinum drugs have been found to overcome resistance in cell lines but have failed in clinics (26).

Cisplatin induced cell death pathways

DNA damage and subsequent induction of apoptosis may be the primary cytotoxic mechanism of cisplatin (27). Apoptosis is a built-in cell suicide program that serves to eliminate cells in the organism that are no longer needed or have sustained severe damage (28). Apoptosis is mediated by caspases, a specialized family of aspartate-specific cysteine proteases. The caspase death effector machinery is negatively controlled by members of the Bcl-2 family. Several independent functions of Bcl-2 have been demonstrated, including the abrogation of the mitochondrial release of caspase-activating factors (such as cytochrome c), the modulation of antioxidant pathways and the regulation of calcium homeostasis (29). There are two separate mechanisms that occur in apoptosis: the extrinsic pathway, activated by pro-apoptotic receptor signals at the cellular surface, and the intrinsic pathway, activated by mitochondrial signals (28). Conventional anticancer therapy seems to stimulate apoptosis primarily via the intrinsic pathway. The Bcl-2 proteins play important roles in the intrinsic pathway, and their elimination resulted in the prolonged survival and rapid loss of leukaemia cells (29).

A critical checkpoint for the activation of the intrinsic pathway is the tumour-suppressor p53 protein, a tran-

scription factor that is considered a 'guardian of the genome'. P53 has multiple functions, including cell-cycle control in response to DNA damage, induction of apoptosis, and DNA repair (30). Recent evidence suggests that genes that regulate apoptotic cell death may play a role in determining the sensitivity of tumour cells to chemotherapy (30). Inactivation of p53 is among the most common mutations, occurring in over half of cancers, and provides a key resistance mechanism that helps cancer to avoid apoptosis (28). Several studies have shown that sensitivity to cisplatin usually correlates with the presence of wild type p53, whereas the lack of functional p53 is related to cisplatin resistance (19).

There are four basic DNA repair pathways: nucleotide excision repair, base excision repair, mismatches repair and double-strand break repairs (31). Each pathway has its own set of proteins that function entirely independently of the other pathways. ERCC1 is an excision nuclease within the nucleotide excision repair pathway. ERCC1 is essential to life, and well preserved through nature (31). Cancer cell that express higher levels of ERCC1, also show higher resistance to platinum drug exposure (31), whereas higher levels of ERCC1 in CLL cells are accompanied with resistance to alkylating agents (33).

CONCLUSION

In spite of the widespread success of cisplatin, the search continues for new platinum drugs. This has been motivated by the desire to improve upon the clinical performance of cisplatin. The side effects associated with cisplatin treatment can be severe and may include toxicity to the kidney and nervous system. The goal is to obtain a compound with better activity and less toxicity than cisplatin (32). Rosenberg`s original experiment identified both platinum (II) and platinum (IV) species as possessing anticancer activity. In spite of this fact, the vast majority of research since then has focused on platinum (II) compounds. Recognition of the enormous potential that platinum (IV) compounds possess as anticancer agents in term of high activity, low toxicity, and perhaps the ability to be effective oral agents, has revived research in this area (32). An interesting therapeutic approach is the combination of cisplatin with two or more non-platinum antitumor drugs. An alternative approach in the treatment of fludarabine-resistant CLL is the inclusion of platinum-based therapy in protocols (34). Cisplatin and oxaliplatin have a synergistic cytotoxic effect with fludarabine on CLL cells (35, 36). However, platinum-based therapy increases the risk of haematological toxicity in patients with hematopoiesis that is already compromised.

Further studies are needed for the development of new generations of more efficient platinum-based anticancer drugs that will show better clinical activity and lower toxicity than the currently used platinum-based agents, even in multidrug-resistant cancers such as CLL.

REFERENCES

1. Jakupec MA, Galanski M, Arion VB, Hartinger CG & Keppler BK. (2008). Antitumor metal compounds: more than theme and variations. Dalton Trans. 14;(2), 183-194.
2. Florea A & Büsselberg D. (2001). Cisplatin as an Anti-Tumor Drug: Cellular Mechanisms of Activity, Drug Resistance and Induced Side Effects. Cancers. 15;3(1), 1351-1371.
3. Oun R. & Wheate NJ. (2013). Platinum Anticancer Drugs. In: Kretsinger RH, Uversky VN & Permiyakov EA. Encyclopedia of Metalloproteins (pp.1710-1714). New York, Heidelberg, Dordrecht, London:Springer.
4. Arnesano F. (2013). Platinum Complexes and Methionine Motif in Copper Transport Proteins Interaction In: Kretsinger RH, Uversky VN & Permiyakov EA. Encyclopedia of Metalloproteins (pp.1714-1723). New York, Heidelberg, Dordrecht, London:Springer.
5. Kostova I. (2006). Platinum Complexes as Anticancer Agents. Recent Pat Anticancer Drug Discov. 1(1), 1-22.
6. Swerdlow SH, Campo E, Haris NL, Jaffe ES, Poleri SA, Stein H, Thiele J & Vardiman JW. (2008). WHO Classification of Tumours of Haematopoetic and Lymphoid Tissues. (4th ed.) (pp 180-184). Lyon: International Agency for Research on Cancer.
7. Aster JC. (1999). Diseases of white blood cells, lymph nodes, spleen, and thymus In: Kumar V, Abbas AK & Fausto N. Robbins and Cotran, Pathologic basis of disease (7th ed.) (pp 661-709). Philadelphia: Elsevier Saunders.
8. Nahi H, Lehman S, Mollgard L, Bengtzen S, Selivanova G, Wiman KG, Paul C & Merup M. (2004). Effects oh PRIMA-1 on chronic lymphocytis leukaemia cells with and without hemizygous p53 deletion. Br J Haematol. 127(3), 285-291.
9. Binet JL, Caligaris-Cappio F, Catovsky D, Cheson Bruce, Davis T, Dighiero G, Döhner H, Hallek M, Hillmen P, Keating M, Montserrat E, Kipps TJ & Rai K. (2006). Perspectives on the use of new diagnostic tools in the treatment of chronic lymphocytic leukemia. Blood. 107(3), 859-861.
10. Chastain EC & Duncavage EJ. (2015). Clinical Prognostic Biomarkers in Chronic Lymphocytic Leukemia and Diffuse Large B-Cell Lymphoma. Arch Pathol Lab Med. 139(5), 602-607.
11. Caligaris-Cappio F. (2009). Chronic lymphocytic leukemia: "Cinderella" is becoming a star. Mol Med. 15(3-4), 67-69.
12. Rai KR, Sawitsky A, Cronkite EP, Chanana AD, Levy RN & Pasternack BS. (1975). Clinical staging of chronic lymphocytic leukemia. Blood. 46, 219-234.
13. Binet JL, Auquier A, Dighiero G, Chastang C, Piguet H, Goasguen J, Vaugier G, Potron G, Colona P, Oberling F, Thomas M, Tchernia G, Jacquillat C, Boivin P, Lesty C, Duault MT, Monconduit M, Belabbes S & Gremy F. (1981). A new prognostic classification of chronic lym-

phocytic leukemia derived from a multivariate survival analysis. Cancer. 48: 198-206.
14. Vujić JM, Kaluđerović GN, Zmejkoviski BB, Milovanović M, Volarević V, Arsenijević N, Stanojković TP & Trifunović S. (2012). Stereospecific ligands and their complexes: Part X: Syntesis, characterization and in vitro antitumoral activity of platinum (IV) complexes with O,O´-dialkyl-(S,S)-ethylenediamine-N,N´-di-2-(4-methyl) pentanoate ligands. Inorganica Chimica Acta. 390, 123-128.
15. Habashi F. (2013) Platinum, Physical and Chemical Properties In: Kretsinger RH, Uversky VN & Permiyakov EA. Encyclopedia of Metalloproteins (pp.1741-1742). New York, Heidelberg, Dordrecht, London:Springer.
16. Palm-Espling ME & Wittung-Stafshede P. (2013) Platinum Interaction with Copper Proteins. In: Kretsinger RH, Uversky VN & Permiyakov EA. Encyclopedia of Metalloproteins (pp.1723-1729). New York, Heidelberg, Dordrecht, London:Springer.
17. Arsenijević M, Milovanović M, Volarević V, Čanović D, Arsenijević N, Soldatović T, Jovanović S & Bugarčić ŽD. (2012). Cytotoxic properties of platinum(IV) and dinuclear platinum (II) complexes and their ligand substitution reactions with guanosine-5`-monophosphate. Transition Metal Chemistry. 37: 481-488.
18. Vujić JM, Kaluđerović GN, Milovanović M, Zmejkoviski BB, Volarević V, Živić D, Đurđević P, Arsenijević N & Trifunović S. (2011). Stereospecific ligands and their complexes: Part VII Syntesis, characterization and in vitro antitumoral activity of platinum(II) complexes with O,O´-dialkyl esters of (S,S)-ethylenediamine-N,N´-di-2-(4-methyl) pentanoic acid. Eur J Med Chem. 46(9), 4559-4565.
19. Cepeda V, Fuertes MA, Castilla J, Alonso C, Quevedo C & Peres JM. (2007). Biochemical Mechanisms of Cisplatin Cytotoxicity. Anticancer Agents Med Chem. 7(1), 3-18.
20. Gately DP & Howel SB. (1993). Cellular accumulation of the anticancer agent cisplatin: A review. Br J Cancer. 67(6), 1171-1176.
21. Bordin DL, Lima M, Lenz G, Saffi J, Meira LB, Paul Me´sange P, Soares DH, Larsen AK, Escargueil AE & Henriques JAP. (2013). DNA alkylation damage and autophagy induction. Mutat Res. 753(2), 91-99.
22. Blair BG, Larson CA & Howell SB. (2009). Copper-Transporter 2 Regulates the Cellular Accumulation and Cytotoxicity of Cisplatin and Carboplatin. Clin Cancer Res. 15(13), 4312-4321.
23. Silaghi-Dumitrescu R & Bischin C. (2013). Platinum-Containing Anticancer Drugs and Proteins, Interaction In: Kretsinger RH, Uversky VN & Permiyakov EA. Encyclopedia of Metalloproteins (pp.1742-1748). New York, Heidelberg, Dordrecht, London: Springer.
24. Kampfenkel K, Kushnir S, Babiychuk E, Inze` D & Van Montagu M. (1995). Molecular Characterization of a Putative Arabidopsis thaliana Copper Transporter and Its Yeast Homologue. J Biol Chem. 270(47), 28479-28486.
25. Eckstein N & Hass B. (2013). Platinum-Resistant Cancer In: Kretsinger RH, Uversky VN & Permiyakov EA. Encyclopedia of Metalloproteins (pp.1748-1751). New York, Heidelberg, Dordrecht, London: Springer.
26. Boulikas T, Pantos A, Bellis E & Christofis P. (2007). Designing platinum compounds in cancer: structures and mechanism. Cancer Therapy. 5, 537-583.
27. Gonzales VM, Fuertes MA, Alonso C & Perez JM. (2001). Is Cisplatin-Induced Cell Death Always Produced by Apoptosis? Mol Pharmacol. 59(4), 657-63.
28. Ashkenazi A & Herbst R.S. To kill a tumor cell: the potential of proapoptotic receptor agonists. The Journal of Clinical Investigation 2008; 118(8): 1979-1990.
29. Del Bello B, Valentini MA, Yunino F, Comporti M & Maellaro E. (2001). Cleavage of Bcl-2 and cisplatin-induced apoptosis of human melanoma cells. Onkogene. 20(33), 4591-4595.
30. Müller M, Wilder S, Bannasch D, Israeli D, Lehlbach K, Li-Weber M, Friedman SL, Galle PR, Stremmel W, Oren M & Krammer PH. (1998). p53 activates the CD95 (APO-1/Fas) gene in response to DNA damage by anticancer drugs. J Exp Med. 188(11), 2033–2045.
31. Reed E. ERCC1 and clinical resistance to platinum-based therapy. Clin Cancer Res.11(17), 6100-6102.
32. Alderden AA, Hall M D. & Hambley TW. (2006). The discovery and Development of Cisplatin. J Chem Educ. 83(5), 728-734.
33. Bou Samra E, Klein B, Commes T & Moreaux J. (2014). Identification of a 20-gene expression-based risk score as a predictor of clinical outcome in chronic lymphocytic leukemia patients. Biomed Res Int. 2014, 423174.
34. Tonino SH, van Gelder M, Eldering E, van Oers MH & Kater AP. R-DHAP is effective in fludarabine-refractory chronic lymphocytic leukemia. Leukemia. 24(3), 652-654.
35. Tonino SH, van Laar J, van Oers MH, Wang JY, Eldering E & Kater AP. (2011). ROS-mediated upregulation of Noxa overcomes chemoresistance in chronic lymphocytic leukemia. Oncogene. 30(6), 701-713.
36. Zecevic A, Sampath D, Ewald B, Chen R, Wierda W & Plunkett W. (2011). Killing of chronic lymphocytic leukemia by the combination of fludarabine and oxaliplatin is dependent on the activity of XPF endonuclease. Clin Cancer Res. 17(14), 4731-4741.