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Research Article

Novel Cyanoximate Pt $(DECO)_2$ as an Anti-Cancer Drug Using ML1 Thyroid Cancer Cells

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Abstract

Although current therapies for treating thyroid cancer offers good prognosis, there is an unmet need for better alternative anti-cancer drugs for treatment. Studies have shown that platinum based chemicals such as Cisplatin offer decent treatment options, but can also lead to severe side effects as well as develop a resistance. Given that cyanoximes are great ligands and that metals such as platinum offer good anti-proliferative and cytotoxic abilities, we investigated the role of a novel platinum-based cyanoximate in the treatment of thyroid cancer. Stage 4 thyroid cancer cell line, ML-1, was used in the present study to test the effects on cell viability and proliferation after treatment of Cisplatin and Pt$(DECO)_2$. Changes in the levels of superoxide were also tested and compared with non-treated cells. Lastly, presentation of cell death was recorded after the treatment. After 24 hours of treatment at concentrations of 1.0 mM of Pt$(DECO)_2$, we noticed drastic reduction in cell viability ($F = 45.77$, $p < 0.0001$), superoxide levels ($F = 28.57$, $p = 0.0001$), and an increase in cell death. These results suggest that this cyanoximate-induced cell death may work similarly to Cisplatin-based cell death. This *in vitro* study demonstrated the efficacy of the novel cyanoximate Pt$(DECO)_2$ in treating thyroid cancer. Further studies are needed to delineate the effectiveness of this treatment *in vivo* and with other cancer cell lines.

Keywords: Cisplatin; Platinum; Pt$(DECO)_2$; Thyroid Cancer; ML-1; Apoptosis; ROS; XTT

Introduction

Thyroid cancer currently presents as the most serious endocrine malignancy worldwide. In fact, incidence of thyroid cancer has been increasing in the past decade and estimated incidence of thyroid cancer in the United States for 2019 is 52,070 [1]. It is predicted that by the year 2030, thyroid cancer will be the fourth leading cause of new cancer diagnosis in the United States [2]. Most thyroid cancer cases respond well to surgery, radioactive iodine, and thyroid stimulating hormone (TSH) suppression; however, many of these treatments result in an overall poorer quality of life for the patient [3]. According to studies by Schlumberger., *et al*. up to 50% of thyroid cancer with metastasis may develop inability to concentrate iodine, and therefore, require more aggressive treatments and even the use of tyrosine kinase inhibitors [4,5]. Even with more aggressive treatments, some of these cancers still progress and become more difficult to treat. Current drug therapies point to combinations of different drugs to combat the cancer. Of these treatment types, platinum-based drugs including Cisplatin are commonly used [6]. Unfortunately for the patient, these therapies could result in multiple toxicities and side-effect profiles [7]. Therefore, the need for better anti-cancer drugs needs to be explored. A cyanoximate is a metal complex of cyanoximes, which are a subclass of oximes knowns to be outstanding ligands with unique properties. Of these properties, detoxification and antimicrobial

abilities have been studied utilizing silver and antimony, but little is known about their potential use as anti-cancer drugs [8]. In a study from Eddings., *et al*. the use of heavy metals such as platinum and palladium ions/nanoparticles combined with one of these cyanoximе ligands marked the start of their potential use in cancer therapy [8]. As such, we have explored the effects of a novel cyanoximate called Pt$(DECO)_2$ on the treatment of a stage 4 follicular thyroid carcinoma cell line, ML-1, *in vitro*. We hypothesized that this new drug will reduce the cell viability of the cancer cells as well as alter levels of oxidative stress and increase programmed cell death more effectively when compared to Cisplatin.

Materials and Methods

Cell line and cell culture conditions

ML-1 (ACC-464) thyroid cancer cells, purchased from the DSMZ (Braunschweig, Germany), are derived from a stage 4 dedifferentiated recurrent follicular thyroid cancer that had originated in a 50 year old patient. Cells were cultured in Gibco Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin and streptomycin antibiotics (Corning). Culture was maintained at 37°C and 5% CO_2. Cells were authenticated by Genetica Lab Corps (Burlington, NC) before submission.

Treatment

Non-commercial chemical agents used in the present study were synthesized from Dr. Nikolay Gerasimchuk in the Chemistry Department at Missouri State University (Springfield, MO). These chemicals, Bis(2-cyano-2-oxiamino-N,N'-diethylaminoacetamide) platinum (II), or Pt $(DECO)_2$, and Bis(2-cyano-2-oxiamino-N,N'-diethylaminoacetamide) palladium (II), or Pd $(DECO)_2$, were solubilized in 100% dimethyl sulfoxide (DMSO) immediately before treatment. As a positive control, the FDA-approved drug, Cis-diamminedichloroplatinum (II), or Cisplatin was used (St. Louis, MO). Due to the poor solubility of Pd $(DECO)_2$, only Cisplatin and Pt $(DECO)_2$ were utilized and treated at concentrations ranging from 0 to 1.0 mM in DMSO.

Cell viability assay

Metabolic activity of the thyroid cancer cells upon treatment of Pt $(DECO)_2$ and Cisplatin were assessed over 24 hours via an XTT assay (Biotium; Fremont, CA). A total of 10,000 cells/well were seeded in triplicate on a 96 well-plate and incubated for 24 hours prior to the treatment.

Activated XTT dye was then added to each well 24 hours after the treatment, and viability was assessed using an ELx808 Absorbance Plate reader (Biotek; Winooski, VT) set to A450-A630 for 7 hours per the manufacturer's guidelines.

Assessment of reactive oxygen species

In order to measure levels of superoxide production, dihydroethidium (DHE) was utilized in this experiment (Biotium; Fremont, CA). DHE was dissolved in DMSO, and a 10 mM stock was made. On the day of the experiment, a 10 µM working solution was made in PBS. A 24-well plate was seeded at a density of 50,000 cells/well and incubated for 24 hours. Wells were then treated with 1.0 mM Cisplatin or Pt $(DECO)_2$ in 2% DMSO. After harvesting cells in each well, pellets were resuspended in 1 mL of the 10 µM DHE working solution and incubated for 30 minutes in the dark. After incubation, cells were analyzed through an Attune NxT flow cytometer (ThermoFisher, US). Excitation and emission wavelengths of DHE were 518 nm and 606 nm, respectively.

Programmed cell death measurements

Programmed cell death, or apoptosis, was measured using an Attune NxT flow cytometer (ThermoFisher, US) and conducted using the manufacturer's protocol. Using a 24-well plate, 50,000 cells/well were seeded and incubated for 24 hours. After incubation, wells were treated with 1.0 mM concentrations of Cisplatin or Pt $(DECO)_2$ for 24 hours. After incubation, wells were harvested and resuspended with 1X Annexin V binding buffer containing Annexin V-APC and propidium iodide based off manufacturer's guidelines (BD Biosciences, US). Cells were incubated with these dyes for 25 minutes and analyzed with flow cytometry. Annexin V-APC has an excitation wavelength of 650 nm and PI has an excitation wavelength of 617 nm.

Statistical analysis

Prism8 statistical software (GraphPad Software Inc.) was used to conduct statistical analyses. One-Way ANOVA was performed assuming Gaussian distribution to compare between anticancer compound-treated groups and non-treated controls (NTC). Post hoc comparisons were done running Dunnett's test to compare values from treated groups to the NTC. All values are expressed as the mean and standard deviation of recorded values. P-values calculated from Dunnett's test were adjusted to account for multiple comparisons. Differences between groups were considered signifi-

cant at a p value of $p < 0.05$. Data was represented as Mean ± S.D. A single *indicates $p < 0.05$, **indicated $p < 0.01$, and ***shows that $p < 0.0001$.

Results

Reduction in cell viability

In these repeated experiments, ML-1 cancer cells were exposed to either Cisplatin or Pt $(DECO)_2$ at concentrations ranging from 0 to 1.0 mM (Figure 1). There was an overall statistically significant reduction in cell viability after 7 hours of incubation of activated XTT dye ($F = 45.77$, $p < 0.0001$). There was an apparent dose-dependent reduction in cell viability with increasing concentration of Cisplatin. Post hoc statistical comparisons using Dunnett's test showed significant reduction at 1.0 and 0.1 mM of Cisplatin treatment compared to the non-treated control or NTC (Dunnett's tests, $p < 0.0001$ and $p = 0.001$, receptively). Statistical comparisons of 1.0 mM of Pt $(DECO)_2$ with NTC showed a significant reduction in cell viability greater than what was seen by Cisplatin at the same concentration ($P < 0.0001$); however, at lesser concentrations of Pt $(DECO)_2$ no significant reduction of cell viability was observed (0.1 mM, $p = 0.9999$; 0.01 mM, $p = 0.9994$). Both chemicals were dissolved in 2% DMSO, which displayed no significant reduction in cell viability when compared with the non-treated control ($p = 0.4376$).

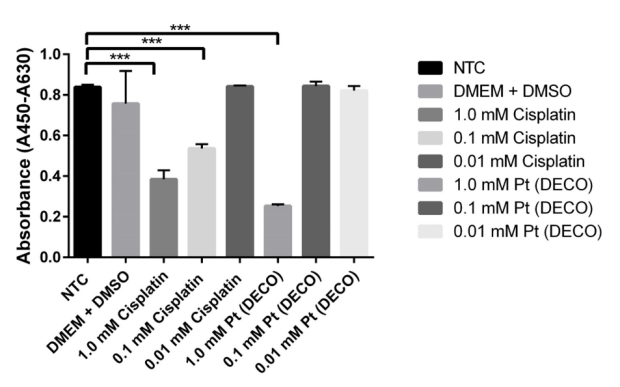

Figure 1: Measurement of cellular viability via reduction of XTT salt at hour 7 post treatment.

Data represents absorbance values (A450-A630) at differing concentrations of Cisplatin or Pt $(DECO)_2$ treatment. A "*" represents $p \leq 0.05$, "**" represents $p \leq 0.01$, and "***" represents $p \leq 0.0001$.

Platinum-based reduction of superoxide

Accumulation of superoxide levels were measured with flow cytometry (Figure 2). Percentages of superoxide detection utilizing the marker DHE are shown in figure 2E. At concentrations of 1.0 mM Cisplatin or Pt $(DECO)_2$, it was observed that levels of superoxide are significantly reduced compared to that of the control ($F = 28.57$, $p = 0.0001$). At 1.0 mM concentrations of Cisplatin, levels of superoxide were reduced by 51.24% when compared with NTC ($p = 0.0006$).

Wells treated with 1.0 mM Pt $(DECO)_2$ displayed a significant reduction at 46.89% ($p = 0.0003$).

Non-treated wells and wells treated with 2% DMSO displayed the highest percent averages of superoxide production (76.75% and 75.27%, respectively; Dunnett's test, $p = 0.9884$).

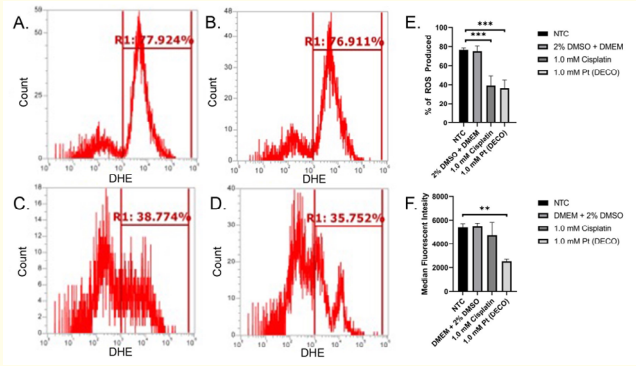

Figure 2: Flow cytometry data of quantification of superoxide production using dihydroethidium (DHE) at 518/606 nm. "A" displays the NTC, "B" shows DMEM + 2% DMSO, "C" 1.0 mM Cisplatin-treated cells, "D" shows cells treated with 1.0 mM Pt $(DECO)_2$, and "E" represents the means of a triplicate series of treatment. "F" shows the mean fluorescent intensity of each group. A "*" represents $p \leq 0.05$, "**" represents $p \leq 0.01$, and "***" represents $p \leq 0.0001$.

Increase of apoptosis

Changes in apoptotic levels due to treatment are seen in figure 3A-3H. After 24 hours of treatment, it is evident that both 1.0 mM of Cisplatin and Pt $(DECO)_2$ treatment increased the levels of programmed cell death (Figure 3C&D, compared to NTC). In regards

to efficacy in killing cells, neither treatment caused increase in necrosis (Figure 3E; F = 1.506, P = 0.2854). However, we observed a significant increase in late apoptosis in cells treated with 1.0 mM Cisplatin and Pt $(DECO)_2$ (F < 0.0001; p = 0.0025, p < 0.0001, respectively) (Figure 3F). Moreover, there was a fourfold increase of late apoptosis with Pt$(DECO)_2$ observed, suggesting more efficient and quicker induction of cell death (Figure 3F). There is a 250% increase in early apoptosis upon treatment with 1.0 mM Cisplatin compared to the control (Figure 3H; F = 16.45, P = 0.0019).

To assess specificity of Cisplatin and Pt $(DECO)_2$ on non-cancerous cells, mouse-derived fibroblasts were treated with 1.0 mM Cisplatin or Pt $(DECO)_2$ for 24 hours to assess changes, if any, to cell death. Figure 4A-4H shows lack of specificity to cell death. An observed increase in late apoptosis and early apoptosis is seen, with no increase in necrosis (Figure 4). The significant increase in late apoptosis due to Pt $(DECO)_2$ treatment suggests swifter cell death (Figure 4F; F = 378.9, P < 0.0001) to the cells. Additionally, viability was reduced significantly in both treatment groups (F = 130.5, P < 0.0001).

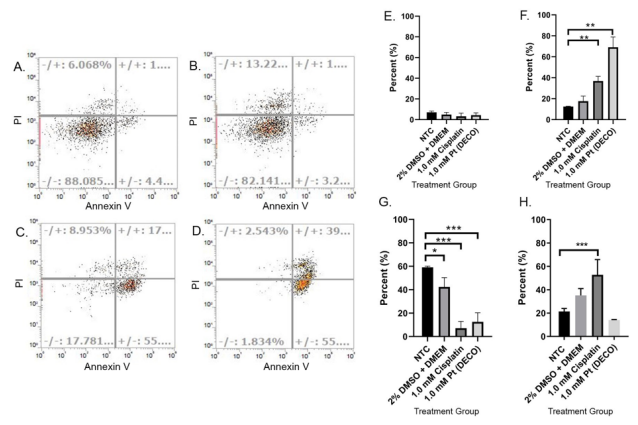

Figure 3: Flow cytometry data of cancer cells presenting at a particular stage of programmed cell death under annexin V-APC and propidium iodide after 24 hours of treatment. "A" displays the NTC, "B" shows DMEM + 2% DMSO, "C" 1.0 mM Cisplatin-treated cells, "D" shows cells treated with 1.0 mM Pt $(DECO)_2$, "E" displays the percent of necrotic cells present, "F" shows cells presenting signs of late apoptosis, "G" displays the percentage of viable cells, and "H" shows cells displaying early apoptosis. A "*" represents p ≤ 0.05, "**" represents p ≤ 0.01, and "***" represents p ≤ 0.0001.

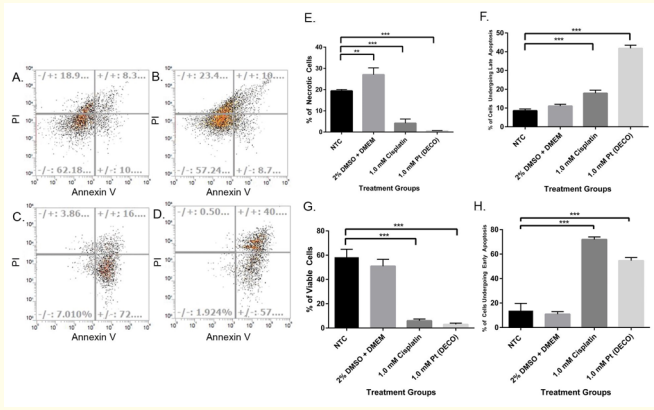

Figure 4: Flow cytometry data of fibroblast cells presenting at a particular stage of programmed cell death with annexin V-APC (650 nm) and Propidium Iodide (617 nm) after 24 hours of treatment. "A" displays the NTC, "B" shows DMEM + 2% DMSO, "C" 1.0 mM Cisplatin-treated cells, "D" shows cells treated with 1.0 mM Pt $(DECO)_2$, "E" displays the percent of necrotic cells present, "F" shows cells presenting signs of late apoptosis, "G" displays the percentage of viable cells, and "H" shows cells displaying early apoptosis. A "*" represents p ≤ 0.05, "**" represents p ≤ 0.01, and "***" represents p ≤ 0.0001.

Discussion

Platinum complexes have been widely used as anticancer agents since the accidental discovery of Cisplatin as a potent anticancer treatment in 1965 [9,10]. Not approved by the FDA until 1978, Cisplatin and its analogs remain the staple treatment for combating many cancers [8]. Cisplatin, Carboplatin, and Oxaliplatin share similar mechanisms of action to confer toxicities, and therefore, also share similar side effect profiles [11]. These side effects include weakening the immune system in patients, anemia, myelosuppression, and organ/tissue damage. Moreover, cancers can learn to build a resistance to Cisplatin treatment; therefore, there is an unmet need to develop newer platinum-based anticancer drugs [8,11]. The present study addressed the effectiveness of a novel platinum-based drug, Pt $(DECO)_2$, on its potential use as an anti-cancer drug, and its potential for reducing cell viability, altering oxidative stress levels, and inducing cell death. Up to this point, little research has been done to test the efficacy of metal cyanoximates and their

use in cancer therapy. In present, the papers from Ratcliff., *et al*. and Dannen., *et al*. studied the efficacy of several different metal cyanoximates, Pt $(MCO)_2$, Pd $(MCO)_2$, Pt $(DECO)_2$ and Cisplatin on reducing cell viability [12,13]. The Dannen paper tested this with a Trypan Blue assay, which determined that both Cisplatin and Pt $(DECO)_2$ were able to reduce cell viability *in vitro* [13]. In the study, viability of cervical cancer cells (HeLa) was reduced by nearly 60 % by Pt $(DECO)_2$ and reduced by 55% in the positive control of Cisplatintreated cells. Additionally, WiDr, a colon cancer cell line, was treated with 1.0 mM Cisplatin or Pt $(DECO)_2$, and an outstanding 95% of colon cancer cells were killed by Pt $(DECO)_2$ and only 12% by Cisplatin. In the Ratcliff and Dannen papers, trypan blue (TB) assay was employed to differentiate between live and dead cells as the dye only penetrates dead cells with damage to their plasma membrane [11-14]. The main disadvantages to this technique include subjectivity judgement of the user to differentiate between stained or unstained cells, inconsistency among different users, the amount of time and labor involved with measuring samples, and the error involved with only measuring single samples at a time [14]. Whereas with XTT, the tetrazolium salt is only getting reduced by metabolically active or viable cells and quantified automatically with a spectrophotometer. Even so, our study utilizing XTT showed similar results to the Dannen paper in that 1.0 mM Pt $(DECO)_2$ reduced viability more effectively (by nearly 59% in thyroid cancer cells), than Cisplatin did (only 45% of cells were killed by 1.0 mM Cisplatin treatment). Based off the Eddings and Dannen papers, Pt$(DECO)_2$ ranks in third place when compared to other platinum or palladium-based cyanoximates when treating cervical or colon cancer cells *in vitro* (Table 1). However, in all three cell lines (HeLa, WiDr, and Thyroid), Pt$(DECO)_2$ is shown to be more effective than Cisplatin at the same concentration. It is thought that the oxime ligand, DECO, alongside the platinum molecule has a more efficient structure to confer lethality than Cisplatin's square planer model, or that the combination of platinum with the oxime molecules in conjunction is more effective than platinum bound to chloride or ammonium as seen in Cisplatin [11,12].

We found that the platinum-based nanoparticles actually reduced the level of superoxide formed upon treatment, similarly to what was seen in the Yusof., *et al*. paper that described the use of platinum nanoparticles as an antioxidant in treating lung disease [15,16]. They assessed the use of platinum-based nanoparticles as an alternative drug carrier due to their ability to produce less of

Cyanoximate (at 1.0 mM)	Viability Reduced by (%):		Rank	
	HeLa	WiDr	HeLa	WiDr
Cisplatin	55/56	12	4	6
Pt$(MCO)_2$	42	73	6	5
Pd$(MCO)_2$	45	81	5	4
Pt$(DECO)_2$	60	95	3	3
Pd$(DECO)_2$	94	98	2	1
Pt$(PyrCO)_2$	95.2	96.4	1	2
Pd$(PyrCO)_2$	-	-	Mixed results (N/A)	

Table 1: Comparison of cyanoximates' effects on cell viability *in vitro*.

an ROS-mediated effect to the system and suggested that platinum may reverse the oxidative imbalance by affecting the epithelial sodium channel, or ENaC. ENaC works by downregulating the Protein Kinase C (PKC) pathway in the lungs and showed great promise in the treatment of lung disease [15]. In a paper by Shibuya., *et al*. it was seen that platinum nanoparticles share similar activity to that of catalase and superoxide dismutase, which are key endogenous antioxidant enzymes found in humans systems [17,18]. Moreover, in a stroke mouse model done by Takamiya., *et al*. there was a significant reduction in the production of superoxide upon platinum treatment [19]. This is in agreement with reduced levels of superoxide observed here in our study and offers insight as to the mechanism behind superoxide reduction.

Programmed cell death, or apoptosis is the body's natural mechanism for killing cells [20,21]. Apoptosis is carried out through pathways that utilize caspases to signal a cell for death [22]. In cancer pathology, these apoptotic pathways are inhibited through overexpression of antiapoptotic proteins and under-expression of proapoptotic proteins [22]. Due to these changes, many cancers can develop a resistance to anticancer chemotherapies. Therefore, there is an unmet need to develop new anticancer drugs that can activate these apoptotic pathways. In our study, it was seen that Cisplatin and Pt $(DECO)_2$ both caused significant amounts of apoptosis. Moreover, it is suggested that Pt $(DECO)_2$ was more efficient in inducing cell death as seen by the increase in apoptosis above (Figure 3). Additionally, it can be concluded that neither Cisplatin

or Pt $(DECO)_2$ had much specificity in the killing of rapidly diving cells, regardless if they were cancerous or not. In the Dannen., *et al*. paper, healthy C_{57}BL/6 Mice were injected with 1.0 mM Cisplatin and Pt $(DECO)_2$ to assess cytotoxicity [12]. Their results suggest a less severe side effect profile compared to Cisplatin; however, enhanced hepatotoxicity was not addressed, even with it being a hallmark of many chemotherapies [23].

Conclusion

Since the discovery of the first platinum-based anti-cancer drug, Cisplatin, a great deal of research has been conducted in elucidating better alternative platinum or metal-based anti-cancer drugs. Due to the adverse side effects associated with treatment, researchers are looking to expand less adverse metal-based anti-cancer drugs for cancer therapy. Presently, in the Dannen study (2020), utilizing healthy mice shows great promise in reducing these chemotherapy induced side effects. However, challenges still remain and more research needs to be done in an animal cancer model to determine how intravenous administration affects the entire organism. There is an ever-growing demand for better compounds to be used in cancer therapy that may offer less severe side effects than existing compounds. Our study demonstrated that Pt $(DECO)_2$ has great potential for use as an anti-cancer drug. Further studies are needed to develop safe and effective treatment against aggressive thyroid cancer using this cyanoximate as an alternative to existing platinum-based anti-cancer drugs. Current research shows great promise of Pt $(DECO)_2$ being more effective than Cisplatin at higher doses. However, more research needs to be done to test efficacy of both at lower doses and their ability to be used in conjunction with each other as a combination therapy.

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Author Disclosure Statement

No competing financial interests exist.

Bibliography

1. Morris LG., *et al*. "The increasing incidence of thyroid cancer: the influence of access to care". *Thyroid* 23 (2013): 885-891.
2. Rahib L., *et al*. "Projecting cancer incidence and deaths to 2030: The unexpected burden of thyroid, liver, and pancreas cancers in the united states". *Cancer Research* 11 (2014): 2913-2921.
3. Nickel B., *et al*. "Health-Related Quality of Life After Diagnosis and Treatment of Differentiated Thyroid Cancer and Association With Type of Surgical Treatment". *JAMA Otolaryngology - Head and Neck Surgery* 3 (2019): 231-238.
4. Schlumberger M., *et al*. "Lenvatinib versus Placebo in Radioiodine Refractory Thyroid Cancer". *The New England Journal of Medicine* 7 (2015): 621-630.
5. Schlumberger M., *et al*. "Long-term results of treatment of 283 patients with lung and bone metastases from differentiated thyroid carcinoma". *The Journal of Clinical Endocrinology and Metabolism* 4 (1986): 960-967.
6. Frezza M., *et al*. "Novel Metals and Metal Complexes as Platforms for Cancer Therapy". *Current Pharmaceutical Design* 16 (2010): 18131825.
7. Yimit A., *et al*. "Differential damage and repair of DNA-adducts induced by anti-cancer drug cisplatin across mouse organs". *Nature Communication* 10 (2019): 309.
8. Eddings D., *et al*. "First Bivalent Palladium and Platinum Cyanoximates: Synthesis, Characterizations, and Biological Activity". *Inorganic Chemistry* 13 (2004): 3894-3909.
9. Dasari S and Tchounwou PB. "Cisplatin in Cancer Therapy: Molecular Mechanisms of Action". *European Journal of Pharmacology* 740 (2014): 364-378.
10. Bergamo A and Sava G. "Linking the future of anticancer metal-complexes to the therapy of tumour metastases". *Chemical Society Reviews* 44.24 (2015): 8818-8835.
11. Ndagi U., *et al*. "Metal complexes in cancer therapy - an update from drug design perspective". *Drug Design, Development and Therapy* 11 (2017): 599-616.

12. Ratcliff J., *et al*. "Part 1: Experimental and theoretical studies of 2-cyano-2-isonitroso- N-piperidynylacetamide (HPiPCO), 2-cyano-2-isonitroso- N-morpholylacetamide (HMCO) and their Pt- and Pdcomplexes". *Inorganica Chimica Acta* 285 (2012): 1-20.

13. Dannen SD., *et al*. "New in vitro highly cytotoxic platinum and palladium cyanoximates with minimal side effects in vivo". *Journal of Inorganic Biochemistry* (2020) :208.

14. Piccinini F., *et al*. "Cell counting and viability assessment of 2d and 3d cell cultures: expected reliability of the trypan blue assay". *Biological Procedures Online* 19.1 (2017): 8.

15. Yusof F., *et al*. "Antioxidants effects of Platinum Nanoparticles: A Potential Alternative Treatment to Lung diseases". *Journal of Applied Pharmaceutical Science* 7 (2015): 140-145.

16. B Halliwell. "Antioxidants in human health and disease". *Annual Review of Nutrition* 6 (1996): 33-50.

17. Shibuya S., *et al*. "Palladium and Platinum Nanoparticles Attenuate Aging- Like Skin Atrophy Via Antioxidant Activity in Mice". *PloS One* 9.10 (2014): 109-288.

18. Li R., *et al*. "Defining ROS in Biology and Medicine". *Reactive Oxygen Species* (Apex) 1.1 (2016): 9-21.

19. Takamiya M., *et al*. "Neurological and Pathological Improvements of Cerebral Infarction in Mice with Platinum Nanoparticles". *Journal of Neuroscience Research* 89.7 (2011): 1125-1133.

20. Goldar S., *et al*. "Molecular mechanisms of apoptosis and roles in cancer development and treatment". *Asian Pacific Journal of Cancer Prevention* 16.6 (2015): 2129-2144.

21. Danial NN and Korsmeyer SJ. "Cell Death: Critical Control Points". *Cell* 2 (2004): 205-219.

22. Pfeffer CM and Singh ATK. "Apoptosis: A Target for Anticancer Therapy". *International Journal of Molecular Sciences* 19.2 (2018): 448.

23. Gong C., *et al*. "Hepatotoxicity and pharmacokinetics of cisplatin in combination therapy with a traditional Chinese medicine compound of Zengmian Yiliu granules in ICR mice and SKOV-3-bearing nude mice". *BMC Complementary and Alternative Medicine* 15 (2015): 283.

Volume 4 Issue 7 July 2021