HHS Public Access

Author manuscript

Inorganica Chim Acta. Author manuscript; available in PMC 2020 October 01.

Published in final edited form as:

Inorganica Chim Acta. 2019 October 1; 496: . doi:10.1016/j.ica.2019.119030.

Beyond Cisplatin: Combination Therapy with Arsenic Trioxide

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Abstract

Platinum drugs (cisplatin, oxaliplatin, and carboplatin) and arsenic trioxide are the only commercial inorganic non-radioactive anticancer drugs approved by the US Food and Drug Administration. Numerous efforts are underway to take advantage of the synergy between the anticancer activity of cisplatin and arsenic trioxide - two drugs with strikingly different mechanisms of action. These include co-encapsulation of the two drugs in novel nanoscale delivery systems as well as the development of small molecule agents that combine the activity of these two inorganic materials. Several of these new molecular entities containing Pt-As bonds have broad anticancer activity, are robust in physiological buffer solutions, and form stable complexes with biopolymers. This review summarizes results from a number of preclinical studies involving the combination of cisplatin and As_2O_3, co-encapsulation and nanoformulation efforts, and the chemistry and cytotoxicity of the first member of platinum anticancer agents with an arsenous acid moiety bound to the platinum(II) center: arsenoplatins.

Graphical Abstract

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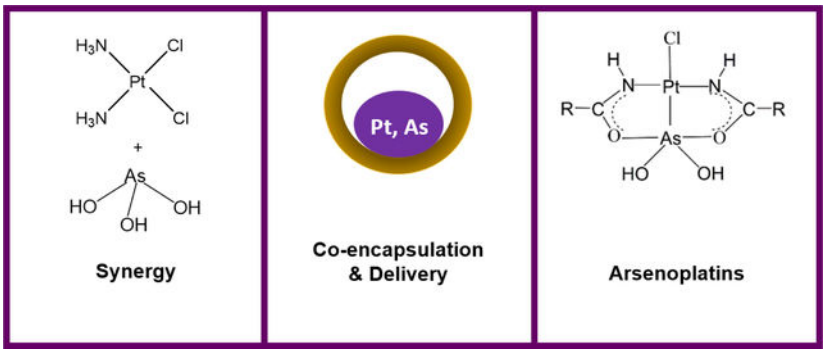
Dedicated to Professor Stephen J. Lippard, a visionary chemist, friend, and an extraordinary mentor

Declarations of interest: none.



Keywords

arsenic trioxide; arsenoplatin; cisplatin; nanobins; nanocomposite; synergy

1. Introduction

Cisplatin was the first platinum drug approved by the US Food and Drug Administration (FDA) in 1978 to treat cancer (Figure 1), and it is still used to treat ovarian, testicular, head and neck, lung, bladder, and colorectal cancers, mostly in combination with other drugs [1–3]. A mechanism of inducing apoptosis in cancer cells [4, 5] through its ability to generate unrepairable DNA lesions is well known [6–8]; however, new evidence suggests that the anticancer activity of cisplatin involves beside a nuclear, also a cytoplasmic component [2]. An investigation of combinatorial regimens of cisplatin with previously approved drugs such as erlotinib [9] and bevacizumab [10] failed to improve the therapeutic profile of cisplatin in randomized clinical trials, and the search for new drug combinations is ongoing.

Arsenic trioxide (As_2O_3, ATO) is another inorganic drug that was approved by the US FDA (Trisenox®) for the treatment of acute promyelocytic leukemia (APL) in 2000 [11]. Arsenic Trioxide is dissolved in saline and administered as a dilute solution of aqueous arsenous acid. Although the mechanism of action is not understood, it is clear that low doses of arsenic trioxide induced de-differentiation of the transformed leukocytes [12, 13]. Arsenic trioxide induces molecular remission and prolongs survival in a high proportion of APL patients when used as a single agent [14]. It has also been shown to act synergistically with ATRA (all trans retinoic acid) [15] to induce degradation of an oncoprotein that drives APL. This oncoprotein results from chromosomal translocation of two genes to form the PML/RARA oncogene (promyelocytic leukemia/retinoic acid receptor alpha), and expression of this fusion gene directly correlates with disease [13]. Unlike anthracycline-based chemotherapy that was until recently the front line treatment for APL [16], the combination of arsenic trioxide and retinoic acid gives rise to significantly less toxicity, particularly in terms of myelosuppression and infections, and decreases both the occurrence of death in remission and development of secondary malignancies [13]. Arsenous acid has been postulated to act by targeting the PML moiety of PML/RARA, where it can induce sumoylation of specific cysteine residues and stimulate proteasome-mediated degradation of the oncoprotein [17]. It has also been shown to induce apoptosis of leukemic cells through

caspase activation and production of ROS [18, 19]. Based on their clear synergy, combination therapy using these two drugs has become a front-line treatment for APL.

A variety of studies in other cell lines have shown that arsenic trioxide causes apoptosis and that the molecular targets are often zinc finger and other thiol reach proteins [20–23]. Arsenic trioxide has been shown to inhibit cancer stem-like cells [24, 25], angiogenesis [26, 27], and importantly to sensitize cancer cells to the activity of other chemotherapeutic drugs [28] and/or radiotherapy [29, 30]. The molecular basis of these activities are thought to involve production of reactive oxygen species (ROS) at levels that subsequently induce mitochondrial membrane depolarization (MMD) [31, 32] and inhibition of DNA repair [33, 34]. Recently, it was discovered that arsenic trioxide and some other commercial chemotherapeutics also exert immunomodulatory activities that can synergistically enhance their anticancer activities [35].

Given the broad anticancer activity of low, non-toxic doses of arsenic trioxide, a number of studies that combine cisplatin and arsenic trioxide have been explored with the goal of developing therapeutic protocols that could be clinically beneficial in cancers resistant to conventional platinum drugs. A clear barrier to these approaches is the rapid renal clearance of arsenous acid, which prevents significant uptake of the drug by solid tumors. While tumors are sensitive to arsenic trioxide, much higher doses of arsenic trioxide are required compared to doses used for APL [36], and off target toxicity becomes dose-limiting. Currently, according to clinicaltrials.gov, only one clinical trial with cisplatin and arsenic trioxide is ongoing: a Phase 2 trial for stage 4 of neuroblastoma. The molecular explanation for the synergistic effect of these two drugs remains poorly understood since only a few papers that evaluate the combinatorial effect of cisplatin and arsenic trioxide have been published. Although there are many reports about successful cisplatin [37–44] or arsenic trioxide [45–52] encapsulation in nanoparticulate form, only two successful co-encapsulations of both drugs were reported. We have developed methods to co-encapsulate cisplatin and arsenic trioxide in the form of Pt,As nanobins (NBPt,As) [53]. Another approach has been recently reported by Xin and co-authors [54]. We have also developed novel compounds in which Pt(II) is coordinated to arsenous acid [55] and demonstrated that these complexes have many properties of small molecule drugs [56]. This review summarizes data on the combinatorial effect of cisplatin and arsenic trioxide, coencapsulation efforts of both drugs and the nature of the Pt-As interaction, and the chemistry and cytotoxicity of the first member of platinum(II) compounds with an arsenous acid moiety-arsenoplatins.

2. Enhanced Cytotoxicity of Cisplatin in Combination Therapy with Arsenic Trioxide

2.1. Experiments in vitro

The significant synergy between cisplatin and arsenic trioxide has been observed in several cancer cell lines including ovarian [57, 58], lung [59, 60], head and neck [61, 62], CNS [63], renal [64], bone [65], and breast [56]. The synergy between these drugs was first reported by Wang and co-authors in 2001 [66]. Arsenic trioxide, known in China as Pishuang [66, 67], a

component of the traditional Chinese medicine Ailing-1[68], regained attention from many Chinese scientists after their success in using arsenic trioxide in the treatment of APL [69, 70]. Weng and co-authors investigated the effect of cisplatin and As_2O_3 in combination on human hepatocellular carcinoma (HCC), which is the leading cause of death among all kinds of cancers in Jiangshu Province. The effect of both drugs in combination *in vitro* was evaluated through the coefficient of drug in interaction (CDI) [71]. The authors found that the inhibition rates of As_2O_3 in combination with cisplatin at various concentrations *in vitro* in HCC Bel-7402 cells were more than that of As_2O_3 or cisplatin alone ($P < 0.01$) with the CDI values less than 1 (the synergistic effect) [71]. The inhibition rate was more pronounced at lower concentrations of the drugs. Combination therapy of arsenic trioxide with another conventional chemotherapeutic agent, so-called “differentiation therapy” [72, 73], was recently proposed as a new approach for treating liver cancers, due to the failure of existing treatments caused by the existence of cancer stem cells which resist apoptosis [74, 75].

Two groups investigated the combinatorial effect of cisplatin and As_2O_3 in ovarian cancers. During the last two decades, platinum based combination chemotherapy has been the first line treatment of advanced ovarian cancer [76], but due to platinum drug resistance, the survival of patients with ovarian cancer in five years is less than 30% [77]. Zhang and co-authors [57] investigated the cisplatin and arsenic trioxide combination therapy in multiple human ovarian cancer cell lines. In the case of COC1 cells in suspension, the combination treatment resulted in higher inhibition growth with combination index (CI) values ranging from 0.93–0.69 (where CI < 1 defines synergism) [78, 79]. The combination also had a favorable dose reduction index (DRI) from 2-fold to 5-fold. The synergistic effect was also obtained in adherent ovarian A2780 and IGROV-1 cancer cell lines that are sensitive to cisplatin (CI 0.44–0.97) or resistant to cisplatin SKOV-3 and R182 (1.5-fold to 13.5 DRI).

Muenyi and co-authors [58] investigated the role of p53 status in epithelial ovarian cancer (EOC) in response to a combination of cisplatin, sodium arsenite, and hyperthermia. Human EOC cells were treated with cisplatin ± 20mM sodium arsenite at 37 °C or 39 °C for 1h. For this study, the authors used wild-type p53-expressing cisplatin-sensitive (A2780) and cisplatin-resistant (OVCA 420, OVCA 429, OVCA 433, and A2780/CP70), p53-mutated (OVCAR-3 and OVCA 432) and p53-null (SKOV-3) human ovarian cancer cells. The results reveal that co-treatment with sodium arsenite and hyperthermia enhanced cisplatin cytotoxicity in cells expressing wild-type p53. Results in A2780 cells after transfection have demonstrated that suppression of p53 abrogates sodium arsenite sensitization to cisplatin, but only hyperthermia sensitized p53-null or p53-mutated cells to cisplatin. The authors also found that this combined treatment also attenuated cisplatin-induced XPC, a DNA damage recognition protein involved in DNA nucleotide excision repair [80]. Cisplatin dependent induction of XPC occurred only in wild-type p53-expressing cells but not in p53-null cells. The attenuation of XPC by sodium arsenite ± hyperthermia is a mechanism for sensitizing wild-type p53-expressing EOC cells to cisplatin. The authors also used inductively coupled plasma-mass spectrometry (ICP-MS) to show that hyperthermia alone or in combination with sodium arsenite enhanced the platinum accumulation and binding to DNA in A2780 and A2780/CP70 cells. Results of these two papers suggest that the combination of cisplatin and arsenic trioxide has significant clinical potential for the treatment of ovarian cancers.

Cisplatin and arsenic trioxide synergy has also been explored in non-small [81] and small cell lung [60] carcinoma. Li and co-authors [81] established a synergistic effect between cisplatin and As_2O_3 in H460 and A549 non-small cell lung cancer (NSCLC) cells. The combination indices obtained for A549 and H460 were CI = 0.5 and CI = 0.6, respectively. Based on FCM and TUNEL staining, the combination of As_2O_3 and cisplatin induced synergistic effects on apoptosis, partially due to the induction of caspase-independent apoptosis. The authors concluded that the death pathway that involves Bax, Bcl-2, and clusterin was the primary mechanism by which arsenic trioxide exerts synergistic effects of cisplatin.

Zheng and co-authors [60] investigated the combined effect of cisplatin and As_2O_3 in five small cell lung cancer (SCLC) cell lines (H187, H526, H69, H 841, and DMS79). Based on CI value, moderate (CI = 0.6–0.8) to strong (CI = 0.2–0.6) synergism was observed in all five SCLC cell lines tested at half the maximal effective dose (ED_{50}). The glutathione (GSH) levels in H841 cells were significantly lowered in As_2O_3/cisplatin combination compared to GSH levels after cisplatin or As_2O_3 treatment alone, which may explain the enhanced cytotoxicity of the drug combination compared to cisplatin alone. In H841 cells a combination of cisplatin and arsenic trioxide enhanced apoptosis through MMD. Besides the suppression of intracellular GSH, the authors also found that the drug combination increased H2AX phosphorylation and thus proposed such phosphorylation as a new mechanism of synergy. The authors concluded that since cisplatin and As_2O_3 have non-overlapping toxicities the combination would be a promising regimen for the treatment of SCLC.

Two research groups investigated the combination therapy in head and neck carcinoma. Kotowski and co-authors [61] investigated the potential of As_2O_3 to enhance the effect of cisplatin in four head and neck squamous cell carcinoma lines (HNSCC) SCC9, SCC25, CAL27, and FADU and also investigated if combination therapy will alter the expression of the anti-apoptotic protein MCL-1 (myeloid cell leukemia protein), a member of the Bcl-2 family involved in inhibiting cell death [82]. The authors found that the two drugs acted synergistically in all investigated HNSCC cell lines but only at high concentrations. No significant change in the expression of MCL-1 in combination compared to the drugs alone was noticed. The combination also did not show a higher apoptosis rate compared to cisplatin or arsenic trioxide alone.

In contrast, Nakaoka and co-authors [62] found that the synergistic effect of As_2O_3 and cisplatin increased apoptosis in oral squamous cell carcinoma (OSCC), which is the most common head and neck neoplasm with poor prognosis [83]. The authors found that the combination significantly suppressed HSC-2 cell growth compared to individual drugs with CI values ranging from 0.78 to 0.90. The cisplatin-As_2O_3 combination reduced the doses of As_2O_3 (ranging from 2.14-fold to 2.44-fold dose reduction) and cisplatin (ranging from 2.15-fold to 2.56-fold dose reduction) in HSC-2 cells. The authors also found that caspase-3/7 activity was significantly higher after 24h incubation with the drug combination compared to incubation with cisplatin alone ($P<0.05$) and also higher after 36h and 48h incubations with the drug combination compared to incubation with As_2O_3 alone ($P<0.05$). JC-1 staining FACS analysis performed indicated that the mitochondrial potential was significantly reduced following 24h of incubation with the cisplatin-As_2O_3 combination

compared to monotherapy ($P<0.005$). Western blot analyses revealed that the levels of mitochondrial cytochrome c, X-linked inhibitor of apoptosis protein (XIAP), and anti-apoptotic B-cell lymphoma 2 (BCL-2) were down-regulated when cells were treated with the cisplatin-As_2O_3 combination. The authors also found that the level of ROS generation was higher in HSC-2 cells after cisplatin-As_2O_3 treatment compared to either monotherapy. Similar effects were seen in other OSCC cell lines, i.e., cisplatin-As_2O_3 combination inhibited the growth of HSC-3 and HSC-4, with CI values ranging from 0.34 to 0.45 and in HSC-4 with CI ranging from 0.60 to 0.92. DRI analysis has shown that the combination reduces cisplatin dose by 3.3-fold to 5.1-fold in HSC-3 and 1.6-fold to 2.5-fold in HSC-4.

Günes and co-authors investigated the effect of co-application of cisplatin and As_2O_3 in CNS (central nervous system) cancers using human SY-5Y neuroblastoma cells [63]. Neuroblastoma is a common cancer in children under five years old, and the five-year survival rate with high-risk neuroblastoma is only 40% [84]. The authors used confocal laser imaging with the calcium-sensitive dye fluo-4 for investigating $[Ca^{2+}]_i$ dynamics. Treatment of cells with cisplatin and arsenic trioxide induces flux of intracellular Ca^{2+} ions, triggering apoptosis. Cisplatin and As_2O_3 modulate the intracellular concentration of calcium ions through different mechanisms: cisplatin triggers an influx of Ca^{2+} ions from extracellular space by an IP3-dependent mechanism while As_2O_3 releases Ca^{2+} from intracellular stores [85, 86]. The SY-5Y cells with an initially low $[Ca^{2+}]_i$ were the most sensitive to the application of drugs when applied alone or in combination. The administration of As_2O_3 first for 1 hour followed by cisplatin for an additional 1 hour resulted in similar cytotoxicity as when cisplatin was administered alone (25.4 ± 7.2%). When cells were exposed first to cisplatin 1h, followed by the addition of As_2O_3 and additional 1hour incubation, threefold higher cytotoxicity (80.3 ± 8.5%) was observed. The effects of As_2O_3 and cisplatin on the sustained $[Ca^{2+}]_i$ increase found to be additive, and the cytotoxicity is synergistically elevated by co-application of the two drugs when cells are incubated first with cisplatin and then with arsenic trioxide.

In 2015, Dogra and co-authors [64] investigated the cytotoxicity of cisplatin in combination with arsenic trioxide in renal cell carcinoma (RCC). They used human tubular cells transformed by HPV-16 E6/E7. In addition, HuH-7 cells and HK-2 cells were also included in this study. Results have shown that As_2O_3 may synergistically enhance cisplatin toxicity via inhibition of the p53 mediator WIP1 (protein phosphatase 2C delta) [87]. HK-2 cells responded well compared to cell growth control when As_2O_3 first downregulated WIP1. The authors concluded that the presence of As_2O_3 synergistically enhanced the cytotoxicity of cisplatin through signaling downstream of p53 and tubulin depolymerization.

Saitoh and co-authors [65] evaluated the effect of arsenic trioxide, a Hedgehog inhibitor [24], in combination with cisplatin in osteosarcoma using CalcuSyn software to examine CI and synergism *in vitro*. The results of a WST-1 assay showed that As_2O_3 and cisplatin inhibited proliferation of 143B and Saos2 cells. The CIs obtained for this drug combination at ED50–90 (the average CI at ED_{50} to ED_{90}) in these two cells were 0.41 and 0.75, indicating a synergistic effect.

There is great interest in the development of new treatment options for breast cancer, which is the most common cancer diagnosed among US women and is the second leading cause of death after lung cancer [88]. We investigated the combinational effect of cisplatin and As_2O_3 in three breast cancer cell lines: MDA-MB-231[56], GILM2, and BT-20, Fig. 2 [89]. The significant level of synergy was established in all breast cancer cell lines tested (CI < 1). The mechanism behind the synergy of these two drugs is proposed based on the investigation of interactions of these drugs with glutathione [89, 90]. Depletion of reduced GSH levels by arsenic trioxide [91] through the inhibition of glutathione reductase and the formation of $As(GSH)_3$ in cells [92] could be one of the causes of synergy [90, 93]. The depletion of GSH then increases cisplatin binding to DNA [52, 94], leading to unrepairable DNA damage and apoptosis. From another point of view in the case of cisplatin binding to glutathione, the export of the glutathione-cisplatin complex from the cells intensifies the role of arsenic trioxide in inducing the formation of ROS and subsequent cell death. Both models tend to increase cisplatin and As_2O_3 cytotoxicity above their normal level when used as single agents.

2.2. Experiments in vivo

Only three research groups investigated a combinatorial effect between cisplatin and arsenic trioxide in animal models. Wang and co-authors [66], after establishing that at low doses cisplatin and arsenic trioxide act synergistically, used low doses of the drugs *in vivo* to treat HepA tumors implanted in mice. They found that the inhibition of HCC tumor growth with cisplatin alone was 46.2 % and with arsenic trioxide alone 30.1 %. These two drugs in combination inhibited the tumor growth by 73.9 % ($P < 0.01$, *vs.* As_2O_3 or cisplatin alone). Since lower doses of both cisplatin and arsenic trioxide were effective in inhibiting tumor growth, low doses of these two drugs in combination may be used to treat hepatic tumors in the future, without increasing toxicity. The mechanism of synergy is not investigated.

Zheng and co-authors [60] confirmed the synergy results obtained *in vitro* in SCLC *in vivo* in tumor xenograft model using an H841 cell line in nude mice. Cisplatin and As_2O_3 acted in synergy in suppressing the tumor growth after 3–4 weeks of treatment compared with the drugs alone (p <0.05. The authors did not investigate the underlying mechanism for the synergy *in vivo*. It is interesting to note that a weight loss or other unfavorable health outcomes were not found in the mice treated with the cisplatin-As_2O_3 combination.

Saitoh and co-authors [65] confirmed the observed *in vitro* synergism between cisplatin and arsenic trioxide *in vivo* in osteosarcoma. 143B osteosarcoma cells were inoculated subcutaneously, and the tumor volume after seven days was set as 1. The tumor volume was then measuredat different time points. The statistical differences in tumor size were observed between the control vehicle and the combination of cisplatin and As_2O_3 ($p < 0.01$), showing that these two drugs act synergistically in mice models of osteosarcoma. The authors found that GLI2 and SMO were overexpressed in human osteosarcoma specimens, and that As_2O_3, which inhibits the GLI expression, could be one of the underlying mechanisms for synergy.

Based on the results of these studies (Table 1), arsenic trioxide enhances the cytotoxicity of cisplatin *in vitro* and *in vivo*. Although mechanisms of causing the synergy in combination therapy are investigated *in vitro*, the mechanism of a synergy *in vivo* is not known. The

synergism established in various cancers and cancer cell lines suggests that this drug combination may be clinically beneficial. The combination therapy might reduce toxicities due to decrease in the effective concentration of cisplatin and arsenic trioxide when used as a single drug. Currently according to clinicaltrials.gov, only one clinical trial with cisplatin and As_2O_3 is ongoing: a Phase 2 trial for stage 4 of neuroblastoma. The molecular explanation for the synergistic effect of these two drugs remains poorly understood, opening a great research potential in this area.

3. Co-Encapsulation Efforts

The established synergy between cisplatin and arsenic trioxide *in vivo* and *in vitro* led to efforts to incorporate both drugs into nanoparticulate formulation. Although there are many reports about successful cisplatin [37–44, 96] or arsenic trioxide [45–52] encapsulation in nanoparticulate form, only two co-encapsulations of both drugs were reported so far: syntheses of Pt,As nanobins (NB-Pt,As) by our lab [53], and Pt,As nanocomposites by Xin and co-authors [54]. The encapsulation of cisplatin into macromolecular, liposomal, or nanoparticle drug delivery systems increases the therapeutic ratio of the cisplatin and enhances the selective accumulation in tumor sites [96]. The encapsulation of arsenic trioxide increases its accumulation at tumor sites, extends the circulation time of the active form of the drug in blood, and diminishes off-target toxicity [51, 97]. The co-encapsulation of both drugs enhances the positive outcomes of the single drug encapsulation and additionally increases cytotoxicity: this is most likely achieved through the established synergism. We consider multidrug formulations that hit multiple targets within the cancer cell will increase anticancer efficacy and combat resistance [98].

3.1. Discovery of Pt,As-Nanobins

Our interest in combining cisplatin with arsenic trioxide stems from arsenic trioxide's inherent tumoricidal activity [99] and a plethora of biochemical interactions inside the cells: inhibition of DNA repair [100, 101], anti-angiogenesis [102, 103], inhibition of cancer stem-like cells [73, 104, 105], induction of oxidative stress through ROS production [100, 106, 107], and induction of apoptosis [108–110]. Our group first developed the platform for stable lipid encapsulation of As_2O_3. The first generation of nanobins contained As_2O_3 co-precipitated with nickel acetate, NB(Ni,As) [111]. The term "nanobins" is used since a nanoprecipitate inside of liposomes contained many arsenic and nickel precipitated particles [51]. NB(Ni,As) were tested in an orthotopic model of triple-negative breast cancer, wherein they demonstrated improved pharmacokinetics, increased tolerability, and greater antitumor effects *in vivo* compared to free arsenic trioxide [52]. The lipid formulation had attenuated cytotoxicity and controlled drug release. After successful encapsulation of arsenic trioxide, we succeeded in co-encapsulation of cisplatin and arsenic trioxide in nanoparticulate form and synthesized Pt,As nanobins (NB-Pt,As) [53]. Different nanoliposomal drug formulations were designed to increase drug circulation time and limit exposure to healthy cells, as both cisplatin and As_2O_3 are highly cytotoxic and As_2O_3 suffers from rapid renal clearance. NB-Pt,As are prepared by first preparing a 300 mM aqua-cisplatin acetate solution (pH 5.1), and then hydrating the dry lipid film (dipalmitoylphosphatidylcholine(DPPC)/ dioleoylphosphatidylglycerol(DOPG)/cholesterol(Chol) = 51.4:3.6:45 mol%) in the aqua-

cisplatin acetate solution followed by extrusion to a diameter of 100 nm (Figure 3a). The addition of concentrated As_2O_3 solution at pH 6.6 resulted in active loading of $As(OH)_3$ into the liposome at 50 °C, giving the final molar ratios of As:lipid of 0.63 ±0.05 and Pt:lipid of 0.48 ± 0.06 after 11 h (n = 7, the number of independent experiments). Transmission electron microscopy (TEM) and energy dispersive X-ray (EDX) analyses revealed the presence of As and Pt cores within NB(Pt, As). The X-ray absorption edge energies agreed well with the assignments of As^{III} and Pt^{II} oxidation states by X-ray photoelectron spectroscopy (XPS). The extended X-ray absorption fine structure (EXAFS) data provided direct evidence for an intimate interaction between $As(OH)_3$ and aqua-cisplatin within the liposome with the Pt-As distance of 2.3 Å (Fig. 3b)[53].

The drug release studies revealed high stability of NB(Pt, As) under physiologically relevant conditions with less than 17% of drugs released over 21-month storage at 4 °C and with less than 20% of drugs released in serum after 72 hours at 37 °C [53]. The bioavailability of the drugs results from the gradual dissociation of NB(Pt,As) into active $As(OH)_3$ and cis-$[(NH_3)_2Pt(OH_2/OH)_2]^{n+}$ species and diffusion from liposomes into cellular environments. Both NB(As,Pt) and NB(Pt) showed attenuated cytotoxicity relative to free arsenic trioxide, aqua-cisplatin, and cisplatin [53]. This indicates that liposome co-encapsulation may lower the general toxicity of both arsenic trioxide and platinum drugs *in vivo*.

The NB(Pt,As) is likely a polycrystalline coordination polymer containing platinum and arsenic in the same ratio as the bulk drug, indicating that most of the drug exists as a solid inert particle, Fig. 4c.

As other nanoparticles designed to target cancer, NB(Pt,As) can promote extravasation in the leaky vasculature of solid tumors, leading to improved accumulation of the drugs due to the enhanced permeability and retention (EPR) effect [112–114]. *In vivo* NB(Pt,As) rely on blood flow to reach the tumor site. NB(Pt,As) are passivated by coating the surface in poly(ethylene glycol) to increase circulation time. “PEGylation” is the most used approach for improving the efficiency of drug and gene delivery to target cells and tissues [115]. PEG coatings on NB(Pt,As) shield the surface from opsonization, aggregation, and phagocytosis [116]. Recently, we established that cisplatin and arsenic trioxide act synergistically in triple negative MDA-MB-231 cancer cell line at all ratios investigated [56]. Our results *in vitro* were confirmed *in vivo*. A series of *in vivo* experiments was designed to investigate the clinical utility of NB(Pt,As). Triple negative breast MDA-MB-231 cancer cells were implanted in the fourth mammary fat pad of female nude mice. Tumors were allowed to grow until they reached an average volume of 50 mm^3, a period of approximately three weeks. Animals were randomized into treatment groups and then treated with either empty (control) nanobins NB(NaCl), cisplatin (2.5 mg/kg), As_2O_3 (4 mg/kg), arsenic trioxide in combination with cisplatin, or the NB(Pt,As) at 4mg/kg arsenic (Table 2). Animals received seven treatments, after which treatment was halted. Tumors were allowed to grow for an additional two weeks. NB(Pt,As) and the cisplatin and arsenic trioxide combination proved effective in reducing tumor growth. The cisplatin in combination with arsenic trioxide significantly reduced tumor growth compared to vehicle control or arsenic trioxide monotherapy after day 31 ($p<0.01$), (Fig. 4) [90]. Arsenic trioxide as a single agent did not suppress the tumor volume, probably due to rapid renal clearance [52].

It is well known that platinum drugs [117–120] and arsenic trioxide [121] react with glutathione and other thiols. In blood, the total glutathione concentration is in 0.81–13.02 μM range [122], and the concentration in plasma is 4–20 μM [123]. We speculate that drug release from NB(Pt,As) will not be initiated under plasma conditions but may be promoted by high intracellular concentration of thiols (0.5–10 mM) [124], after these nanoparticles are taken up by cells within the tumor.

Nanoencapsulation limits the systemic toxicities of cytotoxic therapies while preserving their antitumor effects. Compared with arsenic trioxide, arsenic loaded nanobins may help preserve normal reproductive function for women during therapy, which is especially important for young patients. It was established that female mice treated with NB(Ni,As) displayed a normal estrus cycle compared to free As_2O_3 treatment, wherein 40% of mice had cycling stopped within ten days of initial treatment [52]. We believe that similar effects will be seen with NB(Pt,As). The potential loss of fertility is an important concern, and the design of fetoprotective anticancer drugs is one of the main goals of anticancer research today.

3.2. Discovery of Pt,As-Nanocomposites

Xin and co-authors [54] recently described a facile synthesis of cisplatin and As_2O_3 nanocomposites that are carrier-free. The authors proposed that the main product in the PtAs composite is the salt formed between positively charged $[Pt(NH_3)_2(H_2O)_2]^{2+}$ and negatively charged $HAsO_3^{2-}$ions, Fig. 5.

Cisplatin-As_2O_3 nanocomposites (PtAs) were synthesized via a reverse microemulsion approach shown in Scheme 1. Cyclohexane was mixed with Igepal CO-520 (Igepal = nonylphenol ethoxylate) and a solution of aquo-cisplatin acetate followed with the addition of sodium arsenite solution to form a microemulsion. PtAs nanoparticles (PtAs NPs) were collected by ethanol precipitation and stored in deionized water at 4 °C. PtAs nanocomposites formed were further modified with polyallylamine hydrochloride (PAH) and dextran sulfate (DXS) polymers according to a layer-by-layer assembly method (Scheme 1). The nanocomposite, which is denoted as PtAs@PAH-DXS nanodrugs, was collected by ultrafiltration. The achieved nanocomposites had a high loading capacity and pH-dependent controlled release of the drugs (Fig.5c).

The authors evaluated the cellular uptake of cisplatin, As_2O_3, and PtAs@PAH-DXS nanodrugs (the Pt to As ratio of 1:1.05) *in vitro* after exposing HCC cell lines (SMMC-7721, HepG2, and H22) and human ovarian cisplatin resistant COC-1-DDP and wild type COC-1-WT cells to drugs for 48 hours. IC_{50} values for PtAs@PAH-DXS nanodrugs were lower than IC_{50} values for the drug alone, indicating that the nanodrug combination therapy increased the cytotoxicity. By measuring the cellular uptake using ICP-MS after exposing SMMC-7721, COC-1-WT and COC-1-DDP cancer cells to drugs for 12 hours, the authors found that PtAs@PAH-DXS nanodrugs substantially increased the accumulation of cisplatin and As_2O_3 compared to free drugs accumulation. Western blotting analysis showed a significant increase of γH2AX expression (the phosphorylated minor histone H2A variant) [125] after treating COC-1-DDP cells with nanodrugs compared to cells treated with cisplatin only, suggesting that PtAs@PAH-DXS nanodrugs induced massive DNA damage

compared to free drugs. Based on these results, Xin and co-authors [54] concluded that the inhibition of DNA damage repair could be responsible for the synergistic effect of cisplatin and As_2O_3 in cells treated with PtAs@PAH-DXS nanodrugs. Gene expression profiles demonstrated increased expression of tumor suppressor genes *PTEN*, *VHL*, and *FAS* and reduced expression of proto-oncogenes and DNA damage repair related genes *MYC*, *MET*, and *MSH2* after treatment with PtAs@PAH-DXS nanodrugs.

For studies of PtAs@PAH-DXS nanodrugs *in vivo*, the authors studied male BALB/c mice bearing subcutaneous H22 tumors. Blood samples were collected at various time points after injection to measure the platinum content by ICP-MS. The authors found that nanodrugs had substantially prolonged blood circulation half-lives: 40 min for Pt and 44 min for As, compared to free drugs cisplatin and arsenic trioxide (21 min both), which is essential for passive tumor targeting [126]. The tumor uptake of platinum was 2.9 ± 0.28 % for PtAs@PAH-DXS nanodrugs and 0.7 ± 0.33% for cisplatin of injected dose (ID) per gram, and a large portion of nanodrugs was accumulated in the spleen and liver, suggesting clearance by the reticuloendothelial system (RES) and mononuclear phagocyte system (MPS) [127]. In contrast, the majority of free cisplatin and As_2O_3 was removed from the body. The concentrations of drugs used for intravenous injections were 2 mg Pt/kg body weight for free cisplatin and PtAs@PAH-DXS nanodrugs and 0.8 mg As/kg body weight for As_2O_3. The mean subcutaneous tumor volume had minimal growth at day 18 in mice treated with nanodrugs compared to cisplatin (7-fold increase) and As_2O_3 (10-fold increase) in the same period. The authors concluded that PtAs@PAH-DXS nanodrugs have favorable pharmacokinetics and biodistribution compared to free cisplatin and arsenic trioxide.

Based on the results of these papers, co-encapsulation of both drugs into nanoparticulate form attenuates the cytotoxicity of cisplatin and arsenic trioxide when used as single agents, prolongs the circulation lifetime of the drugs, and allows pH or thiol concentration controllable drug release from the nanoparticles in solid tumors.

4. Discovery of Arsenoplatins

Arsenous acid, $As(OH)_3$, is the active form of arsenic trioxide [70]. The great success of As_2O_3 in treatment of APL [128] was not followed in solid tumors due to rapid renal clearance from the body and the systemic toxicity associated with large amounts of arsenous acid in the blood, which can lead to potentially fatal arrhythmias [129]. Low doses of As_2O_3 are used for treating hematological malignancies; however, the treatment of solid tumors remains a challenge [70]. Inspired by the discovery of the Pt-As bond in nanobins (NB-Pt,As), we successfully synthesized the first platinum(II) compounds with an arsenous acid moiety bound to a Pt(II) center – arsenoplatins (AP) [55]. We hypothesized that small molecules containing both pharmacophores would have improved cytotoxicity compared to free drugs cisplatin and As_2O_3.

Arsenoplatin complexes can be synthesized from cisplatin or $[PtCl_4]^{2-}$ and As_2O_3 in the appropriate solvent mixture (Scheme 2).

Crystal structures of the first two members of arsenoplatin compounds, Fig. 6, as well as an SCN^- derivative of AP-1, were solved by X-ray crystallography [55]. Pt(II) adopts a usual square planar geometry, but As(III) exhibits an unusual trigonal bipyramidal geometry, with As(III) acting simultaneously as a Lewis acid and as a Lewis base. The Pt-$As(OH)_2$ core is stabilized with two five-membered chelate rings. The Pt(II)−As(III) bond length in these complexes is in the 2.269−2.273 Å range, which agrees with the Pt-As bond length of 2.3 Å in Pt,As-nanobins based on EXAFS measurements [53].

The presence of As(III), which exerts a very strong *trans* effect, influences the chemistry of these compounds and differentiates them from cisplatin's and other platinum(II) drugs' reactivities. The substitution of Cl^- with small ligands in aqueous solutions is rapid [55], even without the addition of Ag(I)-salt to remove the Cl^- ion, and during the substitution, the Pt-As bond stays intact (Scheme 2).

The Pt-As bond is also preserved in the reaction of arsenoplatin-1 with small proteins HEWL (Fig. 7) and RNase A. The crystal structures of AP-1-protein adducts revealed that εN atoms of His side chains and the preferred binding sites of AP-1 in both proteins [56]. AP-1 did not show a preference for Met side chains, compared to cisplatin and carboplatin, which bind RNase A through S-atoms of Met side chains [130].

Arsenoplatin-1 is stable in buffered solutions, saline, methanol, and DMSO for at least 48 hours [56]. Unlike cisplatin, which is prone to solvolysis in DMSO [131], solvolysis of AP-1 in DMSO does not occur. This is confirmed by comparison of the ^{195}Pt NMR shifts in DMSO-d_6 [55] and methanol-*d4* solutions of AP-1 [56], revealing a ^{195} Pt chemical shift difference of only 36 ppm [56]. If the substitution of AP-1 chloride with DMSO had occurred, the ^{195}Pt chemical shift would have undergone an upfield shift of 850–1,000 ppm (Table 3).

Since AP-1 is stable in DMSO, DMSO solutions of AP-1 may be used in many biological assays for further studies, including the NCI-60 screen. We compared the anticancer profile of 10 μM arsenoplatin-1 with 10 μM solutions of cisplatin and arsenic trioxide in the NCI-60 screen. Based on the results of this screen, arsenoplatin-1 is more potent than As_2O_3 in all nine indications tested, and more potent than cisplatin in four and comparable in two indications, Fig. 8 [56]. Arsenoplatin-1 was the most lethal in breast and ovarian cancers and had the highest inhibitory effect in leukemia and renal cancers. However, detailed chemical reactivity and biological activity profiles are necessary to fully understand the mechanism of arsenoplatin-1 cytotoxicity.

Investigation of the binding abilities of AP-1 toward DNA has shown some interesting phenomena. AP-1 binding to DNA was confirmed by ICP-MS measurement on AP-1-DNA adducts isolated from triple negative breast MDA-MB-231 cancer cells [56] as well as by DFT calculations by Marino and co-authors [136]. After treatment of MDA-MB-231 cells with 100 μM solution of AP-1 for 4 hours and 8 hours, the platinum and arsenic content in the DNA-adducts was evaluated by ICP-MS. We found that the Pt-As ratio in AP-1- DNA adducts is close to 1 after 4 hours of incubation; however, after 8 hours, a significantly fewer number of moles of arsenic than platinum is present in the adducts ($p < 0.0001$), Fig. 9. This

suggests that the Pt-As bond in AP-1-DNA adducts is intact during the first 4 hours of incubation but breaks afterward, releasing an arsenous acid moiety. We hypothesized that this could be the reason for higher cytotoxicity of AP-1 (IC_{50} = 9.5 ± 0.3 μM) compared to cisplatin (IC_{50} = 22 ± 0.5 μM) in this cancer cell line [55], and we thus proposed that AP-1 acts as a dual pharmacophore anticancer agent [56]. DFT calculations on cisplatin and AP-1 DNA bindings [136] suggest that the hydrolysis of the Pt-Cl bond in AP-1 requires less energy than the hydrolysis of cisplatin due to the *trans e*ffect of the arsenic moiety (27.9 kcal/mol *vs*. 31.8 kcal/mol). Based on the energy calculation for the guanine and adenine platination processes (22.4 kJ/mol *vs*. 31.9 kcal/mol), Marino and co-authors [136] concluded that guanine is the preferred target site of AP-1 in its binding to DNA.

Arsenoplatin compounds are peculiar platinum(II) compounds, and our understanding of chemical reactivity of arsenoplatins is limited so far on interactions of the first member of this family, AP-1, with a small number of simple molecules and biomolecules. Intensive efforts to fully understand the chemistry of the arsenoplatin family and the mechanisms of actios in our lab are ongoing. The synergy between two FDA approved drugs inspired scientists to synthesize triple [137] and quadruple [138] action anticancer agents to combat resistance. The screening approach to discover the effective combinations of platinum drugs to treat malignancies with poor prognosis was recently published [98].

5. Conclusions

There is strong evidence for synergy apparent in the interaction between the two FDA approved drugs cisplatin and arsenic trioxide in cell culture studies. Arsenic trioxide enhanced the cytotoxicity of cisplatin, achieved a significant dose reduction index for cisplatin, and proved that this drug combination may be clinically beneficial. These two drugs can be co-encapsulated using a number of different strategies. The nanoparticulate formulations attenuate the cytotoxicity of both drugs, prolongs the circulation time, and increases efficacy in PDX models of cancer. Physicochemical characteristics of these formulations led to the development of the arsenoplatins: small molecular compounds with a Pt-As$(OH)_2$ core that exhibit activity in cisplatin-resistant cell lines and cells from a variety of tumor types. These and other emerging approaches take advantage of the cisplatin/arsenous acid synergy with the long-term goal of simultaneous delivery of distinct anticancer pharmacophores to the tumor.

6. Future perspective

Cisplatin and arsenic trioxide combination therapy simultaneously delivers two drugs that can cause apoptosis through different mechanisms of action. The increase in cytotoxicity and decrease in toxicity through the dose reduction index is caused by the synergistic effect of these two therapeutics. The synergism established between two anticancer drugs inspired scientists to synthesize the double-, triple-, and even quadruple-action anticancer drugs to combat resistance. Given that cancer is such a complex disease that evolves as a function of therapeutic interventions, we anticipate that new drug combination studies, not only with the FDA approved but also with new candidate anticancer agents will emerge in the earliest stage clinical trials. A variety of research teams are converging on ‘designed combination

therapy' that will deliver several drugs to the specific tumor site. The syntheses, evaluation, screening, and development of new multiple action anticancer drugs and nanoparticles that can deliver multiple drugs is an important next step in realizing more effective and safer cancer treatment.

Acknowledgments

This work was supported by the National Institute of Health [U01CA15146, U54CA193419, and U54CA202995–03 (ChicagoCHEC)].

Biographies

Đenana Miodragović received her Ph.D. in Inorganic Chemistry at the University of Belgrade. She was an Assistant Professor in Inorganic Chemistry at the University of Belgrade School of Chemistry from 2003–2009. She was a representative of Serbia in the European Association for Chemical and Molecular Sciences for the Division of Chemistry in Life Sciences. In 2008/09 she spent a *sabbatical* in Thomas O'Halloran's lab in the Chemistry of Life Processes Institute at Northwestern University, where she worked on synthesizing Pt(II) complexes with arsenous acid, and continued to work later as a Research Associate in 2010/11. Currently, she is a lecturer at Northeastern Illinois University and a Visiting Scholar at Northwestern University, where she continues to work on her Arsenoplatin project.

Elden Swindell received his BS in Chemical Engineering from Syracuse University in 2008 and his Ph.D. in Chemical Engineering from Northwestern University in 2015. His dissertation focused on the delivery and efficacy of targeted liposomes loaded with cisplatin and arsenic trioxide to primary and metastatic tumors. His postdoctoral research was conducted at Northwestern University and Merrimack Pharmaceuticals in Cambridge, MA, and concerned the use of targeted liposomal drugs in single agent and combination therapies in brain tumors, lung cancers, prostate cancers, and bladder cancers. He is currently a Manufacturing Development Scientist at Hitachi Chemical Advanced Therapeutic Solutions LLC in Allendale, NJ.

Zohra Sattar Waxali received her BS in Chemistry with an emphasis in Biochemistry from Northern Illinois University in 2018, where her medicinal chemistry research focused on computational drug design and synthesis of malarial enzyme inhibitors and natural product

extraction from traditional medicinal Kenyan plants. She also completed computational studies in the relative stabilities of low-spin d^6 complexes at Northern Illinois University and the synthesis of covalent peptide inhibitors for the estrogen receptor-cofactor 2 interaction in ER+ breast cancer. She is currently a PhD student at Northwestern University in the O'Halloran laboratory working on characterizing the breast cancer metallome, designing mitochondrial-specific iron chelators for combatting heart disease, and analyzing the chemical activity of Arsenoplatins.

Abraham Bogachkov graduated from Northwestern University in 2011 with a double major in biology and mathematics. He then worked in the O'Halloran laboratory for one year, continuing projects that he began during his undergraduate studies, including work on arsenic and cisplatin susceptibility in multiple cell lines. He subsequently attended medical school at the Northwestern University Feinberg School of Medicine, graduating in 2016 with an MD degree. He is currently a second year radiology resident at Advocate Illinois Masonic Medical Center.

Thomas V. O'Halloran is the Morrison Professor in the Departments of Chemistry and Molecular Biosciences and the Founding Director of the Chemistry of Life Processes Institute at Northwestern University. His research focuses in three areas: the chemistry and mechanism of inorganic drugs, the chemical biology of metal fluxes, and metal receptors that regulate stress responses and developmental pathways. His discoveries have established the functions and structures of two classes of soluble receptors: metalloregulatory proteins that govern metal responsive gene expression and metallochaperone proteins that control intracellular trafficking pathways. He has published over 200 papers on structures and mechanisms of proteins regulating uptake, trafficking, utilization of metal ions, microbiology of zinc and copper, nanoscale drug delivery mechanisms and on the mechanisms of clinically important arsenic, molybdenum and platinum anticancer agents.

Nomenclature

Chemical and biological abbreviations

APL	acute promyelocytic leukemia
ATO	arsenic trioxide
ATRA	all trans retinoic acid
Bax	apoptosis regulator BAX
Bcl-2	anti-apoptotic protein B-cell lymphoma 2
CDI	coefficient of drug interaction
CI	combination index
CNS	central nervous system
Chol	cholesterol
DFT	density functional theory
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DOPG	dioleoyl phosphatidylglycerol
DPPC	dipalmitoyl phosphatidylcholine
DRI	dose reduction index
DXS	dextran sulfate
EOC	epithelial ovarian cancer
ED_{50}	half the maximum effective dose
ED_{90}	ninety-percent of maximum effective dose
EDX	energy dispersive X-ray
EDS	energy dispersive X-ray spectroscopy
EPR	enhanced permeability and retention effect
EXAFS	extended X-ray absorption fine structure
FACS	Fluorescence-Activated Cell Sorting
FAS	cell surface death receptor
FCM	flow cytometry
FDA	Food and Drug Administration

H2AX	H2A histone family member X
γH2AX	phosphorylated minor histone H2A variant
HCC	human hepatocellular carcinoma
HEWL	hen egg white lysozyme
His	histidine
HNSCC	head and neck squamous cell carcinoma
GFP	green fluorescent protein
GLI	C_2-H_2-type ZF transcription factor
GSH	glutathione
IC_{50}	half maximal inhibitory concentration
ICP-AES	inductively coupled plasma-atomic emission spectroscopy
ICP-MS	inductively coupled plasma-mass spectrometry
ID	injected dose
Igepal	nonylphenol ethoxylate
IP3	inositol triphosphate
JC-1	5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimi-dazolylcarbocyanine iodide dye
MCL-1	myeloid cell leukemia protein
MET	tyrosine-protein kinase Met gene
Met	methionine
MMD	mitochondrial membrane depolarization
MPS	mononuclear phagocyte system
MSH2	DNA mismatch repair protein Msh2 gene
MYC	c-myc gene
NB(NaCl)	saline nanobins
NB(Ni,As)	nickel arsenic nanobins
NB-Pt,As	Pt,As nanobins
NB(Pt)	platinum nanobins
NCI-60	National Cancer Institute 60 screen

NSCLC	non-small cell lung cancer
OSCC	oral squamous cell carcinoma
PAH	polyallylamine hydrochloride
PDI	polydispersity index
PDX	patient-derived xenografts
PEG	polyethylene glycol
P53	tumor protein p53
PtAs NPs	PtAs nanoparticles
PtAs@PAH-DXS	PtAs nanoparticles coated in polyallylamine hydrochloride and dextran sulfate
PTEN	phosphatase and tensin homolog protein
RCC	renal cell carcinoma
RES	reticuloendothelial system
RNase A	ribonuclease A
ROS	radical oxygen species
SCLC	small cell lung cancer
SMO	smoothened, frizzled class receptor
STEM	scanning transmission electron microscopy
TEM	transmission electron microscopy
TUNEL	terminal deoxynucleotidyl transferase dUTP nick end labeling
WIP1	protein phosphatase 2C delta
WST-1	cell proliferation assay kit reagent
XIAP	X-linked inhibitor of apoptosis protein
XPC	Xeroderma pigmentosum, complementation group C
XPS	X-ray photoelectron spectroscopy
VHL	gene producing VHL protein

Glossary

List of cell lines

143B	osteosarcoma
A2780	ovarian carcinoma
A2780/CP70	ovarian carcinoma, cisplatin resistant subline
A549	lung carcinoma
Bel-7402	hepatocellular carcinoma
BT-20	breast carcinoma
CAL27	head and neck carcinoma
COC1	ovarian carcinoma
COC1	ovarian carcinoma, cisplatin resistant subline
DMS79	lung carcinoma
H22	murine hepatocellular carcinoma
FADU	head and neck carcinoma
GILM2	breast carcinoma
H69	lung carcinoma
H187	lung carcinoma
H526	lung carcinoma
H 841	lung carcinoma
HSC-2	head and neck carcinoma
HSC-3	head and neck carcinoma
HSC-4	head and neck carcinoma
H460	lung carcinoma
HK-2	renal carcinoma
HuH-7	hepatocellular carcinoma
IGROV-1	ovarian carcinoma
MDA-MB-321	breast adenocarcinoma
OVCA 420	ovarian carcinoma, cisplatin resistant subline
OVCA 429	ovarian carcinoma, cisplatin resistant subline
OVCA 433	ovarian carcinoma, cisplatin resistant subline
OVCAR-3	ovarian carcinoma, cisplatin resistant subline

OVCA 432	ovarian carcinoma, cisplatin resistant subline
Saos2	osteosarcoma
SCC9	head and neck carcinoma
SCC25	head and neck carcinoma
SKOV-3	ovarian carcinoma
SMMC-7721	hepatocellular carcinoma
SY-5Y	central nervous carcinoma
R182	epithelial ovarian carcinoma

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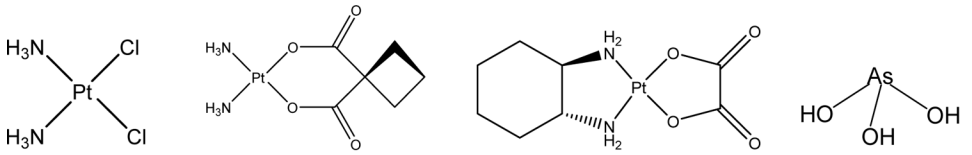
Highlights

- Cisplatin and As_2O_3 act in synergy *in vitro* and *in vivo*.
- Co-encapsulation attenuates the off-target cytotoxicity of both drugs.
- Co-encapsulation increases efficacy in PDX models of cancer.
- Arsenoplatin compounds (AP) contain an $As(OH)_2$ moiety bound to a Pt(II) center.
- Arsenoplatin-1 acts as a dual pharmacophore anticancer agent.



Cisplatin	Carboplatin	Oxaliplatin	Arsenic Trioxide$_{(aq)}$
1978	1989	2002	2000

Fig. 1.

Non-radioactive inorganic anticancer drugs approved by the US FDA with the year of approval.

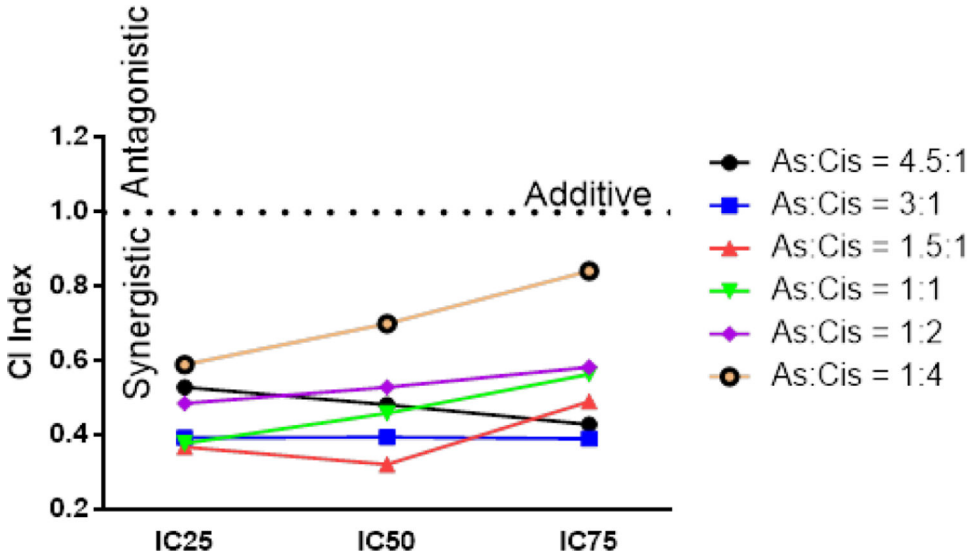


Fig. 2.

Drug combination studies using different cisplatin (Cis) and As_2O_3 (As) ratios in the triple negative breast BT-20 cancer cell line. CI values significantly less than one are found in all cases, confirming strong synergy between the two drugs [89].

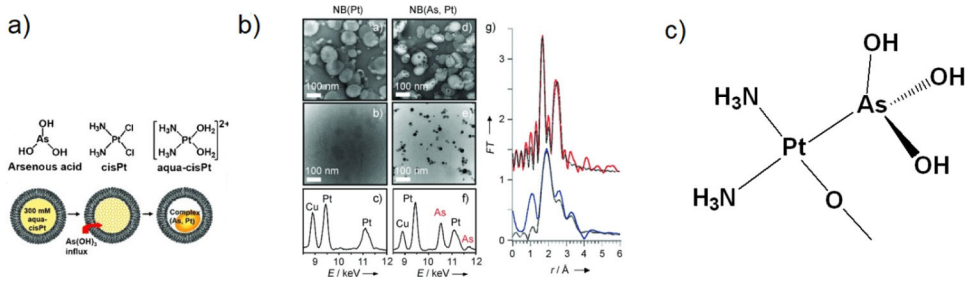


Fig. 3.

a) A method of NB-Pt,As preparation: b) EXAFS shows Pt-As distance of 2.3 Å; c) a Pt-As bond proposed. Modified from Ref. [53] © 2009 Wiley.

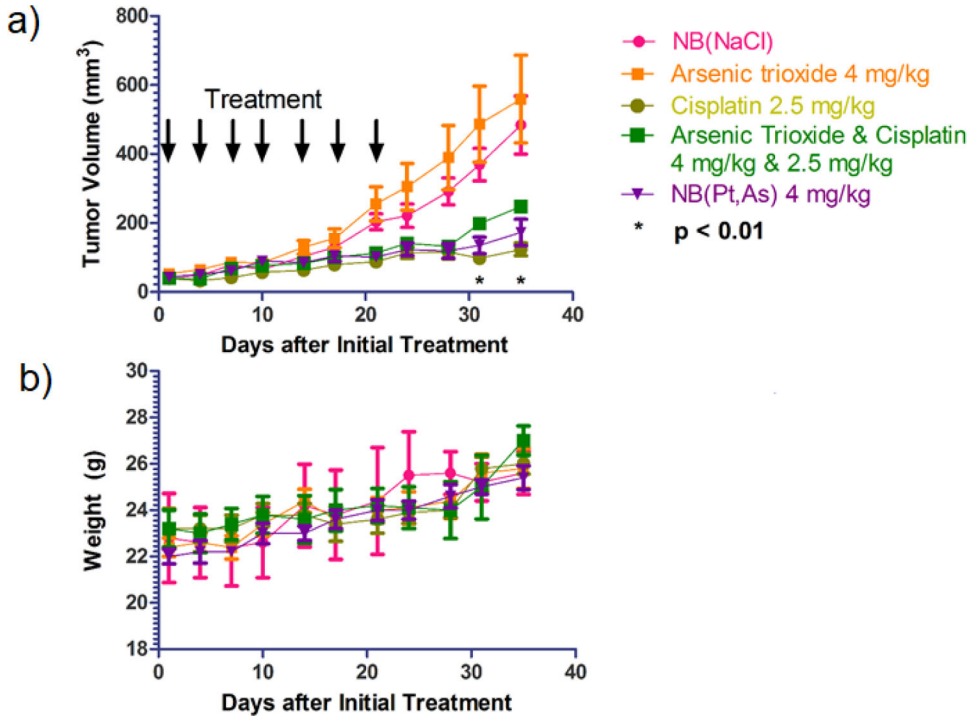


Fig. 4.

NB(Pt,As) show efficacy in a mouse model of triple negative breast cancer: a) Treatment with NB(Pt,As), cisplatin, or cisplatin in combination with arsenic trioxide significantly reduced tumor growth compared to vehicle control or arsenic trioxide monotherapy after day 31($p<0.01$). b) Animal weights were constant or increasing throughout treatment, indicating that treatments were well tolerated [90]

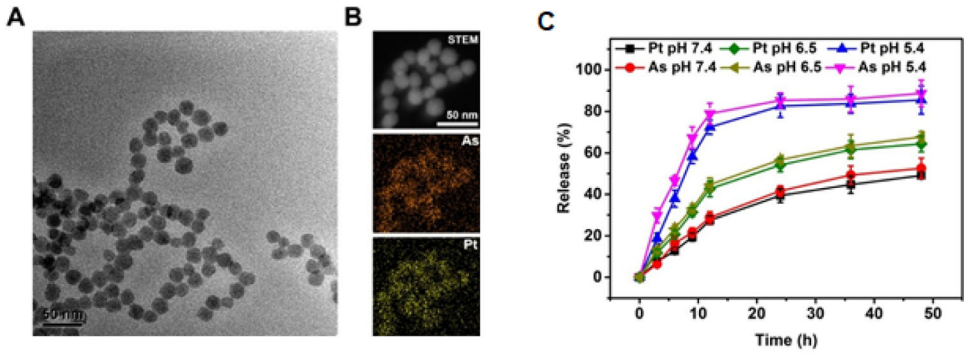


Fig. 5.

(A) A typical TEM image of carrier-free PtAs nanoparticles. (B) STEM and EDS mapping images of PtAs nanocomposites. (C) Releasing profiles of PtAs nanocomposites at pH 5.4, 6.5 and 7.4 at 37 °C (n = 3 per group). Reprinted with permission from Jinhao Gao [54].

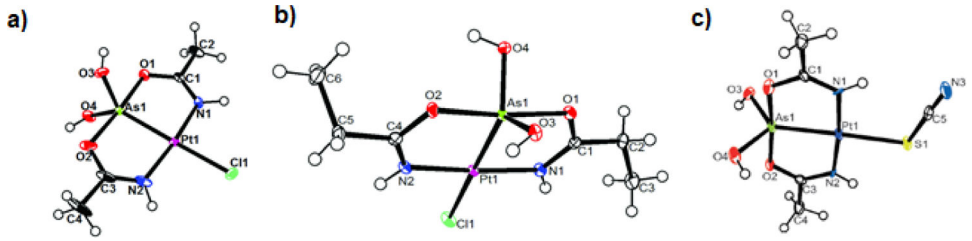


Fig. 6.

Crystal structures of a) AP-1, b) AP-2, and c) SCN^- derivate of AP-1. Modified with permission from Ref. [55] © 2013 Wiley.

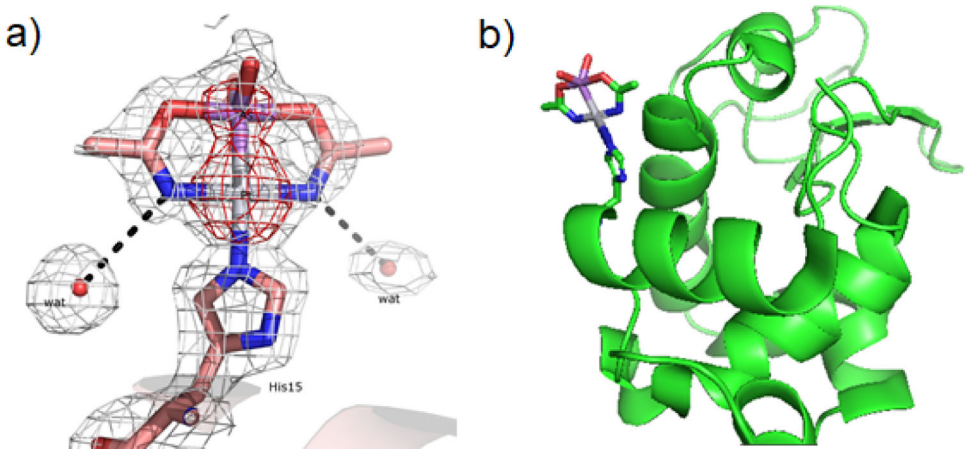


Fig. 7.

a) The surface exposed His side chains are preferred binding site of AP-1 in AP-1-HEWL (shown) and AP-1-RNase A adducts [56], b) AP-1-HEWL adduct (image taken from the PDB, code 5NJ1; the structure was created using PyMol).

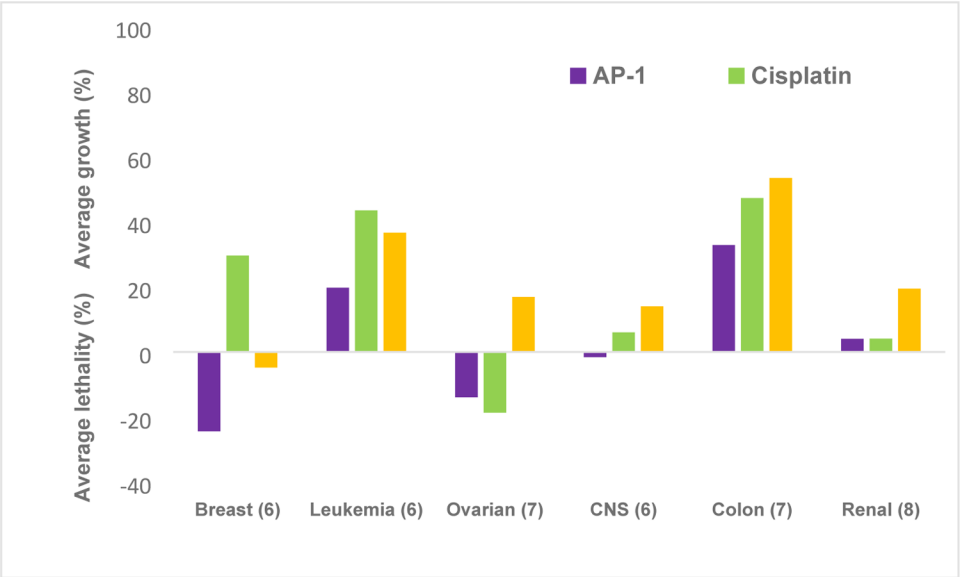


Fig. 8.

Based on the results of the NCI-60 human tumor cell lines screen, AP-1 is more potent than arsenic trioxide in all indications tested. AP-1 is more potent than cisplatin in breast cancer, leukemia, colon cancer, and CNS cancer and is comparable in renal and ovarian cancer cell lines. Numbers in parentheses represent the number of cell lines tested for each indication. Modified from Ref. [56] © 2019 ACS.

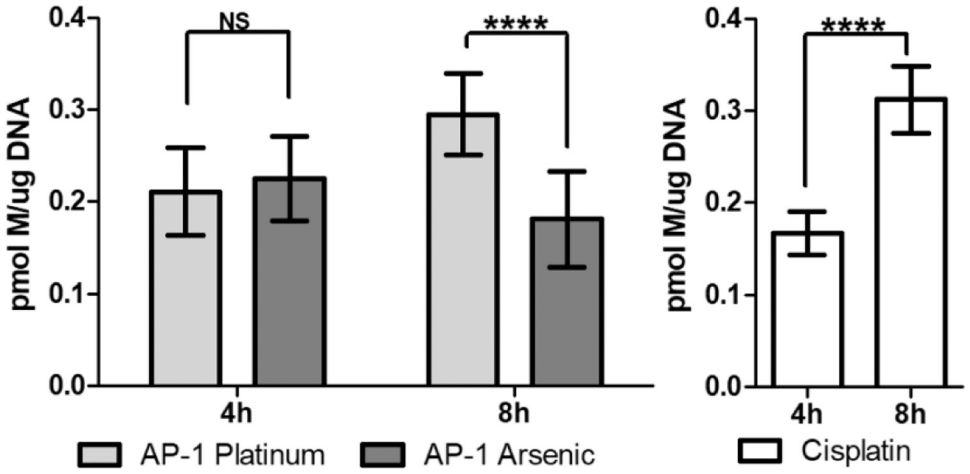
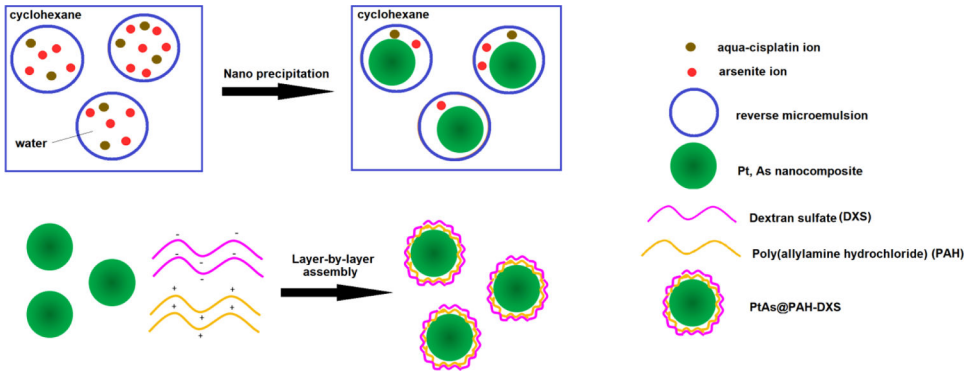


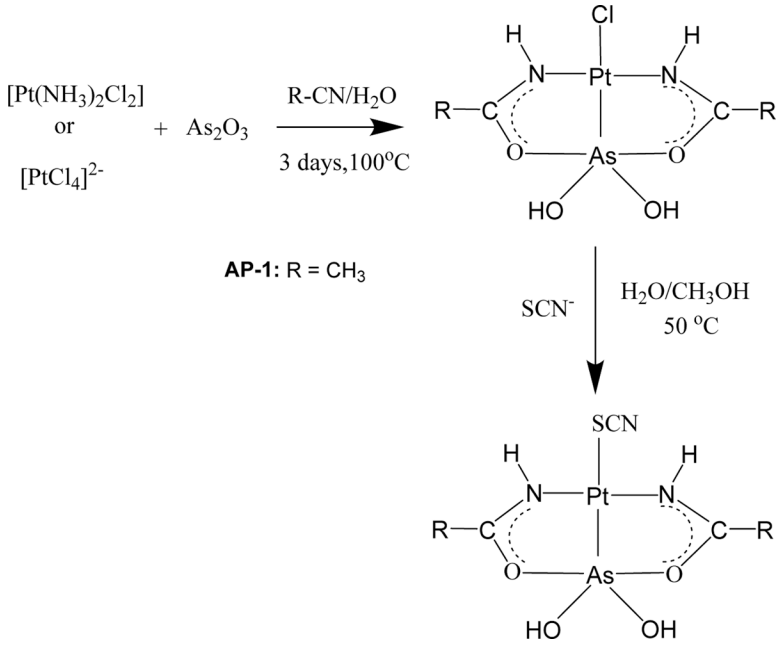
Fig. 9.

MDA-MB-231, triple negative breast cancer cells, were incubated with AP-1 or cisplatin for 4h and 8h, and the extent of adduct DNA formation was determined by ICP-MS, where the amount of platinum and arsenic were in pmolM/μg DNA (M = Pt or As). Results shown are the average of at least five independent experiments. Analysis of one-way-ANOVA using Bonferroni multiple comparisons has shown that the difference in the amount of platinum and arsenic in DNA adducts in an 8-hour experiment is highly significant ($p \leq 0.0001$). Reprinted with permission from Ref. [56] © 2019 ACS.



Scheme 1:

The formation of PtAs nanocomposites and PtAs@PAH-DXS nanodrugs. Modified with permission from Ref. [54] © 2019 RSC.



Scheme 2.

Synthesis of arsenoplatin-1 (AP-1) and its derivative with SCN^-.

Table 1.

Enhanced cytotoxicity of cisplatin in combination with arsenic trioxide

Cancer/Cancer Cells	Effect on cells/cancer	CI/CDI	DRI	Study
Liver/HCC: Bel-7402	Synergistic activity *in vitro* 73.9% tumor growth inhibition *in vivo vs.* 46.2 %	< 1		Wang, *et al.* 2001 [66]
Ovarian/COC1(in suspension), A2780, IGROV-1, SKOV-3, R182	Synergistic activity	0.69–0.93 0.44–0.97	2–5 1.5–13.5	Zhang, *et al.* 2009 [57]
Ovarian/A2780, OVCA 420, OVCA 429, OVCA 433, A2780/CP70, OVCAR-3, OVCA 432, SKOV-3	Enhanced platinum accumulation, DNA binding, and cytotoxicity in WT p53 cells			Muenyi, *et al.* 2012 [58]
Lung/NSCLC: A549, H460	Induction of caspase independent apoptosis	0.5 0.6		Li, *et al.* 2009 [59]
Lung/SCLC: H841, H187, H69, H526, DMS79	Enhanced apoptosis through MMD and reduction of intracellular GSH	0.2–0.8		Zheng, *et al.* 2013 [60]
Head and neck/SCC9, SCC25, CAL27, FADU	Increased apoptosis			Kotowski, *et al.* 2012 [61]
Head and neck/OSCC: HSC-2, HSC-3, HSC-4	Increase in apoptosis, increase in ROS and reduction of MMP, down-regulated mitochondrial cytochrome C, XIAP, BCL-2,	0.78–0.90 0.34–0.45 0.60–0.92	2.15–2.56 3.3–5.1 1.6–2.5	Nakaoka, *et al.* 2014 [62]
CNS/Neuroblastoma SY-5Y	Increased cytotoxicity and apoptosis caused by the flux of intracellular Ca^{2+}			Günes, *et al.* 2009 [63]
Renal/HPV-transformed human tubular cells: HPV-16 E6/E7, HuH-7, HK-2	Enhanced cytotoxicity through downstream of p53 mediator WIP1 and tubulin depolymerization			Dogra, *et al.* 2015 [64]
Bone cancer/143B, Saos2	Synergy in *vitro* and *in vivo*	0.41 0.75		Saitoh *et al.* [95]
Breast/MDA-MD-231, GILM2, BT20	Enhanced apoptosis through reduction of intracellular GSH	0.4–0.9 0.5–0.9 0.40.0.8		Miodragovic, *et al.* [56], Bogachkov [89]

Table 2.

Physical Properties of NB(Pt,As) used *in vivo* experiments

Batch	Elemental Composition[a] (mM) [As]	[Pt]	[P]	Diameter[b] (nm)	PDI[b]	Zeta Potential[b](mV)
1	13.4±0.16	8.1±0.16	16.2±1.5	92.3±3.1	0.02	−5.3
2	15.3±0.59	9.4±0.6	19.47±1.7	89.3±1.4	0.01	−4.6

[a]Measured by ICP-AES;

[b]Measured by dynamic light scattering; PDI = polydispersity index [90].

Table 3.

^{195}Pt NMR shifts (δ)

Complex	δ	Δδ (shift upfield upon Cl^- substitution with DMSO)	Reference
AP-1 (in DMSO-d_6)	−3589		[55]
AP-1 (in CH_3OH-d_4)	−3625		[56]
Cis-[$Pt(NH_3)_2Cl_2$]	−2087		[132]
Cis-[$Pt(NH_3)_2Cl(S\text{-}DMSO)]^+$	−3145	1058	
trans-[$Pt(NH_3)_2Cl_2$]	−2086		
trans-[$Pt(NH_3)_2(S\text{-}DMSO)Cl]^+$	−3112	1026	
[$Pt(en)Cl_2$]	−2345		[133]
[$Pt(en)(S\text{-}DMSO)Cl]^+$	−3307	962	
cis-[$PtCl_2$(1,4-(i-Pr)$_2$dab]	−2300		[134]
cis-[PtCl (1,4-(i-Pr)$_2$-dab)(S-DMSO)] Cl	−3150	850	
[$PtCl_2(H_2bim)$]	−2319		[135]
[$PtCl(S\text{-}DMSO)(H_2bim)]^+$	−3161	842	