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Complete List of Authors:	Marmion, Celine; Royal College of Surgeons in Ireland, Pharmaceutical and Medicinal Chemistry Parker, James; Royal College of Surgeons in Ireland, Pharmaceutical & Medicinal Chemistry Ude, Ziga; Royal College of Surgeons in Ireland, Pharmaceutical & Medicinal Chemistry

Exploiting Developments in Nanotechnology for the Preferential Delivery of Platinum-Based Anti-Cancer Agents to Tumours: Targeting Some of the Hallmarks of Cancer

James P. Parker[a], Ziga Ude[a] and Celine J. Marmion[a]*

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Abstract

Platinum drugs as anti-cancer therapeutics are held in extremely high regard. Despite their success, there are drawbacks associated with their use; their dose-limiting toxicity, their limited activity against a large array of common cancers and patient resistance to Pt-based therapeutic regimes. Current investigations in medicinal inorganic chemistry strive to offset these shortcomings through selective targeting of Pt drugs and/or the development of Pt drugs with new or multiple modes of action. A comprehensive overview of strategies involving the employment of liposomes, nanocapsules, polymers, dendrimers, nanoparticles and nanotubes as vehicles to selectively deliver cytotoxic Pt payloads to tumour cells is provided.

1. Cancer and its Treatment

Cancer is not one disease, but an umbrella term for over 100 different types of distinctive diseases. All forms of cancer are characterised by abnormal cell growth resulting from spontaneous, inherited, or environmentally induced genetic mutations. These cells can then invade adjoining parts of the body and spread to other organs, a process referred to as metastasis which is the major cause of death from cancer.[1] Identifying differences between cancer cells, cancerous tumours, normal cells and normal tissues is of paramount importance if one is to rationally design more efficacious cancer therapies to overcome drawbacks associated with those currently in use.

Numerous strategies have been investigated to combat cancer. These include but are not limited to hormonal therapies and monoclonal antibodies to antibody drug candidates and oncolytic viruses, to molecular therapies such as angiogenesis inhibitors or agents that interfere with the immune system (e.g. immune check point inhibitors and adoptive cell therapy) or the use of multi-targeted or drug combination regimes. [2] An alternative approach is to employ small-interfering RNA or siRNA as a therapeutic platform to modulate the expression of disease-related genes. [3] The purpose of this review however is to showcase how different types of nanotechnologies may be exploited to selectively deliver Pt drugs to tumour cells. While the earliest reports on the therapeutic use of transition metal complexes in cancer date from the sixteenth century it was, however, the serendipitous discovery of the anti-cancer properties of cisplatin, *cis*-$[PtCl_2(NH_3)_2]$, by Barnett Rosenberg in 1965 and its subsequent clinical introduction in 1978 that propelled the scientific community into conducting expansive studies on an array of metals and their respective complexes for therapeutic gain. Now half a century since the anti-cancer properties of cisplatin were first discovered and despite the large number of metals available, the exploitation of Pt for cancer treatment dominates other metal complexes with nearly 50 % of all anti-cancer therapies being Pt based. [4] While clinically very successful, it is surprising to note that only three Pt drugs boast worldwide clinical approval for the treatment of cancer, namely cisplatin, **1**, carboplatin, **2**, and oxaliplatin, **3,** while three others have gained regional limited approval, namely nedaplatin, **4**, heptaplatin, **5**, and lobaplatin, **6**, in Japan, South Korea and China respectively, Figure 1. [4-6]

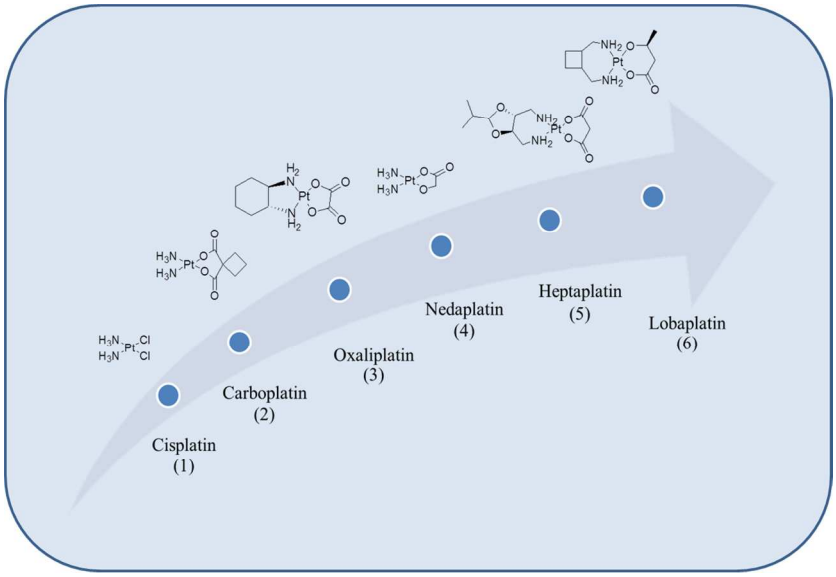


Figure 1: Chemical structures of Pt-based anti-cancer agents that have received worldwide clinical approval, namely cisplatin (1), carboplatin (2) and oxaliplatin (3) and regionally approved drugs nedaplatin (4), heptaplatin (5) and lobaplatin [6]

In total, 22 Pt drug candidates have reached clinical trials.[7] This is surprising given the number of anti-cancer agents that have been investigated. The clinical evaluation of 14 of these drugs was discontinued, Figure 2, because of severe and/or unpredictable side effects, because of a lack of activity in Phase II/III trials, or for economic reasons. [7]

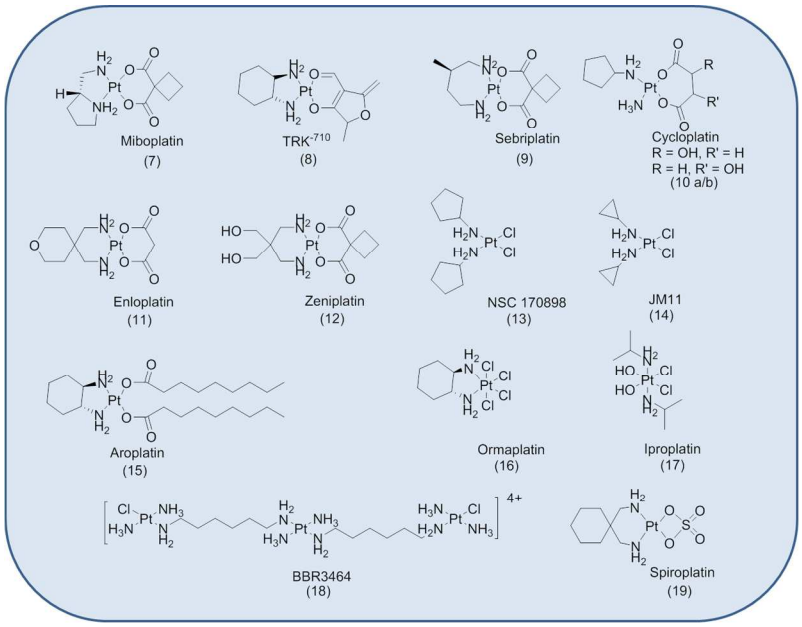


Figure 2: The Pt-based anti-cancer drugs discontinued from clinical trials.[7] Not shown is the liposomal formulation of SP1-77 with Aroplatin™

Currently there are 4 drugs in various phases of clinical trials, namely the Pt(IV) satraplatin, **20** and the liposomal formulation Lipoplatin™, the polymeric delivery system ProLindac™, **21,** and picoplatin, **22**, Figure 3. [6, 7] No new small molecule Pt drug has entered clinical trials since 1999.[7] That said, Pt drug development still remains the subject of many current investigations with a clear shift in focus from drug discovery towards targeted drug delivery in the quest to add to the existing armamentarium of chemotherapeutic agents.

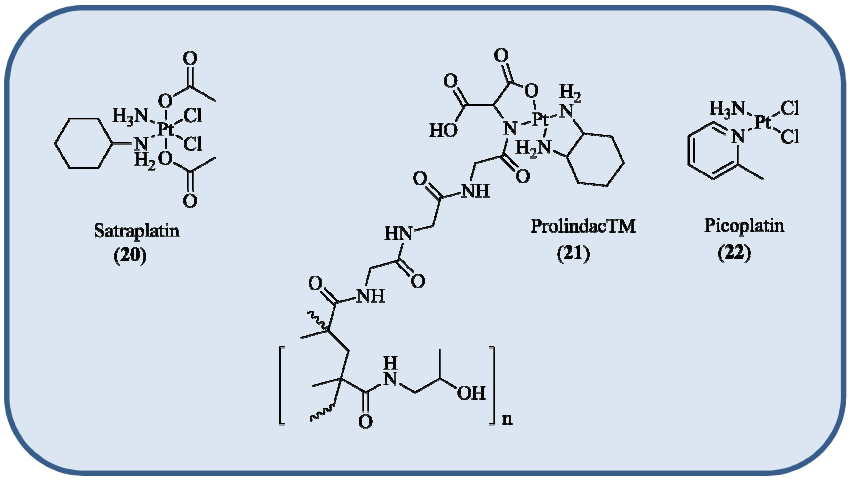


Figure 3: The Pt-based drugs currently undergoing clinical trials as anti-cancer therapeutics. Not shown is the liposomal formulation Lipoplatin™ [7]

2. Recent Advances in Platinum-Based Cancer Chemotherapy

The search for Pt drugs which (i) target cancer cells and/or (ii) have a different mode of action to classical Pt drugs remains the subject of intense investigation. By targeting characteristics unique to cancer we can hope to reduce unwanted side-effects brought about by non-selective targeting while new modes of action may circumvent the intrinsic and/or acquired resistance associated with Pt drugs.

2.1 Platinum Drug Delivery Systems

Extensive studies into tumour vasculature revealed abnormal molecular and fluid transport dynamics. Certain molecules of particular sizes (typically liposomes, nanoparticles, and macromolecular drugs) were identified as having the ability to accumulate in solid tumour tissues much more so than in normal tissues.[8-10] This is not possible for low molecular-weight molecules because of rapid washout by capillary blood flow. This seemingly selective accumulation, coined as the enhanced permeability and retention (EPR) effect, subsequently stimulated efforts into tumour targeting using macromolecular delivery systems such as liposomes, polymers and dendrimers. Such molecular nanostructures with well-defined particle size and shape capable of targeting tumours may overcome some of the shortcomings of existing therapies, including but not limited to poor drug bioavailability, non-specific systemic drug distribution and inadequate drug concentrations reaching the tumour. While this targeting and delivery

strategy clearly offers many advantages, there have as yet been no mainstream drug delivery/targeting technologies approved to date for Pt drugs although some are currently undergoing clinical evaluation.

2.1.1 Liposomes

Liposomes, Figure 4, discovered in the 1960s, are currently one of the most successful and developed macromolecular methodologies used in delivering anti-cancer agents.[11] A liposome is an artificially prepared, self-assembled structure composed of phospholipids in which an outer lipid bilayer surrounds a central aqueous space.

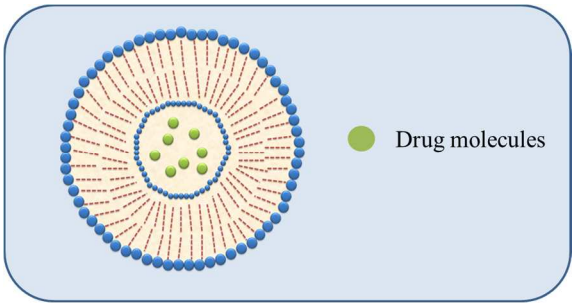


Figure 4: An illustration of a liposome as a macromolecular carrier for the selective delivery of drug molecules

Liposomes offer the advantage of being able to carry hydrophilic as well as hydrophobic drugs; water soluble drugs can be trapped in the interior of the liposome-enclosed aqueous core while the encapsulating bilayer can be used to deliver hydrophobic drugs. Liposomes have several advantages such as biocompatibility, versatility allowing encapsulation (and thus protection from metabolic degradation of biologically active drugs) and the capacity to deliver drugs to a desired location reducing side effects. Their surface properties, size and charge are easily modified during formulation. Such systems have been clinically approved for the anti-cancer therapeutics doxorubicin and paclitaxel.[11, 12] The liposomal formulation of doxorubicin and albumin-based delivery systems for paclitaxel utilise the EPR effect to allow better permeation in cancer tissue, as well as improved retention.[12]

Exploitation of liposomes as delivery vehicles to selectively deliver Pt drugs to tumours is not a new phenomenon. For example, Asefa *et al.* demonstrated time-dependent enhanced cytotoxicity of Pt drugs when loaded into mesoporous silica nanoparticles, MCM-41 and SBA-15.[13, 14] There are now several liposomal formulations of Pt drugs under evaluation with some currently in clinical trials and one already receiving orphan drug status. [15-21] Lipoplatin™, which is a promising cisplatin-liposome formulation, is currently in advanced stages of clinical trials for the treatment of non-small cell lung cancer (NSCLC),[19] HER2/neu negative metastatic breast cancer [18] and advanced gastric cancer.[17] The macromolecular targeting entity, comprising ~9% cisplatin to ~91% lipids (w/w) and having a particle size of approximately 110 nm, displays preferential uptake and retention in cancerous tissue compared to surrounding non-cancerous tissue. The formulation also shows reduced toxicity lacking the serious side effects associated with cisplatin treatment while retaining the same efficacy of cisplatin. [15-21] Lipoplatin™ was granted orphan drug status by the European Medicines Agency for the treatment of pancreatic adenocarcinoma.[20] The molecular mechanisms of Lipoplatin, together with pre-clinical and clinical data, are comprehensively reviewed by

Staphopoulos and Boulikas. [16]

LiPlaCis, another cisplatin-liposomal system, reached phase I clinical trials but the trials were terminated as results indicated no additional benefit over standard cisplatin treatment.[22] SPI-77, another liposomal formulation encapsulating cisplatin, advanced to several phase II studies of patients with inoperable head and neck cancer,[23] advanced NSCLC[24] or Pt-sensitive recurrence of ovarian cancer, but failed to progress despite being less toxic compared to cisplatin, most likely due to slow and inefficient release of its Pt payload.

More recently, the cationic modification of liposomes using the transfection agent polycation polyetheylenimine (PEI), rarely used to generate liposomes for anti-tumour drugs, was employed to successfully deliver cisplatin to A549 cells. [25] A follow up *in vivo* study on a H22 hepatoma-bearing mouse model indicated the liposomal system retained the efficacy of cisplatin and demonstrated reduced nephrotoxicity. [26]

Replacement of the chlorido ligands of cisplatin with either one or two caprylate ligands generated caprylate-cisplatin analogues which were successfully loaded into liposomes with an encapsulation efficiency in the region of 96% demonstrating unprecedented drug loading (0.21 mg cisplatin/mg of lipids) and comparable efficacy to cisplatin against A549 tumour cells.[27]

Liposomes, with their capacity to deliver water insoluble drugs, have also been investigated *in vitro* as potential delivery vehicles for the water insoluble Pt(II) complex, 2-(4-(tetrahydro-2H-pyran-2-yloxy)-undecyl)-propane-1,3-diamminedichloroplatinum (II). Of the seven tumour cell lines evaluated, the lipsomomal formulation was found to be more effective than cisplatin against cisplatin resistant TGCT 1411HP and anaplastic thyroid carcinoma SW1736 cell lines.[28]

Several phase I and phase II clinical studies of a liposomal formulation of an oxaliplatin analogue, *cis-bis*-neodecanoato-*trans-R,R-1,2*-diaminocyclohexane platinum(II) or Aroplatin™ have been undertaken; for example phase I studies in patients with pleural mesothelioma, ovarian cancer and peritoneal carcinomatosis and sarcomatosis and in phase II for mesothelioma and advanced colorectal cancer. [15] In the latter case, in patients with advanced colorectal cancer resistant to 5-fluorouracil/leucovorin, capecitabine or irinotecan, the study indicated good tolerability but limited tumour response. [21] Interestingly, the liposomal carrier itself is thought to play an instrumental role in the cytotoxicity and anti-tumour activity of Aroplatin™.

Lipoxal, another liposomal oxaliplatin formulation, in contrast, was found to be well tolerated by patients with advanced disease of the gastrointestinal system with peripheral neuropathy being the most common toxic side effect.[29]

The transferrin (Tf)-conjugated glutaryl phosphatidylethanolamine liposomal formulation of oxaliplatin, MBP-426, has also demonstrated promise. It binds to transferrin receptors which appear to facilitate preferential tumour targeting. It is currently in phase II clinical trials.[30-32]

Pre-clinical studies of polyethylene glycol (PEG)ylated carboplatin liposomes on SGC-7901 gastric cell bearing mice indicated promising anti-tumour and anti-metastatic effects.[33]

Oxaliplatin is currently used to treat certain (wild-type KRAS) metastatic colorectal cancers expressing epidermal growth factor receptor (EGFR) in combination

with the monoclonal antibody Cetuximab. Garrido *et al.* successfully linked Cetuximab to oxaliplatin-loaded EGFR-targeted liposomes in a pioneering study. This facilitated the selective delivery of both drug entities in a single therapeutic approach. The liposomes demonstrated enhanced tumour drug accumulation as compared to free oxaliplatin or a non-targeted liposome as well as having improved efficacy in mice inoculated with a colorectal cancer cell line which over expresses this receptor. This approach was also shown to overcome resistance associated with classical Pt drugs in that targeted delivery was also demonstrated in oxaliplatin resistance cell lines.[34]

The reader is directed to a series of reviews on the use of liposomes to selectively deliver Pt drugs to cancer cells. Liu *et al.* provide a comprehensive review of the lipid compositions, physical properties, loading methods and drug-to-lipid ratios of Aroplatin, SPI-77, Lipoplatin, Lipoxal and LiPlCis together with their pharmacokinetic, biodistribution and toxicity profiles and therapeutic efficacies both in pre-clinical animal studies and in patients.[35] Wang and Guo review different drug targeting and delivery (DTD) strategies for enhancing efficacy of Pt drugs whilst reducing side effects. [36] Despite the advancement of some liposomal formulations of Pt drugs in clinical trials, challenges remain as outlined in reviews by Kieler-Ferguson et al. [37] and Zalba and Garrido.[38] The use of liposomes, polymeric nanocarriers and carbon nanotubes to selectively deliver oxaliplatin to tumours are reviewed by Lila *et al.* [39]

Despite the aforementioned success in exploiting liposomes as Pt-drug nanocarriers, there remains a need to overcome the existing drawbacks with liposomal formulation. These include poor storage stability, rapid clearance from the bloodstream, and non-specific uptake by the mononuclear phagocytic system. Furthermore, the limited volume of the lipid bilayer makes the delivery of hydrophobic drugs highly inefficient as well as the current lack of functionalisation for targeted delivery to non-tumour based cancers. In contrast, other nanostructures such as polymers and dendrimers offer a higher degree of functionalisation accommodating the possibility of creating specific cell targeted structures.

2.1.2 Nanocapsules

Lipid-coated nanocapsules, Figure 5, offer an advantage over liposomes in that they are capable of carrying a much greater drug load. They are vesicular systems consisting of a polymeric membrane which encapsulates an inner liquid core at the nanoscale level.

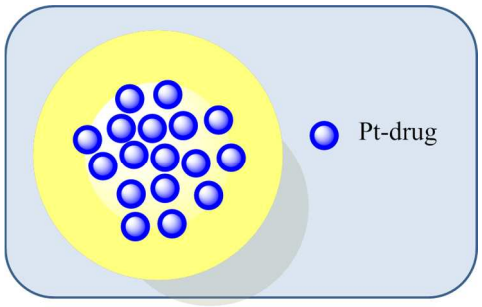


Figure 5: An illustration of a nanocapsule as a macromolecular carrier for the selective delivery of Pt drug molecules

In 2002, Burger *et al.* utilised this technology to encapsulate cisplatin with high encapsulation efficiency resulting in the formation of small aggregates of cisplatin enveloped by a single lipid bilayer. The resulting Pt-loaded nanocapsules had unprecedented drug to lipid ratio and up to 1000-fold greater *in vitro* cytotoxicity as compared to the free drug.[40] Building on this work, the same group examined the molecular architecture of these Pt-loaded nanocapsules using solid state NMR spectroscopy and demonstrated that the nanocapsule core consisted of solid cisplatin (with ~90% present as the dichloro species) and was devoid of water. [41] A further study demonstrated that while Pt loading in nanocapsules was highly efficient, stability was a potential issue. Adjusting the formulation to include a poly(ethylene glycol 2000) (PEG)-derivatised phosphatidylethanolamine and cholesterol in the bilayer coat was shown to extend the lifetime of the cisplatin nanocapsules in mouse serum.[42] The high cytotoxicity associated with the cisplatin nanocapsules in ovarian cancer cells was later shown to require caveolin-1-dependent endocytosis. [43] An *in vivo* study was conducted in nude mice bearing human ovarian carcinoma OVCAR-3 xenografts to investigate the anti-cancer efficiacy and biodistribution of PEGylated cisplatin nanocapsules as compared with those of the free drug. The Pt-nanocapsules and cisplatin were found to inhibit the growth of the OVCAR-3 xenografts in nude mice to a similar extent and the concentration of Pt in both plasma and tumour were found to be similar for both formulations. The Pt derived from the nanocapsules was however shown to rapidly accumulate in the liver (4.5-fold higher accumulation as compared to cisplatin alone), and was also shown, albeit at a slower rate, to accumulate to a high concentration in the spleen. Rapid clearance from circulation appeared to be a factor contributing to the efficacy of these Pt-nanocapsules. [44]

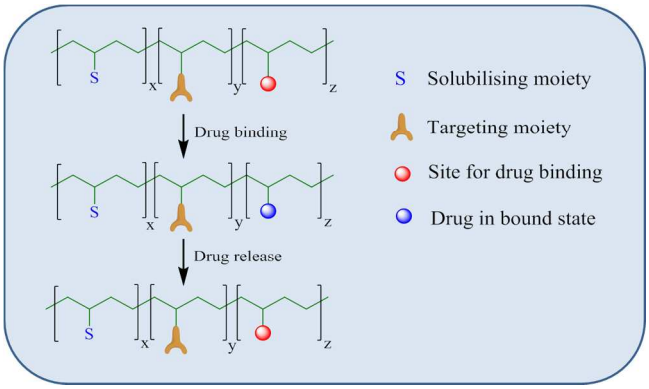
Nanocapsules incorporating carboplatin have also been developed and have been found to exhibit 1,000-fold greater cytotoxicity against a range of tumour cell lines as compared to carboplatin alone. This significant increase in cytotoxicity is thought to be associated with greater Pt accumulation in tumour cells resulting from uptake of the formulation by endocytrosis. [45]

Bryde and de Kroon provide a comprehensive review of Pt nanocapsules and their potential as chemotherapeutic agents. [46]

2.1.3 Polymers

Multi-component polymer-based drugs and delivery systems have been investigated with a view to overcoming the inherent instability and degradation associated with liposomal formulations. Polymeric systems exhibit an array of attractive properties as therapeutic agents through their high degree of variable functionalisation, including; high stability *in vivo,* allowing for prolonged time in the circulatory system; a slower rate of dissociation that allows retention of the therapeutic payload for a longer period of time and a high drug loading capability. [12] Typically, polymeric systems have as a minimum a tripartite design, Scheme 1, with (i) a tri-block polymer containing the polymer, (ii) the linker and (iii) the payload (drug) which is subsequently released at the target site. Sometimes targeting moieties, antibodies, proteins, peptides and other small molecules and/or imaging agents are also included. The linker is often designed to release the drug through hydrolytic or enzymatic cleavage. In contrast to the cleavable linker, non-degradable spacers are also often used to allow attachment to, for example, targeting or solubilising units that need to remain adhered to the polymer. The non-toxic and water soluble properties of the polymer *N*-(2-hydroxy)propylmethacrylamide (HPMA) in addition to its biocompatible profile and its previous use as a plasma expander made this polymer a potential candidate as a macromolecular delivery system for therapeutic agents.[47] The first macromolecular pro-drug system to utilise HPMA was

PK1 which conjugated the polymer to doxorubicin *via* a tetrapeptide spacer, the latter of which is subject to enzymatic cleavage within the tumour tissue with subsequent release of the therapeutic payload. [48]



Scheme 1: A schematic representation of the binding of a drug to a polymer and its release at its intended target. Adapted from Neuse *et al*. [49]

Early studies focussed on conjugating carboplatin to HMPA *via* a malonate end group of the polymer (AP5280).[50] Although progressing to clinical trials, it is the nanoparticulate polymer pro-drug bound to the Pt anti-cancer agent, ProLindac™ (AP5346), **21**, Figure 3, which is dominant in this domain. It utilises a 25 KDa delivery vehicle based on hydrophilic HMPA to target the active form of oxaliplatin to tumour cells. [51, 52] The oxaliplatin analogue was covalently bound to the polymer *via* a pH sensitive amidomalonate linker that releases the active form of oxaliplatin in the more acidic environment associated with many solid tumours, compared to surrounding normal tissues. This macromolecular system was designed to be relatively non-toxic while in general circulation. Pre-clinical data in 10 tumour models showed that the polymeric delivery system was never inferior to oxaliplatin and often markedly superior and capable as evident in the B16 melanoma tumour model of the study. Studies extended into phase I and phase II clinical trials for patients with reoccurring ovarian cancer and these indicated that ProLindac™ exhibited equal or greater efficacy than oxaliplatin. [51] The patients also demonstrated a higher tolerability attributed to the ability of ProLindac™ to deliver the oxaliplatin directly to the tumour. However, its clinical evaluation was subsequently discontinued due to inconsistencies in its formulation. Several Phase II combination studies in which ProLindac™ is administered in combination with other chemotherapeutics such as paclitaxel and gemcitabine in patients with solid tumours including colorectal and ovarian cancer are currently underway.[15]

Exploitation of the lower critical solution temperature (LCST) i.e. the critical temperature below which the components of a mixture are miscible for all compositions is another avenue receiving attention of late. This property has been exploited in the development of thermosensitive Pt(II)-cyclotriphosphazenes in which (diammine)Pt(II)–cyclotriphosphazenes were conjugated to alkoxy polyethylene glycol. These were found to exhibit variable LCST in the wide range of 12 to 92 °C and were subsequently assessed for their anti-cancer activity both *in vitro* and *in vivo*. [53, 54] These conjugates offer several advantages over unconjugated cancer drugs or those physically loaded in polymeric matrices such as liposomes. For example, the conjugates with LCST below

body temperature offer the possibility of direct application of the nanoparticulate to the tumour *via* intra-tumoural injection where degradation of the phosphazene ring results in the release of the (diammine)Pt(II) cytotoxic agent *in vivo*. Another advantage is the reproducible formulation of the Pt-polymer conjugates as well as their high degree of functionality enabling modification of their LCST and solubility. They are also capable of carrying and delivering high quantities of the therapeutic agent. Each investigated conjugate showed a markedly higher accumulation within the chosen cells when compared to cisplatin demonstrating potent anti-cancer activity both *in vitro* and *in vivo* against murine L1210 with the effective dose (ED_{50}) comparable to cisplatin and a mean survival time for the mice much greater than those treated with cisplatin or carboplatin.

Other polyphosphazene-Pt derivatives have also been synthesised and their *in vivo* potential evaluated.[53, 55] One of the more promising results involved the modification of an amphiphilic polyphosphazene using pegylation and its conjugation to the anti-tumour diaminocyclohexane Pt(II) moiety. The resulting Pt-polymer conjugate was shown to accumulate in tumour tissue to a much greater extent as compared to normal tissue (tumour/tissue ratio > 4) and exhibited high *in vitro* cytotoxicity against human cancer cell lines.[56] Amphiphilic polyphosphazene-Pt conjugates capable of assembling into stable nanoparticles with a mean diameter of approximately 90–200 nm have also more been reported.[57] These conjugates exhibited good activity against a selection of human tumour cell lines but had lower *in vitro* cytotoxicity as compared to cisplatin.

Jain *et al.* developed orally active hyaluronic acid coupled chitosan nanoparticles bearing oxaliplatin encapsulated in Eudragit S100 for colonic delivery of oxaliplatin. Superior efficacy was demonstrated in a HT-29 murine tumour model with high local accumulation of the nanoparticles in colonic tumours over a prolonged period of time.[58]

Lippard *et al.* loaded the Pt(IV) prodrug of cisplatin into poly(D,L-lactic-co-glycolic acid) (PLGA)-PEG-functionalized polymers decorated with prostate-specific membrane antigen (PSMA) targeting aptamers for enhanced targeted delivery to and in vitro cytotoxicity against prostate cancer cells. Release of the produg was facilited upon cell entry whereupon reduction to cisplatin occurred with subsequent formation of cisplatin 1,2-intrastrand d(GpG) cross-links on nuclear DNA. The efficacy of the Pt-loaded NPs against against the PSMA(+) LNCaP cells was found to be an order of magnitude greater than cisplatin alone. [59] A later *in vivo* study confirmed the therapeutic efficacy of these Pt-PLGA-b-PEG-Apt-NP with improved pharmacokinetics, biodistribution, tolerability and efficacy when compared to cisplatin. [60]

The expression of integrins, transmembrane proteins involved in cell adhesion and cell signalling, is commonly upregulated in inflammatory diseases and in cancers. The α(v)β(3) integrin in particular is differentially upregulated on angiogenic endothelial cells in addition to many tumour cells. As such, integrins have also been the subject of investigation for targeted drug delivery. Lippard *et al.* rationally designed cisplatin prodrug loaded PLGA-PEG NPs with differential targeting to the α(v)β(3) integrin on cancer cells using the cyclic pentapeptide c(RGDfK). These nanoparticles, as compared to cisplatin, had enhanced efficacy against prostate and breast cancer epithelial cells as well as being more efficacious and better tolerated in an orthotopic human breast cancer xenograft model *in vivo*. [61]

Sadhukha and Probha likewise successfully exploited PLGA nanoparticles but, in their case, for the targeted delivery of carboplatin. The greater cytotoxicity of the carboplatin-loaded nanoparticles against several cell lines as compared to carboplatin

alone was attributed to enhanced and more rapid intracellular accumulation and greater distribution within the cell nucleus. [62]

While much research has focused on exploiting pH or the reduction environment of tumour cells to mediate drug release from nanocarriers, there are only a few examples of systems specifically responsive to physiological levels of H_2O_2 (50-100 mM). [63-65] Guo, He *et al.* have recently developed an innovative H_2O_2-responsive PLGA-based nanocarrier incorporating cisplatin and catalase, the latter acting as an O_2-generating agent. Upon intracellular H_2O_2 penetration, catalysed by catalase, O_2 is released which results in an increase in pressure and generation of high levels of ROS species. The increase in pressure causes rupturing of the NP and release of the drug payload. An enhancement in cytotoxicity was observed for the carrier system as compared to cisplatin. Moreover, release of O_2 was found to overcome hypoxia-induced multi-drug resistance in cancer cells. This system is the first of its kind to integrate chemotherapy and oxygen therapy in a synergistic manner. [66]

A rationally designed biodegradable beta-casein–chitosan nanocarrier system loaded with the bipyridine morpholine dithio-carbamate Pt(II) nitrate developed by Razmi *et al.* has also shown promise for targeted oral delivery applications. This study investigated the influence of pH on the formation of these stable colloidal systems with a pH of 5.7 being optimal for particle formation. This carrier system was found to have enhanced cellular uptake into and greater efficacy against colorectal carcinoma HCT116 cells as compared to the free Pt complex. [67]

Condensation polymerisation was employed to generate backbone Pt(IV)-coordination polymers using either diamminedichlorodihydroxyplatinum or its dicarboxyl derivative diamminedichlorodisuccinatoplatinum as comonomers. [68, 69] *In vitro* studies confirmed that these polymers were cytotoxic against a range of tumour cell lines and an *in vivo study* demonstrated that, in comparison to the monomer diamminedichlorodisuccinatoplatinum, the polymers had slower blood clearance, enhanced tumour accumulation and lower levels of Pt were found in most normal organs. [69]

Self-assembly, under aqueous conditions, of linear-brush grafted copolymers forming inter-polymer complexes by H-bonding via pH control afforded robust cross linked polymers capable of conjugating cisplatin with high loading efficiency and steady release rate. [70]

Combining drug delivery with biomedical imaging can be highly advantageous as you can not only selectively deliver your drug payload to its intended target but you can track its journey along the way. This is precisely what Lin *et al.* set out to achieve. They rationally designed and developed multifunctional upconversion nanocrystals/polymer nanocomposites for delivery of the Pt(IV) prodrug of cisplatin to tumours by linking the Pt(IV) prodrug to the amphiphilic tri-block copolymer, methoxyl-poly(ethylene glycol)-block-poly(3 caprolactone)-block-poly(L-lysine) or mPEGb-PCL-b-PLL. Rhodamine B was linked to the same polymer to form conjugates which could co-assemble into fluorescent miscelles, facilitating both *in vitro* and *in vivo* imaging. Entry into HeLa cells and tumour tissue was shown to be via endocytosis whereupon release of the cytotoxic Pt load took place. This study is an excellent example of how theranostics may be exploited to not only deliver cytotoxic payloads but to gain a deeper insight into the delivery route and mechanism of action of the drug molecules. [71] Guo, Wang et al. have likewise developed fluorescent theranostic maghemite Nps incorporating cisplatin that display high cytotoxicity towards cisplatin-resistant cell lines.

They too were able to track drug distribution both *in vitro* and *in vivo* using confocal fluorescence imaging. [72]

Jing, Zhang *et al.* developed camplatin, a Pt(IV) hybrid prodrug derived from cisplatin and the medicinal plant camphor. Conjugation of camplatin onto the amine groups of the amphiphilic biodegradable polymer MPEG-b-PCL-b-PLL afforded a macromolecular prodrug which was found to have superior efficacy as compared to cisplatin to both cisplatin sensitive and cisplatin resistant cell lines. This enhanced cytotoxicity was attributed to the ability of the macromolecular prodrug to efficiently and effectively enter the tumour cells via endocytosis and, upon release, down-regulate the anti-apoptotic gene Bcl-2. There was little effect on the pro-apoptotic gene Bax. [73]

Kim *et al.* have reviewed progress over the past five years in relation to the application of polymeric NPs for the delivery of Pt-based chemotherapeutics. [74]

2.1.4 Dendrimers

Repetitively branched molecules known as dendrimers (derived from the Greek 'dendron' meaning tree, and 'meros' meaning part), Figure 6, have been shown to accumulate more selectively in cancerous cells over normal cells due to the EPR effect. As such, they exhibit a myriad of possibilities for anti-cancer drug delivery as their structure allows for functionalisation, encapsulation into the dendrimer interior, or conjugation of numerous molecules on the surface or at the core of the dendrimer *via* chemical attachment or physical adsorption.[53, 54] Dendrimers present a high level of control over their architectural design; including their size, shape, branching length/density, and their surface functionality making these structures unique and optimal particulates for therapeutic exploitation. In conjunction with this, the *exo* presented surface groups allow for the attachment of targeting groups, thus enhancing the biological profile of these multifaceted agents, Figure 6.

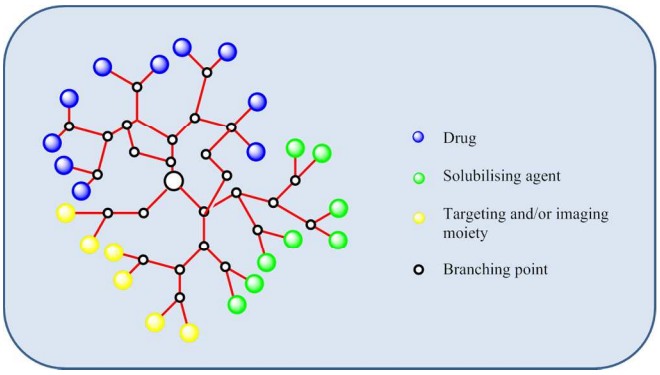


Figure 6: An illustration of a possible design for a dendritic drug carrier which displays the flexibility and versatility that dendritic systems provide.

The most frequently reported dendrimers are polyamidoamines (PAMAM).[75] These have been conjugated to cisplatin *via* a functionalised sodium carboxylate surface.[76] The conjugates demonstrated increased solubility, high loading capacity, decreased systemic toxicity, selective accumulation in solid tumours and the ability to slowly release cisplatin *in vitro*. Of note was the ability of this Pt-dendrimer to retard the growth of the subcutaneous B16F10 murine melanoma in contrast to cisplatin alone

which failed to demonstrate any anti-tumour activity. An investigation by Wheate *et al.* into the use of anionic PAMAM dendrimers as delivery vehicles for the passive targeting of Pt drugs to solid tumours by the EPR effect demonstrated that only a fraction of the Pt drug is released, most likely a function of non-reversible coordinate bond formation between the Pt moiety and the amine and amide groups within the dendrimer branches. Whilst the dendrimer exhibited no cytotoxicity in A2780 ovarian tumour cell lines, moderate cytotoxicity was observed for the Pt-dendrimer conjugate. *In vivo* studies using an A2780 tumour xenograft showed more promising results.[77]

The encapsulation efficiency, *in vitro* cytotoxicity and cellular accumulation of cisplatin-loaded biotinylated PAMAM dendrimers have also been reported. Encapsulation efficiency ranged from 5-21%. The cisplatin-dendrimers exhibited enhanced cytotoxicity as compared to cisplatin alone against a range of ovarian cancer cell lines and improved cellular accumulation. [78]

Oxaliplatin-pegylated dendrimer conjugates as pH responsive drug delivery vehicles have also been recently investigated.[79] These pH-sensitive conjugates demonstrated greater efficacy as compared to oxaliplatin alone in a SKOV-3 human ovarian xenograft without inducing toxicity.

Optimisation of a carboxylate-terminated Pt-PAMAM dendrimer formulation has very recently been reported with respect to varying dendrimer core, generation, drug entrapment, purification, yield, reproducibility, stability, storage and *in-vitro* release.[80]

2.1.5 Nanotubes

The unique and intrinsic physical, mechanical and chemical properties of carbon nanotubes (CNTs), Figure 7, have also stimulated efforts into exploring their potential medical applications. CNTs are allotropes of carbon with a cylindrical, tubular structure in the nanoscale range. Depending on the number of layers they can be either single-walled or multi-walled nanotubes (SWCNT or MWCNT respectively). Their application spans many fields due to their dynamic properties and have proved valuable in nanotechnology, electronics, optics, and other fields in material sciences and technology. Typically, they are constructed with diameters of 1~2 nm, and lengths ranging from as short as 50 nm up to 1 cm, i.e. a length-to-diameter ratio of up to 132,000,000:1. [81]

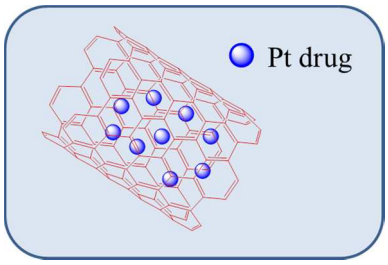


Figure 7: An illustration of a SWCNT as a Pt drug carrier

CNTs provide very high surface area per unit weight facilitating high drug loading. Plenty of inner spaces facilitate incorporation of drugs while the tube walls allow for drugs and other functional molecules (imaging/targeting agents) to be physically adsorbed. Also, the ends and side holes can be oxidised to afford functional

groups where covalent attachment of useful moieties is possible. The surface holes can be also plugged with drugs.

Pioneering work by Ajima *et al.* demonstrated that cisplatin could be incorporated into and subsequently released from single walled carbon nanotubes (SWCNTs).[82] They later found that incorporation and release of cisplatin from these SWCNTs could be enhanced through chemical modification of the SWCNTs structural holes [83] or by changing the solvent system during the preparation of these 'nanohorns' [84] with promising *in vitro* and *in vivo* results.

A separate molecular modelling study conducted by Hilder and Hill demonstrated that in order for a nanotube to host cisplatin, its radius must be a minimum of 4.8 Å while the maximum uptake of cisplatin was observed when the radius of the nanotube was approximately 5.3 Å.[85]

Cisplatin encapsulation into ultra-short SWNTs wrapped with Pluronic-F108 surfactant (used to control cisplatin release) resulted in nanotubes which exhibited enhanced cytotoxicity over free cisplatin against two different breast cancer cells lines, MCF-7 and MDA-MB-231 after 24 hours.[86]

Lippard *et al.* utilised an amine-functionalised SWCNT as an effective tool to deliver a Pt(IV) prodrug, *cis*,*cis*,*trans*-$[Pt(NH_3)_2Cl_2(OEt)(O_2CCH_2CH_2CO_2H)]$ to tumour cells. *cis*,*cis*,*trans*-$[Pt(NH_3)_2Cl_2(OEt)(O_2CCH_2CH_2CO_2H)]$ was tethered to the nanotube *via* a peptide linkage. The resulting water soluble nanotube was shown, by atomic absorption spectroscopy, to carry an average of 65 Pt(IV) centres per nanotube. They anticipated that once inside the reducing environment of tumour cells, the Pt(IV) prodrug would be reduced to the cytotoxic *cis*-$Pt(NH_3)_2Cl_2$ or cisplatin. The Pt(IV) precursor and the amine-functionalised SWCNT alone were shown to be relatively non-toxic as compared to cisplatin against the testicular carcinoma cell line NTera-2. The SWCNT-Pt(IV) conjugate, in contrast, was significantly more cytotoxic as compared to the free Pt(IV) prodrug and was shown to surpass that of cisplatin when compared on a per Pt basis.[87] They also exploited the use of folic acid (FA) as a means of drug targeting given that many cancer cells overexpress the folate receptor. The Pt(IV) complex *cis,cis,trans*-$[Pt(NH_3)_2Cl_2(O_2CCH_2CH_2CO_2H)(O_2CCH_2CH_2CONH\text{-}PEG\text{-}FA)]$, bearing the folate derivative in the axial position, was tethered to the surface of an amine-functionalised SWCNT through multiple amide linkages. Release of the Pt payload was facilitated upon reduction of the Pt(IV) to Pt(II) inside tumour cells whereupon the Pt(II) adduct was shown to form 1,2-intrastrand cross links with nuclear DNA. This is the first example of a construct containing both the targeting and delivery moieties in one molecule.[88]

Lippard *et al.* subsequently exploited Au-NPs as an alternative delivery system in which the Au-NPs were functionalised with thiolated 28mer oligonucleotides containing a terminal dodecyl amine for conjugation. *cis,cis,trans*-$[Pt(NH_3)_2Cl_2(OH)(O_2CCH_2CH_2CO_2H)]$, an inactive Pt(IV) cisplatin prodrug, was tethered to an amine functionalised DNA-Au NP surface *via* amide linkages which could then be activated by reduction in the acidic environment in cancer cells.[89] *In vitro* studies confirmed the parent Pt(IV) complex to be relatively inactive but subsequently made active against several cancer cell lines when attached to Au-DNA NPs. Fluorescence spectroscopy revealed HeLa cells incubated with the conjugate displayed its localisation within cell vesicles after 6 hours and within the cytosol after 12 hours. The cytotoxicity profiles of Pt-DNA-Au NPs in human lung carcinoma A549, human prostate cancer PC3, cervical cancer HeLa, and human osteosarcoma U2OS cells showed the conjugate to be more toxic relative to cisplatin.

Liu *et al.* employed pegylated Au nanorods conjugated to the Pt(IV) prodrug of cisplatin whereupon cell entry, the Pt(IV) is reduced to Pt(II) with subsequent release of cisplatin from its carrier, Figure 8. [90] The rationale for choosing pegylated Au nanotubes was based on the premise that they have been proven to be highly stable and relatively non-toxic *in vivo*. In addition, such carriers can mask the pegylated agent from the host's immune system thus reducing immunogenicity and antigenicity. [91] These Pt-loaded nanotubes were found to be stable under physiological conditions, demonstrated enhanced cellular accumulation of the Pt prodrug and had a significantly higher cytotoxicity profile as compared to cisplatin against a range of cancer cell lines. [90] Building on this work, the same group demonstrated that this same Pt(IV)-carrier system avoided the types of drug resistance associated with cisplatin use. For example, endocytosis was found to be the route of entry for these carriers in contrast to the resistance-associated uptake mediated by the copper transport protein Ctr1. Utilising the more inert Pt(IV) prodrug of cisplatin overcame issues around deactivation by glutathione-S-transferase and metallothioneins, found in high concentrations in the resistant A549R cell lines tested. These pegylated Au nanorods conjugated to the Pt(IV) prodrug of cisplatin were found to be highly cytotoxic to these resistant cells. [92]

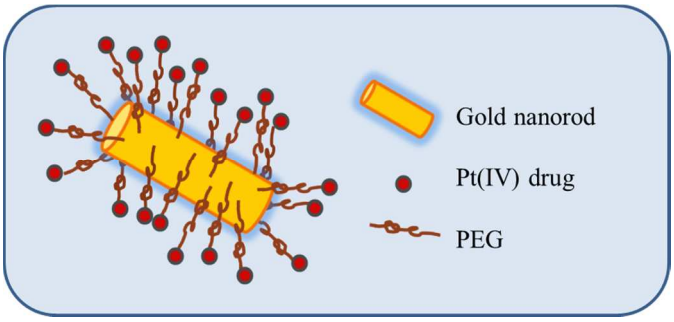


Figure 8: An illustration of a Au NP as a delivery vehicle for Pt(IV) prodrugs of cisplatin

A nanotube consisting of a modified SWNT attached to cisplatin and epidermal growth factor (EGF) was reported by Rusling, Gutkind, Patel *et al.* This bioconjugate capitalises on the specific affinity for the EGF for its cognate cell surface receptor, expressed on most squamous cancer cells. *In vitro* and *in vivo* imaging and cytotoxicity studies indicated that the targeted drug delivery system selectively and effectively targeted squamous cancer cells that over express EGF receptors with cell entry occurring *via* a receptor-mediated endocytosis pathway, accompanied by a less specific and less efficient secondary cell-internalisation.[93]

In another approach to selectively target cancer cells, Liang, Wang, Zhang *et al.* developed neuropilin-1-targeted Au NPs to enhance tumour penetration of Pt(IV) drugs, thereby increasing their therapeutic efficacy. Neurophil-1 (Nrp-1) is a transmembrane glycoprotein which is expressed by a large variety of tumours. They constructed glutathione-stabilised Au NPs together with a Pt(IV) prodrug of cisplatin and functionalised with the targeting neuropilin-1 targeting peptide CRGDK in a single platform. Glutathione was chosen because of its well established anti-oxidant properties which can lead to tumour regression. These Au NPs were found to be more cytotoxic towards prostate cancer cells that overexpress Nrp-1 receptors due to greater cell penetration and internalization efficiency as compared to the NPs without the targeting peptide. [94]

Nanotubes have also been exploited as carriers of carboplatin with activity against urological tumour cell lines enhanced following treatment with the carboplatin-loaded nanotubes as compared to the free drug. [95]

The use of PEGylated MWCNTs for encapsulation and sustained released of oxaliplatin has also been investigated and compared with non-PEGylated CNTs by Wu *et al.* After 20 hours, 80% of oxaliplatin had been released from the non-PEGylated CNT in contrast to 50% of the PEGylated derivative. These findings were consistent with relative cytotoxicities measured over this timeframe. Cytotoxicities were greatly enhanced after 48 and 96 hours most likely due to increased cellular accumulation of oxaliplatin.[96]

In another study, the influence of functionalised CNTs encapsulating either cisplatin or an inert Pt(IV) complex, *cis,cis,trans*-$[Pt(NH_3)_2Cl_2(O_2CC_6H_5)_2]$, on the biodistribution of the Pt complexes was investigated. Interestingly, Pt accumulation in vital organs suggested that the functionalised CNTs did not affect cisplatin distribution but significantly enhanced that of the Pt(IV) derivative in certain tissues. Enhanced accumulation was observed for example in lung tissue with a reduction in accumulation in both kidney and liver tissues thus demonstrating their potential to safely and efficiently deliver Pt drugs to target organs.[97]

Whilst nuclear DNA is thought to be the major target of classical Pt drugs, Yoong *et al.* investigated the use of CNTs functionalised with the mitochondrial targeting lipophilic cation rhodium-110 (Rho-110), to selectively deliver a Pt(IV) prodrug of cisplatin to the mitochrondria. The CNT-Rho110 alone was neither cytotoxic to cells nor detrimental to mitochondrial function. In contrast, the Pt-encapsulated CNT-Rho110 augmented cytotoxicity relative to cisplatin. Co-encapsulation of the Pt(IV) prodrug with 3-bromopyruvate, a chemo-sensitiser, resulted in a synergistic effect in the cell lines tested.[98]

More recently, tetrameric nanotubes, formed through self-assembly from α-helical right handed coiled coils (RHCC), encapsulating a Pt(IV) derivative have shown promise in that they exhibit superior *in vitro* and *in vivo* chemotherapeutic efficacy and an improved selectivity towards human malignant glioblastoma cells when compared to the free Pt(IV) compound.[99]

The use of nanotubes for targeted and 'remote control' dual drug of doxorubicin and a cisplatin prodrug has also been explored. Shanmugam *et al.* developed Au nanorods in which the 5' thiol ends of single stranded DNA were conjugated to the nanorods, following which the complimentary DNA strands were hybridised. In so doing, this new entity was able to bind doxorubicin through intercalation into the CG base pairs of a DNA duplex. The complimentary DNA was designed such that it incorporated a 5' amine functional moiety free to tether the Pt(IV) prodrug of cisplatin through the formation of an amide bond upon reaction of this amine with a carboxylate functionality in one of the axial ligands. The other axial ligand housed folic acid and served as a targeting agent in its own right for folate receptors overexpressed on cancer cells. Upon cell entry, exposure of these gold nanorods to NIR radiation resulted in doxorubicin and Pt prodrug release. The Pt prodrugs were subsequently reduced, under the reducing environment of tumour cells, yielding the cytotoxic Pt(II) species. This novel 'external stimulus combination drug delivery system' was found to be highly effective both *in vitro* and *in vivo*. [100]

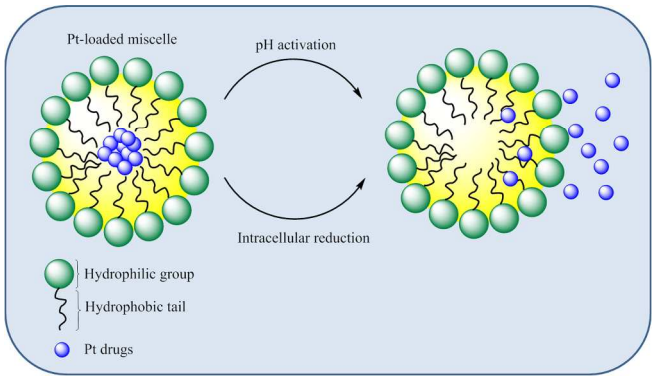
Tuning the diameter of nanotubes to selectively deliver Pt drugs has also been investigated. The incorporation of a hydrophobic Pt(IV) complex within the inner chamber of two different diameter functionalised nanotubes resulted in nanotubes with

differing cytotoxicity profiles as compared to the free drug. While both induced cell death after 72 hours, it was the larger diameter nanotubes which proved more efficacious due to more prolonged release of the drug cargo. Interestingly, these nanotubes, regardless of diameter, were only poorly cytotoxic on macrophages. No pro-inflammatory cytokine production nor cell activation was observed. This study demonstrated that fine-tuning the diameter of nanotubes can lead to effective drug delivery systems without inducing an inflammatory response. [101]

Wong *et al.* provide a comprehensive review of the use of CNTs for the delivery of small molecule drugs including Pt-based drugs. [102]

2.1.6 Polymer micelles

Polymer micelles are known for their ability to entrap drugs, usually within their micelle core, Scheme 2. This incorporation can greatly enhance drug solubility thus increasing bioavailability while also reducing adverse side effects.[103, 104] Possible delivery routes for polymer micelle-based structures of various drugs have been investigated for parenteral, oral,[105-107] nasal, [108, 109] and ocular [110, 111] administration.



Scheme 2: An illustration of Pt-loaded micelles and their intracellular mode of activation

Hydrophobic drugs tend to distribute into the micelle core while more polar drugs tend to occupy peripheral positions. Those which are peripherally located tend to be predisposed to release. The use of block ionomers which have the ability to form polymer-metal complexes have been exploited for Pt drug delivery. Pt drugs form coordinate bonds with the polyion block of the copolymer which also facilitates micelle formation. [112] The use of polycarboxylates as ionic blocks has been particularly exploited given the propensity of polycarboxylates to substitute anionic ligands such as chlorido ligands in, for example, cisplatin. [113] Pt drug release can be affected by external conditions such as salt concentrations, pH, reductants, and overall micelle stability, Scheme 2. In addition, biologically abundant counter ions can further enhance the promotion of drug release by ligand exchange. [112]

The most widely used copolymers for Pt drug delivery are poly(amino acid) based, such as poly(aspartic acid), PAsp and poly(glutamic acid), PGlu.[114] Pioneering studies by Kataoka *et al.* [113, 115, 116] in which cisplatin was complexed to PEG PAsp copolymers resulted in the spontaneous formation of stable polymer micelles with sizes in the range of 20-100 nm and high drug loading. They also demonstrated that release of the Pt drug from the micelles was dependent on the PAsp block length and occurred *via*

chlorido ion exchange. [113] Joining the PEG-PAsp block ionomers with PAsp homopolymer was shown to alter micelle size, micelle decay and cisplatin release.[116] Moreover, studies in mice demonstrated that incorporation of cisplatin into such polymer micelles prevented kidney toxicity in contrast to cisplatin treatment alone which is highly nephrotoxic, and enhanced exposure of the drug in tumours.[115]

Incorporation of cisplatin into PGlu-based block copolymers resulted in micelles with enhanced stability as compared to PAsp derivatives. Preclinical studies demonstrated prolonged blood circulation and a 20-fold higher accumulation in solid tumours (Lewis lung carcinoma cells) as compared to cisplatin alone. While both cisplatin and the cisplatin-loaded miscelles had significant *in vivo* cytotoxicity in C26 bearing mice, the miscelles demonstrated complete regression of tumours with no significant body loss in contrast to cisplatin alone which did result in tumour survivals and body weight loss.[117] These miscelles were also found to have a safer toxicity profile as compared to cisplatin in a guinea pig model.[118] Phase I clinical studies demonstrated that these miscelles, under the development name NC-6004, were well tolerated by patients with a range of advanced solid tumour types. However, hypersensitivity reactions induced by NC-6004 were more frequent than those caused by cisplatin. [119] This formulation is currently undergoing phase III clinical trials in Asia (Nanoplatin; Nanocarrier Co., Ltd.; Japan). [15] A Phase II study in which patients with locally advanced or metastatic pancreatic cancer were treated with NC-6004 in combination with gemcitabine demonstrated that Pt hypersensitivity was not an issue if prophylactic treatment was used with no need for pre-hydration. [15, 120] Survival rates were better (12.3 months) using this combination compared to the overall median survival (7.5 months) reported for cisplatin/gemcitabine combination.[121] An FDA application for an investigational new drug (IND) based on this combination was submitted in 2013 in the US. [15]

Another approach incorporating cisplatin using polymer micelles was based on the biodegradable polyester block polymer PEG-*b*-polycaprolactone (PEG-*b*-PCL). Anti-tumour activity of such micelles was demonstrated *in vitro* and *in vivo* with high encapsulation efficiency.[122] pH sensitive miscelles incorporating cisplatin have also been developed with rapid endosomal cisplatin release.[123] *In vivo* studies of cisplatin-loaded core cross-linked micelles of poly(ethylene glycol)-b-poly(methacrylic acid) demonstrated prolonged blood circulation, enhanced tumour accumulation, a decrease in renal exposure as well as enhanced efficacy as compared to cisplatin alone.[124]

The use of graft copolymers for Pt drug delivery has also been exploited. Carboxylic acid-functionalized poly(beta-aminoester)graft-poly(ethylene glycol) copolymers were complexed to cisplatin resulting in 100-200 nm negatively charged nanogels with a PEG outer layer. Whilst these demonstrated significantly lower *in vitro* cytotoxicity against SKOV-3 ovarian cancer cells as compared to cisplatin alone, they exhibited similar anti-cancer activity toward SKOV-3 tumours xenografted to immunocompromised mice.[125] Micelles incorporating mPEG-g-alpha,beta-poly [(N-amino acidyl)-DL-aspartamide] (mPEG-g-PAAsp) complexed to cisplatin were not found to be as cytotoxic as compared to cisplatin against Bel-7402 hepatoma cells.[126] The development of carboxylic acid conjugated, hydrophobically derivatized, hyperbranched polyglycerols as nanoparticulate drug carriers for cisplatin has also been described. These biocompatible carriers were found to inhibit proliferation of KU-7-luc bladder cancer cells.[127] Folate-decorated nanogels incorporating cisplatin have also been developed as targeted therapeutic agents for the treatment of ovarian cancer where folate receptors are overexpressed. These cisplatin-containing nanogels were found to possess superior anti-tumour properties *in vivo* as compared to the free Pt drug.[128]

Whilst the use of micelles as drug delivery vehicles has been extensively investigated, their dynamic nature can often lead to disassembly *in vivo* which can in turn negatively impact on their biodistribution and cellular uptake. Cross-linking can overcome this in many ways by locking the micelle into its desired spherical form. [129]

An interesting *in vitro* and *in vivo* study by Zhang, Chen *et al.* describes cisplatin cross-linked pH-sensitive dextran-nanoparticles as efficient vehicles for the selective delivery of doxorubicin to cancer cells. Here the cisplatin is employed as a cross linker and this cross linking appears to significantly enhance the surface charge and stability of the NPs leading to improved tolerability, *in vivo* pharmacokinetics, biodistribution and anti-cancer efficacy. In the A549 xenograft model investigated, a reduction in tumour size as well as drug-related multi-organ toxicity was observed. [130]

In another study, complexation of cisplatin to the pendant carboxyl groups on the poly(e-caprolactone) core of methoxy poly(ethylene oxide)-block-poly-(a-carboxylate-e-caprolactone) or PEO-b-PCCL generated pH-responsive micelles in which cisplatin release was triggered upon exposure to electrolytes and/or pH change mimicking that of the extracelleular tumour microenvironment or intracelleular organelles. These demonstrated promising *in vitro* activity against breast cancer cell lines. [131]

The potential of methoxy poly (ethylene glycol)-block-poly (glutamic acid) NPs as carriers of cisplatin for the treatment of solid tumours has already been highlighted in numerous studies including but not limited to those by Nishiya *et al.* [119] and Yamasoda et al.[118] and Chen et al. [132] Rapid release of cisplatin facilitated by the higher chloride ion concentration in blood plasma compared to chloride ion concentration inside tumour cells has been a challenge. To overcome this, Tang, Shah, Chen *et al.* reported the first example of a miscellar methoxy poly (ethylene glycol)-block-poly (glutamic acid) carrier incorporating a hydrophobic moiety poly (L-phenylalanine), [mPEG-b-P (Glu-co-Phe)], for targeted delivery of cisplatin. Cisplatin loading was facilitated through metal conjugation with the carboxyl groups of the poly (L-glutamic acid) block while the hydrophilicity of the poly (ethylene glycol) shell protected the carrier from phagocytosis. The presence of the poly (L-phenylalanine) afforded the system hydrophobicity to control cisplatin drug release. Two cisplatin-loaded [mPEG-b-P (Glu10-co-Phe10) (PGlu10) and mPEG-b-P (Glu20-co-Phe10) (PGlu20)] were developed and their drug release, cell viability, plasma clearance, and pharmacokinetic prolfile compared. Both nanoparticles demonstrated controlled and sustained release at physiological and lysosomal pH. Both showed dose and time-dependent cytotoxicity against the human breast cancer cell line ZR-75-30. The *in vivo* pharmacokinetic profile for both demonstrated prolonged blood circulation times in contrast to cisplatin alone. [133]

Wang *et al.* rationally designed core shell corona polyion complex NPs for Pt drug delivery utilising positively charged and Pt(IV)–prodrug-conjugating micellar NPs and the pH responsive negatively charged pegylated diblock copolymer PPC-DA. pH activation led to release of the positively charged micellar NPs which further facilitated NP internalisation and subsequent release of cisplatin upon exposure to the intracellular reducing environment. Prolonged circulation times and tumour growth inhibition in an A549 tumour xenograft model were observed. [134]

Antibody fragment-installed polymeric miscelles have also recently been investigated as a potential means to selectively deliver Pt drugs to pancreatic tumours. The Pt-loaded miscelles demonstrated more than 15-fold increased cellular binding within the first hour and rapid cellular internalisation compared to non-targeted miscelles

which ultimately led to enhanced *in vitro* cytotoxicity. These Pt-loaded miscelles were also found to significantly suppress the growth of pancreatic tumour xenografts.[135]

Polymer-albumin micelles loaded with Pt drugs have also been developed. These conjugates self-assemble in water with a nanoparticulate size of approximately 80 nm attributed to the hydrophobic nature of the Pt drugs. These albumin coated polymers were taken up readily by ovarian cancer cell lines and were found to have superior efficacy over a control sample without albumin coating. [136]

Micelles incorporating oxaliplatin have also demonstrated promise. [137, 138] Their relatively small size results in deep tumour penetration even in poorly permeable tumours such as intractable pancreatic [139] and scirrhous gastric cancers.[140] Their ability to selectively dissociate within late endosomes and thus enhance delivery of the Pt drug to DNA relative to oxaliplatin alone, results in these micelles generally exhibiting greater efficacy as compared to oxaliplatin alone even against oxaliplatin-resistant tumours.[141] For example, chitosan-based polymer miscelles encapsulating oxaliplatin, formed by stearic acid-grafted chitosan oligosaccharide, resulted in *in vitro* anti-tumour activity against drug sensitive SGC-7901, SKOV3, BEL-7402, K562 and MCF-7 and the multidrug resistant cells MCF-7/Adr. Significantly enhanced cytotoxicity was observed for the oxaliplatin-loaded miscelles over oxaliplatin alone. They were also active against the multidrug resistant cells tested.[142]

Cross-linked miscelles carrying oxaliplatin, generated by using block ionomer complexes of poly(ethylene oxide)-b-polymethacrylate (PEO-*b*-PMA) copolymer and divalent metal cations as templates, have also been investigated and were found to be not only stable but also exhibited pH-dependent sustained release of the Pt drug. Up to 25% w/w% loading was achieved as a result of the core's ionic character. The drug loaded miscelles demonstrated significantly higher *in vitro* cytotoxicity as compared to oxaliplatin alone which increased with exposure time.[143] The core cross-linked block ionomer micelles were utilised by the same group as pH-repsonsive carriers for cisplatin but with more efficient loading (up to 42% w/w%). This core cross-linking helped to stablise the micelles against structural disintegration while also preventing premature drug release.[144]

Amphiphilic biodegradable polymers bearing pendant carboxyl groups, mPEG-b-P(LA-co-MCC), have been utilised to bind the Pt of dichloro(1,2-diaminocyclohexane)platinum(II) resulting in the assembly of water soluble miscelles incorporating the oxaliplatin analogues. They demonstrated dose-dependent cytotoxicities against breast cancer EMT6 cells which were comparable to free oxaliplatin.[145] Another amphiphilic biodegradable copolymer, mPEG-b-P(LAco-MAC/TMA), bearing 1,2-dicarboxyl groups capable of chelating the (DACH)Pt of oxaliplatin has also been developed. This system too self-assembles into miscelles with desirable acid-responsive drug release kinetics and *in vitro* cytotoxicity against SKOV-3 and MCF-7 cancer cells. They were also found however to display reduced toxicity to HeLa cells as compared with oxaliplatin. [146]

Biodegradable polymers have also been exploited to co-deliver oxaliplatin and daunomycin. Polymers with a similar backbone were conjugated to oxaliplatin analogues bearing axial carboxyl groups and to daunomycin and these were able to co-assemble into composite miscelles. Release of the oxaliplatin derivative was facilitated upon reduction in tumour cells and daunomycin release was facilitated via acid hydrolysis. *In vitro* and *in vivo* results demonstrated that these composites exhibited reduced systemic toxicity and enhanced synergy over the combination of both drugs. [147]

More recently, an *in vivo* study using a transgenic model of spontaneous pancreatic cancer indicated that these micelles loaded with oxaliplatin prolonged mice survival for more than 100 days preventing the onset of intraperitoneal metastasis in contrast to those treated with oxaliplatin alone where 50% of the mice were dead after 50 days.[148] The anti-tumour efficacy of these micelles and their association with peripheral neuropathy has also been evaluated given that the latter is a primary dose-limiting factor in oxaliplatin therapy. Their efficacy was found to be superior to that of oxaliplatin in an *in vivo* rat model bearing the human carcinoma KB. The animals did not experience acute cold hypersensitivity, often felt by patients following oxaliplatin administration.[149] This micelle formulation is being developed under the name NC-4016 (NanoCarrier Co., Ltd.; Japan) and is advancing to Phase I/II clinical trials in the US against a range of solid tumours. [15] Incorporation of the oxaliplatin motif into cross-linked block copolymer micelles have also shown potential both *in vitro* and *in vivo* against with improved efficacy as compared to oxliplatin alone against A2780 ovarian cancer cells and in an ovarian tumor xenograft model respectively.[150]

Yong *et al.* utilised mPEG-Ad@β-CD-7PLGA/CDDP nano-sized supramolecular micelles fabricated from β-CD-7PLGA/CDDP and mPEG-Ad as vehicles for cisplatin but compared to cisplatin alone, the micelles demonstrated decreased cytotoxicity against KB cells. [151]

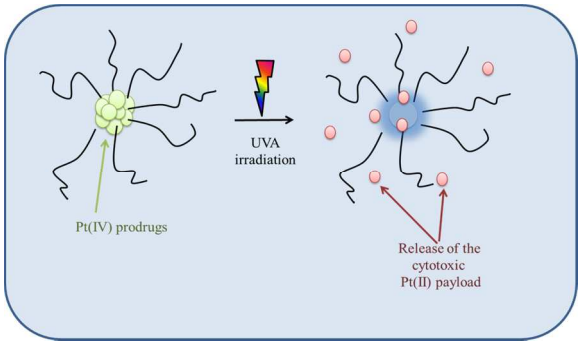
Whilst many Pt-loaded micelles developed to date target solid tumours, the ability of micellar NPs as oxaliplatin transporters for the treatment of liver metastases of bioluminescent murine colon adenocarcimona C-26, during overt and pre-angiogenic metastatic stages has also recently been investigated. These oxaliplatin-loaded micelles effectively inhibited tumour growth in the metastases models investigated. Anti-tumour activity in the overt model was associated with selective accumulation of the micelles in cancerous tissues having neovasculature. In contrast, a correlation was found between the ability of the micelles to target preangiogenic metastases and the inflammatory microenvironment of the niche. [152]

Whilst most studies to date have focussed on micelles encapsulating cisplatin and to a lesser extent, oxaliplatin, Duong *et al.* reported novel micelles incorporating a Pt(IV) derivative of cisplatin. Isocyanate groups in the poly(oligo(ethylene glycol) methyl ether methacrylate)-*block*-poly(styrene-*co*-3-isopropenyl-*R,R*-dimethylbenzyl isocyanate) (POEGMA-*block*-P(STY-*co*-TMI)) miscelle core reacted with amine groups in the Pt(IV) derivative to generate stable miscelles with promising *in vitro* activity. The rationale behind the approach was that the Pt(IV) would be reduced in the reducing environment of tumour cells releasing the active cisplatin and the resulting non-toxic micelle subsequently excreted.[153]

The incorporation of bioactive ligands in the axial positions of the Pt(IV) analogue of cisplatin is not a new phenomenon. For example, Lippard *et al.* developed mitaplatin, a dual-functioning Pt(IV) prodrug incorporating the DNA-binding drug cisplatin and the mitochondria-targeting drug dichloroacetate (DCA) which demonstrated cytotoxicity comparable to cisplatin.[154] Zhang *et al.* developed paclitaxel-cisplatin(IV) conjugates but these, in contrast, were found to be non-toxic and inactive against tumour cells, most likely due to their inability to enter cancer cells.[155] Xiao *et al.* sought to overcome drawbacks such as instability, poor solubility and bioavailability, short blood circulation time, etc., often associated with small molecular drugs such as these by designing micelles capable of encapsulating mitaplatin [156] and the paclitaxel-cisplatin(IV) conjugates[157] with a view to enhancing both efficacy and tolerance. They employed succinic acid as a linker between the Pt(IV)-DCA complex and the carrier biodegradable and amphiphilic copolymer MPEGb-PCL-b-PLL, forming a polymer-

Pt(IV) conjugate and its miscelles. Under simulated intracellular conditions, the miscelles rapidly released the drug and demonstrated higher cytotoxicity towards SKOV-3 human ovarian cancer cells than its precursors alone. [156] The same group co-assembled the Pt(IV) cisplatin prodrug and paclitaxel into single carrier composite miscelles. Release of the cisplatin prodrug was facilitated upon cellular reduction and paclitaxel via acid hydrolysis following entry into tumour cells. Moreover, a synergistic effect was observed *in vitro*. *In vivo* studies provided evidence that the miscelles carrying the two drug cargos displayed safer and more efficacious inhibition towards U14 tumour growth as compared to the drugs administered in combination. [157] Scarano *et al.* likewise employed a dual-drug delivery approach to transport curcumin and Pt(IV) prodrugs in polymeric miscelles. When tested against A2780 ovarian cancer cells, co-administration of curcumin and the Pt drug without the carrier demonstrated synergy with a combination index from 0.4-0.8. This synergy was enhanced when the two were co-delivered in these miscelles resulting in a combination index of 0.2-0.35. [158]

Site-specific activation of photosensitive Pt(IV) prodrugs is now possible through the use of lasers and fibre optics capable of reaching any tissue in the body, thus minimising the severe toxic side effects associated with Pt(II) drugs. Sadler *et al.* were the first to develop photo-sensitive Pt(IV) prodrugs whereby the prodrug would only be activated upon exposure to visible light releasing the highly reactive Pt(II) species capable of binding rapidly and stereospecifically to nucleotides forming established cisplatin-nucleotide cross-links. These Pt(IV)-azide complexes offer a distinct advantage over photodynamic therapy in that they do not require photosensitizing catalysts or oxygen-rich environments. [159-161] Recognising the potential of these Pt(IV)-photosensitiser systems, Bilgicer, Jing *et al.* recently developed a novel series of cisplatin and oxaliplatin Pt(IV)-photosensitiser produgs in micellar nanoparticle formulations with a miscellar diameter of 100–200 nm, sizes which are known to induce EPR effects *in vivo*. These formulations, which were stable in the dark, exhibited high sensitivity towards UVA irradiation, releasing their cytotoxic Pt(II) agents which were subsequently found to form DNA cross links, Scheme 3. These photosensitiser prodrugs were found to be up to 8-fold and 13-fold more effective *in vitro* as compared to cisplatin and oxaliplatin respectively. They were also found to be highly effective in an *in vivo* H22 murine hepatocarcinoma model upon UVA activation with enhanced blood circulation times, greater tumour growth inhibition and reduced systemic toxicity. [162]



Scheme 3: A schematic to illustrate how photosensive Pt(IV) prodrugs, upon irradiation with UVA light, release their cytotoxic Pt(II) payloads

Stenzel *et al.* conducted a comprehensive *in vivo* investigation into the effectiveness of folate decorated cross-linked micelles for the delivery of the Pt(IV) prodrug of cisplatin. They used a selection of fluorophore-labelled miscelles (with and without folate) cross-linked with 1,8-diaminooctane with sizes ranging from 75 to 200 nm and with both spherical and worm-like conformations. Using optical imaging, they established that accumulation in organs, especially in the liver and kidneys, was enhanced when the micelles were cross-linked and decorated with folic acid while micelles that were not conjugated with folate and not cross-linked were rapidly clearly from the mice. The micelles with worm-like conformations had a slower clearance rate as compared to the spherical micelles. [129]

A Pt(IV)-thermo gel polymer, capable of self-assembling into miscelles in water has recently been investigated in which a Pt(IV) prodrug was covalently linked to the hydrophobic end of two methoxyl poly(ethylene glycol)-b-poly(d,l-lactide) (mPEG-PLA) copolymer chains, resulting in the formation of Bi(mPEG-PLA)-Pt(IV). This novel conjugate was shown to accumulate in cells via endocytosis with sustained release of its Pt cargo up to 2 months. Bi(mPEG-PLA)-Pt(IV) was also found to have enhanced *in vitro* cytotoxicity as compared to cisplatin against MDA-MB-231cancer cells.[163]

In vitro activity of miscelles (NP-UVA-Pt2) incorporating a photosensitive platinum(IV) prodrug (UVA-Pt2) conjugated to a biodegradable polymer (PE, methoxyl-poly(ethylene glycol)-block-poly(lactide-co-2-methyl-2-carboxyl-propylene carbonate-ethanol amine) demonstrated improved cytotoxicity against SKOV-3 cells as compared to cisplatin. As anticipated, cellular accumulation appeared to be facilitated via endocytosis rather than passive diffusion, and did not involve the use of copper transporter protein (Ctr1). NP-UVA-Pt2 was found to be highly responsive to photo-irradiation while the miscelles were stable at physiological pH in the dark.[164]

Cyclotriphosphazene micelles incorporating a hydrophobic and water insoluble Pt(II) complex cis-bis(cyclohexylamine) dinitratoPt(II) demonstrated both potent *in vitro* and *in vivo* cytotoxicity as well as a favourable pharmacokinetic profile *in vivo* as compared to carboplatin. The miscelles were shown to be particularly cytotoxic to stomach tumour cells (SNU638), which is one of the least responsive cancers to chemotherapeutics currently in clinical use.[165] An amphiphilic polyphosphazene-Pt conjugate designed to selectively deliver oxaliplatin to tumours has also been developed. The (dach)Pt[HEDM] where dach is trans-(±)-1,2-diaminocyclohexane and HEDM is 2-hydroxyethoxydiethylmalate was designed such that the HEDM could serve as a linker between the Pt and the polyphazene backbone generating a novel amphiphilic polyphosphazene-Pt conjugate, [NP(MPEG550)(dach)Pt(EM)]n [MPEG550: methoxy poly(ethylene glycol) which could self-assemble into stable polymeric micelles of a mean diameter of 130 nm, suitable for passive tumour targeting by the EPR effect. This novel polyphosphazene-Pt conjugate was found to have a superior pharmacokinetic and cytotoxicity profile as compared to oxaliplatin.[166]

A strategy employed to overcome some of the drawbacks associated with classical Pt drugs was the development of non-classical Pt drugs capable of binding DNA in a different manner to cisplatin and its analogues. BBR-3464 (18), Figure 2, is an example of the first and only 'non-classical' Pt drug to undergo clinical evaluation. [167] This is a tri-nuclear positively charged Pt(II) drug which can form flexible and non-directional and long range DNA adducts resulting in conformational changes to both A- and Z-type DNA. [168-170]

Inspired by the success of cisplatin and oxaliplatin and more recently that of multi-nuclear Pt drugs, polymer-di-Pt(IV) conjugates derived from cisplatin and

oxaliplatin were developed which were assembled into miscelles, the rationale being that these new miscelles would be internalised by tumour cells via endocytosis increasing drug concentrations and reducing dose-limiting systemic toxicity. Once inside the reducing environment of tumour cells, the prodrugs would be reduced releasing the cytotoxic dinuclear Pt(II) adducts free to bind DNA. These miscelles did indeed demonstrate reduced systemic toxicity, relatively long blood circulation and enhanced tumour efficacy as anticipated [171]

The synthetic, biodegradable, and water soluble polypeptide methoxy-polyethylene glycol-block-poly(glutamic acid) (MPEG-PGA) bearing pendant negatively charged carboxyl moieties has been exploited as a drug carrier due to its biodegradability and biocompatibility. The presence of the negatively charged carboxyl groups are ideally suited to interact with positively charged drug molecules via electrostatic interactions. Xiao *et al.* have used this strategy to develop novel micellar NP incorporating positively charged multi-nuclear Pt drugs. Drug loading can be adjusted by varying the stoichiometric ratios of the multi-nuclear Pt drugs to the negatively charged carboxyl groups present on the polypetide. These multi-nuclear Pt-loaded miscelles exhibited not only efficient Pt loading but improved cellular accumulation and *in vitro* as well as *in vivo* activity. [172]

Malzert-Fréon *et al.* specifically review nanocarriers including polymeric conjugates, dendrimers, inclusion molecules: cyclodextrines, polymeric miscelles, nanogels, nanoparticles and liposomes for the targeted treatment of ovarian cancers with a particular emphasis on their use in preclinical development. [173] Lippard *et al.* describe recent advances in Pt(IV) prodrugs and the development of Pt(IV) drug nanoconstructs for their selective *in vivo* delivery. [174] Liang, Gottesman *et al.* focus on abnormal membrane protein trafficking in their review of nanoscale drug delivery platorms to overcome Pt-based resistance in cancer cells. [175] Cabral and Kataoka provide a comprehensive review of polymeric micelles and their performance in human studies.[176] Guo, Wang and Wang review the functionalization of Pt complexes for both targeted drug delivery and as theranostic agents. [177] Mumper *et al.* draw from the expertise of leaders in biology, chemistry, materials science, pharmaceutics, toxicology, chemical engineering, imaging, physiology, oncology and regulatory affairs and provide an insightful commentary into the 'six tennets' of biotargeted cancer nanomedicines required to translate basic science into clinical applicaiotns. [178] Other, more general reviews are provided by Oberoi *et al.*,[15] Sadler *et al.*,[179] and Kieler-Ferguson *et al.*[37]

3. Conclusion

The rational design and development of innovative anti-cancer Pt drug candidates to overcome drawbacks associated with those currently in the clinic has produced an inspiring armamentarium of possible chemotherapeutics. However, in the 50 years since the discovery of the anti-cancer properties of cisplatin, none to date have been as successful as cisplatin, carboplatin or oxaliplatin. Recent advances in this field have included the search for nanotechnologies to essentially protect the Pt from deactivation until such time as the drug reaches the tumour site whereupon the technology is capable of releasing its Pt payload. An alternative approach is to incorporate vectors onto existing nanotechnologies to serve as homing devices with a view to enhancing drug targeting. Exploiting the use of nanotechnology to preferentially deliver and deposit Pt drug payloads to tumours has resulted in the employment of liposomes, nanocapsules, polymers, dendrimers, nanoparticles, nanotubes and others as exciting vehicles for this purpose. We have attempted to provide an overview of progress in this exciting domain. There has undoubtedly been much success in this field with some technologies already

undergoing clinical evaluation. Future work should focus on more fully understanding the complexity of the mechanisms underlying nanoparticle targeting. The advent of nanotechnologies with theranostic applications will certainly help to improve our understanding in this regard and we look forward to further developments in this field. There remains a need however, through multi-disciplinary research, to further optimise the synthesis (homogeneity), characterisation and scale-up, physicochemical profiles, stability in systemic circulation, and delivery and therapeutic efficacy of these systems as well as reflecting on data generated to date to inform the future design of these systems if such technologies are to successfully cross the interface between pre-clinical and clinical application for cancer treatment.

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[a] *Centre for Synthesis and Chemical Biology, Department of Pharmaceutical & Medicinal Chemistry, Royal College of Surgeons in Ireland, 123 St. Stephen's Green, Dublin 2, Ireland. Tel: 353 1 4022161; E-mail: cmarmion@rcsi.ie*

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