Malignant Pleural Mesothelioma: state of the art and advanced cell therapy

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ABSTRACT: Malignant Pleural Mesothelioma (MPM) is an aggressive malignancy highly resistant to chemotherapy, with a response rate of 20% of patients and for this reason an efficient treatment is still a challenge. Platinum-based chemotherapy in association with a third-generation antifolate is the front-line standard of care whereas any second-line treatment was approved for MPM thus making it a pathology that evokes the need for new therapeutic agents. Different platinum-drugs were synthesised and tested as an option for patients who are not candidates to cisplatin-based therapy. Among these, monofunctional cationic antineoplastic platinum compounds received a special attention in the last decade. Alternative strategies to the commonly used combination-therapy resulted from the use of Mesenchymal Stromal Cells (MSC) widely used in the field of regenerative medicine and recently proposed as natural carriers for a selective delivery of chemotherapeutic agents and from the use of immune checkpoint and kinase inhibitors. The present short review shed light on the recent state of art and the future perspectives relative to MPM therapy.

Malignant Pleural Mesothelioma (MPM)

MPM is a rare, fatal, asbestos-associated malignancy originating in the mesothelial cells of the pleura. It tends to grow over the serosal surface and finally encases the lung, causing death by asphyxiation.[1, 2] The association between asbestos exposure and the later development of MPM has been widely documented.[3] Whilst the incidence of MPM in the USA peaked in 2004, incidence rates in Europe and Japan are projected to peak in 2020 and 2025 respectively.[4, 5] There are three major histopathologic types of MPM: epithelioid, sarcomatoid and biphasic, being the epitelioid form the most prevalent type.[6] Biologically, MPM is difficult to defeat because it spreads aggressively within the affected hemothorax, is highly resistant to conventional chemotherapeutic agents and exhibits a propensity to

recur at local surgical margins following resection.[1] Trimodal therapy with chemotherapy, surgery and radiation has produced overall median survivals in the range of 1-2 years; a platinum-based doublet containing a third-generation antifolate (pemetrexed (PMX) or raltitrexed) is the front-line standard of care whereas there are no approved second-line treatments for MPM which remains a disease setting to test the efficacy of new therapeutic agents.[7]

New platinum complexes for MPM treatment

New drugs and tailored treatments are highly desired to improve the outcome of MPM patients. Thus, the design of new platinum compounds was conducted and realised for obtaining drugs with stronger pharmacological properties, less toxicity and more favourable therapeutic indices if compared to cisplatin. The family of platinum complexes binds directly to DNA, resulting in the formation of DNA-platinum adducts, *i.e.* intra- and interstrand DNA crosslinks, that impede a proper cell division.

The lead compound, cisplatin, is a widely used chemotherapeutic agent which acts primarily *via* DNA damage and subsequent induction of apoptotic cell death including mitochondrial depolarization and characteristic membrane changes. Several studies have examined the functional inhibitory effects of surviving in mesothelioma cell lines depending on apoptotic morphology and in caspase-independent pathways in response to cisplatin. Platinum resistance is considered multi-factorial and includes both mechanisms that limit the formation of platinum-DNA adducts as well as mechanisms that prevent cell death following drug-induced damage.[8]

In this context, different platinum(II) and (IV) complexes were synthesised and evaluated because optimal delivery of cytotoxic Pt(II) agents is of paramount importance to widen their therapeutic index. Reduced cellular accumulation of platinum either by impaired uptake or increased efflux is often found in cells selected for cisplatin resistance, both *in vivo* and *in vitro*, and it is often considered as one of the most consistent characteristics of platinum resistant cells. Previously, passive diffusion through the cellular lipid bilayer was the dominant process involved in drug uptake and distribution. However, more recently, the concept of carrier mediated and active uptake of commonly prescribed drugs, has become rule rather than exception. Cisplatin passes through the cellular lipid bilayer by both passive diffusion and facilitated transport involving the copper transporter proteins, in particular Ctr1.[9] Membrane transporters and channels, collectively known as the transportome, could be exploited for predicting the platinum sensitivity/resistance of the tumour and the

critical pharmacokinetic parameters and determining the severity of platinum-associated adverse events.

On the contrary, its Pt(IV) congeners are believed to cross cellular membrane by passive diffusion only, since their reactivity towards transport proteins has been found negligible. The intracellular reduction from Pt(IV) to Pt(II) oxidation state resulted in the formation of the cisplatin metabolites subsequent to the diffusive pathway due to their high lipophilicity. The fine tuning of the reduction potential and the lipophilicity of the complex can be modulated by introducing differently long saturated and unsaturated chains.[9, 10] Considering that the six ligands around Pt(IV) offer major possibilities of structural variations over the four ligands as in Pt(II), some bifunctional Pt(IV) conjugate complexes based on the cisplatin square-plane with two axial valproate (2-propylpentanoate, VPA) (**I**) or ethacrynoate (ethacrynic acid, EA) (**II**) ligands were tested and showed an extraordinary cytotoxic effect on cells derived from MPM. (**Figure 1a**) In these cases, the remarkable cytotoxicity of the complexes was interpreted as the result of the increased cellular accumulation of the complex in reason of its increased lipophilicity and of the synergy between the pharmacologic activity of VPA or EA and the well-established efficacy of cisplatin/pemetrexed combination.[11, 12]

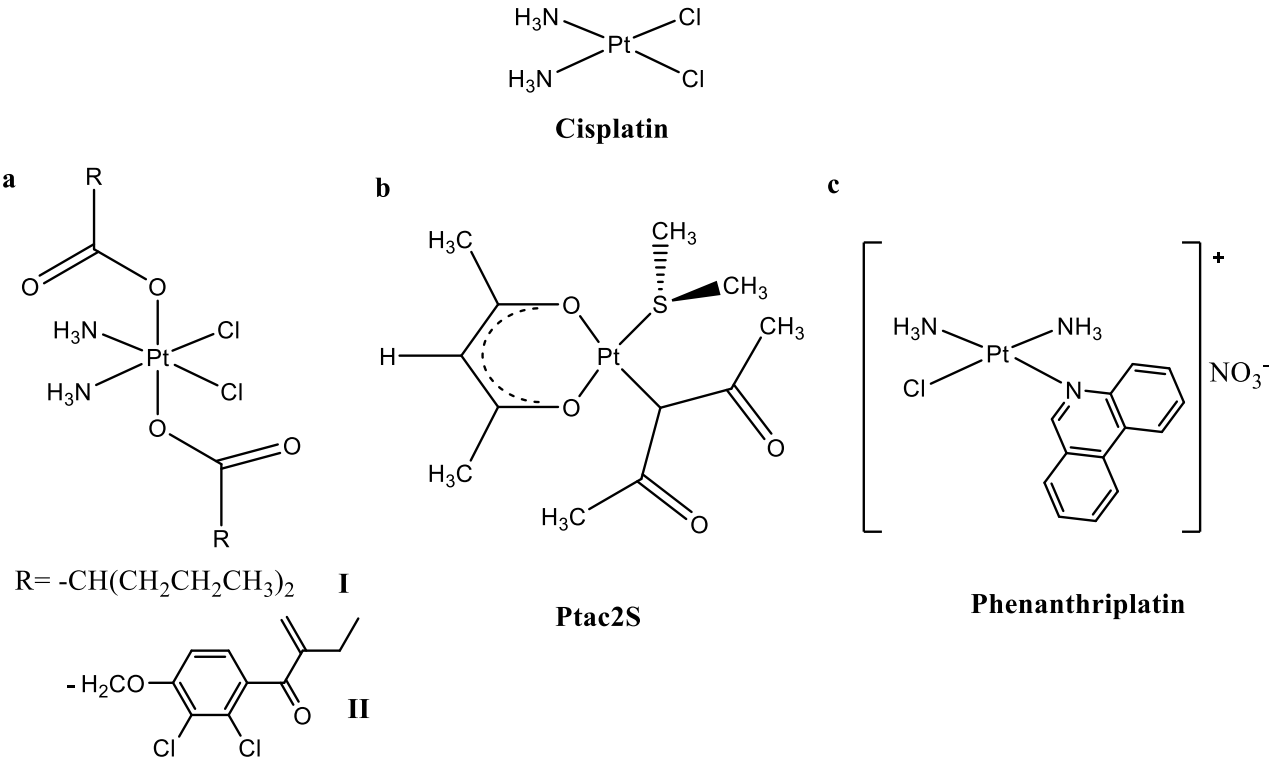

Figure 1: Pt(IV)-bis(carboxylate) (**I** and **II**), ([Pt(*O*,*O*'-acac)(γ-acac)(DMS)]) (**Ptac2S**) and (*SP*-4-3)-diamminechlorido (phenanthridine)platinum(II) nitrate) (**phenanthriplatin**).

Recently, the outcome that platinum cross-link formation is not essential for anticancer activity, shed a light on the possibility to bypass chemoresistance by using platinum(II)

complexes with different pharmacological targets than cisplatin. For example, Ptac2S ([Pt(*O*,*O*'-acac)(γ-acac)(DMS)]) revealed a characteristic reactivity with sulphur ligands, largely represented in amino acid residues instead of a small reactivity with nucleobases indicating that the cellular targets could be of protein nature. One of the important reasons responsible for mesothelioma strong resistance to chemotherapy seems to be the overexpression of anti-apoptotic proteins of the Bcl-2 family. Ptac2S resulted more efficacious than cisplatin also in inducing apoptosis by increasing bax expression, its cytosol-to-mitochondria translocation and *vice versa* by decreasing Bcl-2 expression.[13] (**Figure 1b**)

Major features of the cisplatin mechanism of action involve cancer cell entry, formation of mainly intrastrand cross-links that bend and unwind nuclear DNA, transcription inhibition and induction of programmed cell-death while evading repair.

Monofunctional platinum compounds such as phenanthriplatin ((*SP*-4-3)-diamminechlorido (phenanthridine)platinum(II) nitrate),[14] (**Figure 1c**) which makes only a single bond to DNA nucleobases, could be far more active and effective against a range of tumour types than dichloro platinum derivatives. Without a cross-link-induced bend, monofunctional complexes can be accommodated in the major groove of DNA.[15] The resulting adducts potentially inhibit transcription, while the low distortion of the DNA significantly eludes repair. Recently, phenanthriplatin was loaded on negatively charged Dextran Sulfate (DS) as a model vector for drug delivery *via* electrostatic interactions. Free complex and conjugated with DS revealed higher antiproliferative activity in many tumour cell lines and could bypass acquired cisplatin resistance on MPM cells.[16]

Pemetrexed (PMX)

In accordance with the combination therapy established for MPM, FDA approved PMX (Alimta®) on February 4, 2004 in association with cisplatin. Pemetrexed (*N*-[4-[2-(2-amino-3,4-dihydro-4-oxo-7H-pyrrolo[2,3-d]pyrimidin-5-yl)-ethyl]-benzoyl]-L-glutamic acid) disodium salt belongs to the class of antifolic agents but it's distinguished from the other antifolates for its novel structure, possessing a unique 6-5 fused pyrrolo-[2,3-d]pyrimidine nucleus instead of the classical 6-6 core structure (pteridine or quinazoline) as in methotrexate or in raltitrexed.[17]

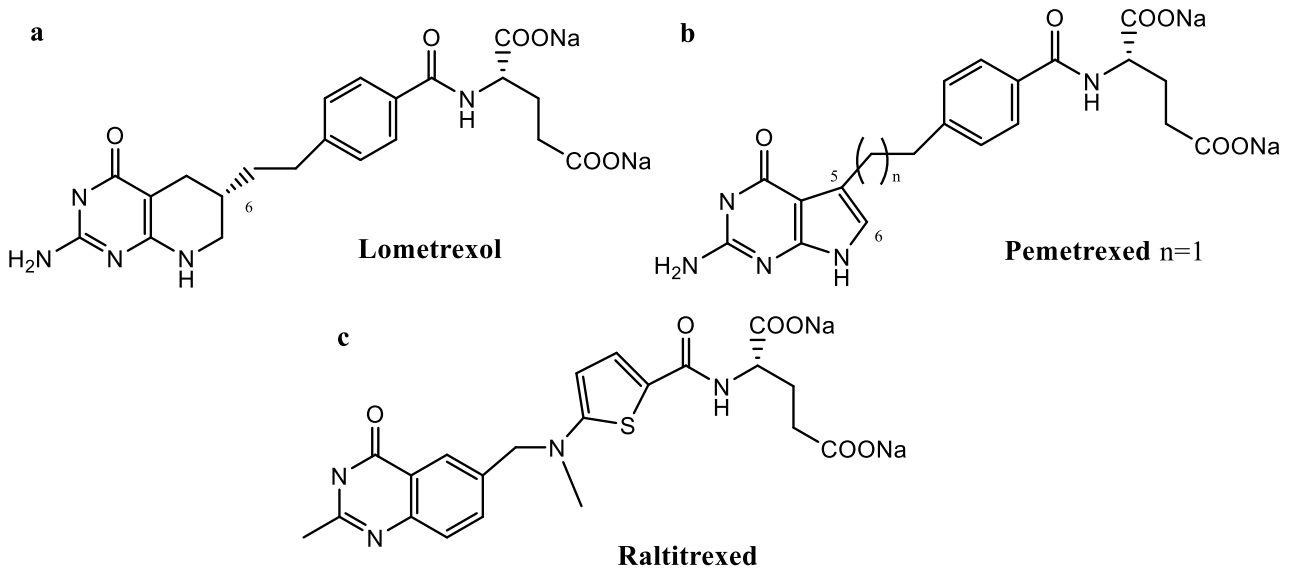

Figure 2: Lometrexol (**a**), **pemetrexed** (**PMX**) and its derivatives (**b**) and **raltitrexed** (**c**).

It was firstly synthesised in an attempt to study the biochemical consequences of removing the C-6 stereogenic centre from its precursor lometrexol (**Figure 2**) but anyway maintaining the hydrogen-bonding NH function close to the C-6 pyrimidine ring, resulted essential in lometrexol inhibitory activity of Glycinamide Ribonucleotide Formyltransferase (GARFT), the key enzyme involved in the purines biosynthesis.[18] The lack of the stereogenic centre in PMX structure makes it 30-200 times a more potent inhibitor of Thymidylate Synthase (TS) than GARFT thus blocking more selectively the synthesis of deoxythymidine monophosphate necessary for DNA assembly. Exploiting the Reduced Folate Carrier (RFC), it enters the cells in its active pentaglutamate form, a reaction catalysed by the Folypolyglutamate Synthase (FPGS) and in this form is 60 times more potent in inhibiting TS.[19] Polyglutamation reaction of PMX takes place 6-13 times more efficiently than of its precursor lometrexol increasing its cellular retention and thus explaining its every 3-week administration schedule. By binding to the folate receptor α (FRα) with an affinity similar to that of the physiological ligand, it also inhibits Dihydrofolate Reductase (DHFR). These targets, however, are the major responsible of the toxicity of pemetrexed (myelosuppression, gastrointestinal and cutaneous toxicities) as thymidine and hypoxanthine are substrates necessary to overcome the massive cellular death caused by pemetrexed used at the recommended doses.

A recent study showed that in comparison with the 5-substituted pyrrolo-[2,3-d]pyrimidine pemetrexed the corresponding 6-substituted analogues with a carbon side chain of

increasing length were still efficiently uptaken by tumour cells through FRα but the affinity for RFC was completely lost, although maintaining the same antitumor efficacy depending on the inhibition of AICARFTase, 5-Aminoimidazole-4-Carboxamide Ribonucleotide Formyltransferase. When the same approach was applied to the synthesis of pemetrexed analogues, the 4-carbon bridge one showed the greatest FRα potency and a cytotoxicity mostly dependent on ATP pool depletion that it's secondary to its inhibition activity towards both GARFTase and AICARFTase.[20]

In several European countries in alternative to PMX, raltitrexed (**Figure 2c**) is in use in association with cisplatin for MPM therapy due to its better toxic profile although sharing the same mechanism of action based on the inhibition of TS.[21]

For MPM the FDA recommended dose of PMX is 500 mg/m^2 administered as i.v. over 10 minutes on day 1 of each 21-day cycle followed by cisplatin at a dose of 75 mg/m^2 infused over 2 h, 30 minutes after the beginning of PMX administration. A supplementation of folic acid (350-1000 µg orally and daily administered) and Vitamin B_{12} (1000 µg i.m. started 1-3 weeks before the therapy and repeated every 9 weeks) is necessary in order to reduce pemetrexed toxicities.[17]

Although the combination of cisplatin with the PMX antifolate showed remarkable results in comparison with single drugs used alone, the half of patients are primary resistant and the benefits are not satisfactory above all as well as the biological basis are not so clear. The investigation of new combining therapeutic strategies is an effective and urgent need. An alternative drug combination could be the use of cisplatin with phenethyl isothiocyanate (PEITC). ITCs, arising from glucosinolate hydrolysis in vegetables, are known for antiproliferative properties. PEITC and cisplatin caused cellular death by apoptosis in different manner involving distinct pathway of the DNA damage response (DDR). Thus, their combination therapy allowed potentiation of both compounds' cytotoxicity by apoptotic mechanism fully dependent on ROS production and, thanks to the difficulty for a cell to set up contemporary two pathways of DDR, preventing cell resistance. Interesting was the possibility of a local administration of cisplatin-PEITC combination in pleural cavity for the treatment of MPM considering its lack of toxicity on primary mesothelial cells.[22]

Drug loading and drug delivery by Mesenchymal Stromal Cells (MSC)

Another potential therapeutic strategy is the use of mesenchymal stem cells.

MSC are undifferentiated multipotent adult cells defined as plastic-adherent, fibroblast-like cells possessing extensive self-renewal properties and the *in vivo* and *in vitro* ability to

differentiate into osteogenic, chondrogenic and adipogenic lineages when cultured in specific inducing media.[23] After exposure to high doses of chemotherapeutic drugs like paclitaxel, compound known for eliciting its activity by stabilization of the β-subunit of tubulin in microtubules,[24] MSC have been shown to accumulate intracellularly and deliver the antineoplastic agents without any genetic modifications, thereby decreasing tumour proliferation.[25] Many different methods of drug delivery have been described in the last decade, including immunoconjugates for targeting tumour-specific antigens, nanoparticles and genetically modified stem cells. However, non-modified MSC are probably the best choice for anticancer drug delivery as they readily adapt themselves to culture conditions and home to pathological tissues when injected *in vivo* in addition to their intrinsic antineoplastic activity.[26] On one hand, MSC hold great promise for oncology because they release active soluble factors and play an effective immunomodulatory role. They can also cross the blood brain barrier, thus representing a potential therapeutic tool for adult and paediatric brain tumours; on the other, the issue of whether MSC cross-talk with the tumour microenvironment boosts tumour suppression or instead favours tumour growth remains unsettled.[27]

IMMUNE CHECKPOINT AND KINASE INHIBITORS

The standard first-line chemotherapy for MPM with proven evident efficacy is the platinum-containing drugs and pemetrexed combination, indeed. Emerging immunotherapy based on an altered expression of genetic pool, makes some genes an excellent antigen target for antibody-based therapy.[28] As a significant T-cell inflammatory expression pattern is recently highlighted in MPM, as well as a high infiltration of lymphocites and macrophages, the addition of immune check-points inhibitors to cisplatin plus pemetrexed could result in a better clinical outcome. PD-1 (programmed cell death protein 1) and CTLA-4 (cytotoxic T-lymphocyte associated protein 4) are two key negative regulators of the immune system. Tremelimumab[29], is a fully human IgG2 monoclonal antibody against CTLA-4 unfortunately accompanied by several severe side effects while pembrolizumab [30] is currently under investigation as an inhibitor of PD-1, demonstrating to be safe and tolerable. A main feature of MPM pathogenetic development is the loss of tumor suppressor genes instead of a hyper-regulation of oncogenes. In particular, CDKN2A/ARF tumor suppressor gene resulted the most frequently down-regulated or silenced one. Its expression envisages encoding two proteins involved in cell cycle regulation: cyclin dependent kinase inhibitor 2A (p16/INK4a) and alternate reading frame (p14/ARF).

By binding to CDK4/6, the former (p16/INK4a), whose expression is associated with chemotherapy efficiency,[31] prevents the formation of a complex with cyclin D1 thus resulting in the activation of the retinoblastoma protein (Rb) responsible of G1 cell cycle arrest; the latter binds to the oncogenic protein MDM2 leading to an up-regulation of p53-related cell apoptotic death.

Palbociclib (PD-0332991) (**Figure 3**), whose pyrido-[2,3-d]pyrimidin-7-ones ring proved critical for specificity and activity for binding ATP pocket,[32] represents a valid alternative therapeutic approach in MPM patients with inactivated CDKN2A/ARF suppressor gene. By inhibiting Rb phosphorylation, palbociclib inhibits selectively the cellular DNA synthesis with the consequence of a G1 to S phase cell cycle arrest. Another important feature that makes palbociclib extremely relevant in MPM patients treatment among other kinase inhibitors, is its ability to activate the serine-threonine kinase mTOR because of an increased PI3K phosphorylation of AKT. In this regard, an association of palbociclib with PI3K inhibitors (NVP-BEZ235[33] and NVP-BYL719[34]) might result in a new favorable approach in MPM therapy due to an irreversible inhibitory effect on tumor cell proliferation alongside with an increase in the number of senescent cells.[35]

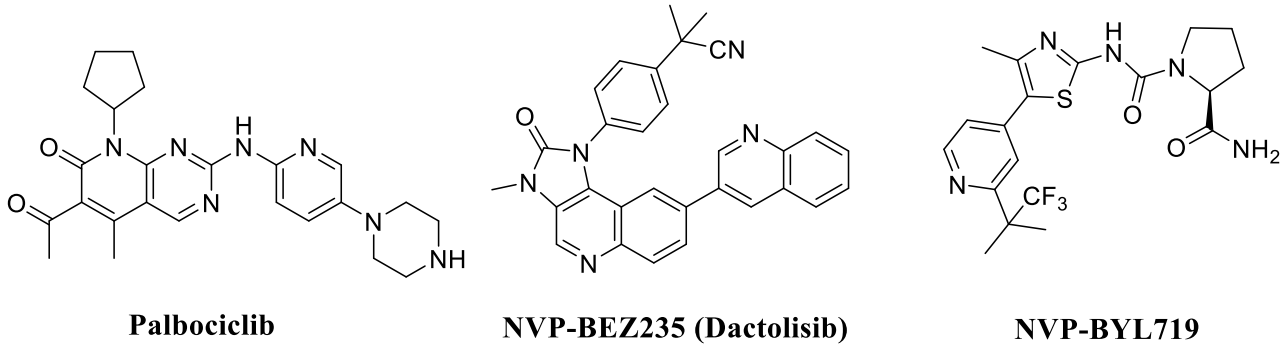

Figure 3: **Palbociclib**, CDK4/6 kinase inhibitor, **NVP-BEZ235** and **NVP-BYL719**, PI3K inhibitors.

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