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Review

Drug resistance reversal—are we getting closer?☆

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Abstract

Clinical drug resistance is a major barrier to overcome before chemotherapy can become curative for most patients presenting with metastatic cancer. Rational attempts to tackle clinical drug resistance need to be based on an understanding of the mechanisms involved; these are likely to be complex and multifactorial, and may be due to inadequate drug exposure or alterations in the cancer cell itself. This article reviews a number of strategies used to tackle drug resistance, focussing on work in our institution related to the treatment of ovarian cancer and resistance to platinum and taxane-based chemotherapy. Further progress towards drug resistance reversal will require a three-pronged approach, namely: the development of novel cytotoxics which exploit selectively expressed targets; modulation of resistance to conventional agents and, most importantly, a serious attempt to understand resistance mechanisms in tumour samples taken both pre- and post-chemotherapy.

Keywords: Drug resistance; Ovarian cancer; Carboplatin; Paclitaxel; Platinum compounds; Chemotherapy; DNA repair; Thymidylate synthase; Signal transduction; DNA methylation

1. Introduction

Clinical drug resistance is a major barrier to overcome before chemotherapy can become curative for most patients presenting with metastatic cancer. In many common cancers (for example, non-small cell lung, colorectal and ovarian cancers), substantial tumour shrinkage can be expected in more than 50% of cases with conventional chemotherapy. In other cases, response rates are lower; 10–20% of patients with renal cell carcinoma, pancreatic and oesophageal cancers respond to treatment. In almost all, drug resistance eventually develops within a few years and is universally fatal. If this could be prevented, the impact would be substantial.

Clinical tumour resistance to chemotherapy can be intrinsic or acquired. Intrinsic resistance is present at the time of diagnosis in tumours that fail to respond to first-line chemotherapy. Acquired resistance occurs in tumours that are often highly responsive to initial treatment, but on tumour recurrence, exhibit an entirely different phenotype. They become resistant to both previously used drugs, and new agents with different structures and mechanisms of action. Clinical resistance to a wide range of anticancer agents should not be confused with experimental 'multidrug resistance', whereby cross-resistance to structurally-unrelated compounds in experimental models is associated with increased expression of P-glycoprotein (P-gp) [1].

Rational attempts to tackle clinical drug resistance need to be based on an understanding of the mechanisms involved. Given the high level of genomic instability and mutations seen in cancer cells, these mechanisms are likely to be complex and multifactorial, allowing the cancer cell many escape routes to survival. This article reviews a number of strategies used to tackle drug resistance, with an emphasis on work carried out at our institution. Particular emphasis will be placed on the need to further describe the mechanisms of resistance through the laboratory analysis of sequential clinical samples.

1.1. Causes of drug resistance

The causes of drug resistance fall into two groups; firstly, those leading to inadequate drug exposure and, secondly, alterations in the cancer cell itself that affect drug sensitivity (Table 1). Factors leading to inadequate

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drug exposure include: giving an insufficient dose, low bioavailability, poor drug distribution, increased metabolism and excretion and impaired diffusion of drug to tumour cells (sanctuary sites, poor vascular supply). Alterations in the cancer cell itself may be: increased drug efflux, decreased drug influx, activation of detoxification systems, alterations of the drug targets and evasion of apoptosis.

1.2. Focus on ovarian cancer

This review is focussed primarily on ovarian cancer, but the general approach to the study of drug resistance and strategies employed to overcome it may be relevant to cancers arising in other sites. Ovarian cancer affects approximately 1 in 75 women in the developed world, and is metastatic at presentation in over 75% of cases. It is one of the most chemo-sensitive cancers, with response rates of approximately 70% to combination chemotherapy. Randomised controlled trials over the last 10 years have established paclitaxel–carboplatin as the standard treatment for stage III-IV disease. However, drug resistance often develops quickly, with tumours recurring in most cases within 2 years of the initial treatment. Median survival for these patients is 2–3 years, with only 30% surviving 5 years from diagnosis.

Ovarian cancer provides a good opportunity to study clinical drug resistance for two further reasons. Firstly, there are considerable experimental data available on resistance to platinum and taxanes. Secondly, and crucially, tumour cells are often easily accessible both pre- and post treatment from patients with ascites (Fig. 1).

1.2.1. Data available in ovarian cancer—how to establish clinical relevance?

So what causes clinical drug resistance in ovarian cancer, and how can we tackle it? These questions are usually addressed in studies which use stored tumour

Table 1
Causes of clinical drug resistance

Reason for clinical drug resistance	Mechanisms involved	Therapeutic manoeuvres to tackle drug resistance
Inadequate drug exposure		
Insufficient dose	Low plasma drug concentration	Increase dose
Poor drug distribution	Low drug solubility High degree of plasma protein binding Low level of tissue binding	Pegylation •Pegylated liposomal doxorubicin Polymer–drug conjugates •HMPA–drug conjugates
Impaired diffusion to tumour cells	Sanctuary sites Poor vascular supply	
Alterations in the cancer cell itself		
Increased drug efflux	ATP-dependent pumps •P-Glycoprotein (P-gp, MDR1) •Major vault protein (LRP)	 •P-glycoprotein inhibitors •PSC 833
Evasion of apoptosis	Impaired DNA damage response •Mismatch repair deficiency •Loss of hMLH1 expression via promotor hypermethylation •Platinum resistance Dysfunctional p53 •Platinum resistance Proteins regulating apoptosis •Bcl-2, BAX, surviving •CDK1A (p21, CIP1) •CDK1B (p27, KIP1) PI3 kinase pathway (PTEN, PI3K, AKT)	Hypomethylating agents •Decitabine Signal transduction inhibitors •HSP90 inhibitors (17AAG) •PI3 kinase inhibitors
Activation of detoxification systems	Cytoplasmic inactivation by -thiols •Glutathione/metallothionein •Platinum resistance	Target cytotoxics Novel platinum analogues
Alteration of drug targets	β-Tubulin mutations •Paclitaxel resistance	Alternative antimicrotubule agents •Epothilones

ATP, adenosine triphosphate; PI3 kinase, phosphoinositide-3-kinase; MDRI, multi-drug resistance; HSP90, heat shock protein 90.

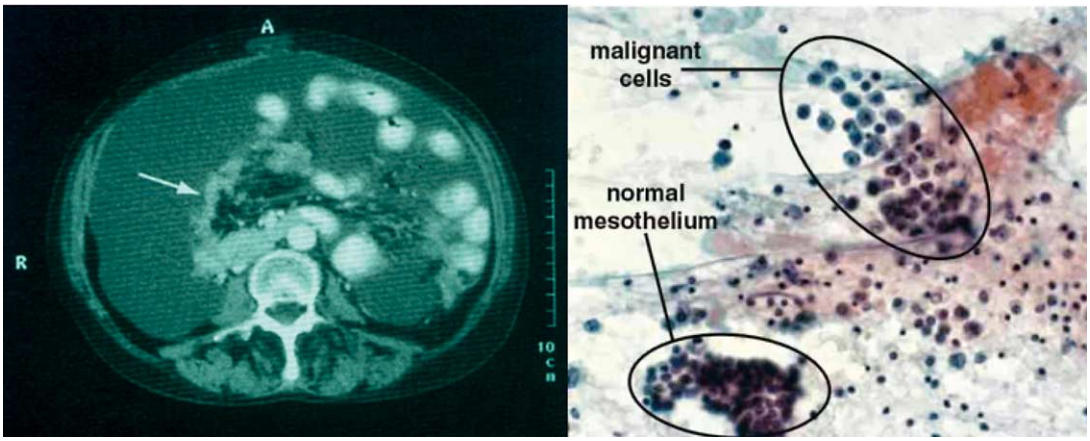

Fig. 1. Ascites in ovarian cancer.

material obtained prior to therapy. The levels of DNA, RNA or protein expression are then correlated with a clinical database, preferably from prospective phase III clinical trials in which treatment is controlled. The problem is that any positive correlation between outcome and expression levels can be attributed either to drug resistance or to some other biological property in cells expressing high levels of the protein in question, e.g. increased propensity for invasion and metastasis in the case of cells expressing P-gp [2]. The only way to distinguish between these two interpretations is to obtain information from tumour material after chemotherapy as well as beforehand. There are few examples of this approach, but the presence of ascites in many patients with relapsed ovarian cancer is an opportunity to study evolving drug resistance in a tumour cell population over time. We are therefore engaged in a systematic approach to the collection of ascites in ovarian cancer patients in our unit. Using magnetic beads coated with the BerEP4 epithelial antibody, tumour cell separation and enrichment is feasible in most cases. Our aim is to utilise this resource to perform gene expression profiling on tumour samples from patients with varying degrees of clinical drug resistance. One molecular marker of potential importance is AKT, overexpression of which has previously been noted in ovarian cancer. In a preliminary immunohistochemical study in the first 12 samples, 100% of samples were positive. This observation will now be followed-up with our gene expression studies.

2. Drug resistance—mechanisms and reversal

2.1. Inadequate drug exposure

Inadequate drug exposure may result from: giving an insufficient dose, poor drug distribution, increased metabolism and excretion and impaired diffusion of the drug to tumour cells (Table 1).

2.1.1. Dose of chemotherapy

Chemotherapy should, by definition, be given at the maximum tolerated dose (MTD) to achieve maximum tumour cell kill. Retrospective studies suggest that efficacy is proportional to the degree of myelotoxicity encountered. However, the question of dose has been addressed by a number of studies in different tumours comparing the effectiveness and toxicity of higher-dose chemotherapy regimens [3–6]. One such study randomised patients with advanced ovarian cancer to receive six cycles of cyclophosphamide 750 mg/m^2 and either 50 or 100 mg/m^2 cisplatin [5,6]. The higher dose of cisplatin indeed resulted in a significantly improved median survival, with a relative death rate of 0.52 for the high-dose group. However, this was at the cost of increased toxicity, in particular, neurotoxicity. The survival benefit also reduced over time, with the relative death rate slipping to 0.68 after a median follow-up of 4 years and 9 months, whilst the toxicity persisted. Ultimately, the survival curves come together, indicating the eventual regrowth of a population of tumour cells which have survived despite higher dose treatment. Much higher doses of chemotherapy (i.e. 4-fold or more increases in the dose of the most active drug—carboplatin) are currently being explored in prospective randomised trials. An alternative way of achieving improved tumour drug exposure is intraperitoneal (IP) chemotherapy [7]. The role of IP chemotherapy remains to be firmly established, although overall the data do appear promising.

2.1.2. New drug-delivery systems

Inadequate drug exposure can also be caused by poor drug delivery to the tumour. This can occur because of:

low bioavailability, extensive first-pass metabolism, high plasma protein binding and low tissue binding (Table 1). One approach is to use novel drug delivery systems, which include pegylation [8–10] and polymer-drug carriers [11].

2.2. Alterations in the cancer cell itself

2.2.1. Increased drug efflux

Experimental models demonstrate that multidrug resistance can be caused by increased expression of adenosine triphosphate (ATP)-dependent efflux pumps [1]. These pumps actively transport drugs out of the cell, in particular natural product hydrophobic drugs, for example: vinca alkaloids, anthracyclines and taxanes. The *ABC1* multi-drug resistance (*MDR1*) gene produces the transporter P-gp [12], which is overexpressed in many multidrug resistant cells; other ABC genes encode different multidrug-resistance-associated proteins, for example MRP1 (ABCC1).

ABC transporters, like MDR1 and MRP1, are overexpressed in a number of human cancers including leukaemias and solid tumours. In ovarian cancer, up to two-thirds of tumour specimens have been found to overexpress P-gp on immunohistochemistry [13–17], and this overexpression has been shown in some cases to correlate with poor overall survival. Once again, most of these studies are based on a single tissue snapshot taken before chemotherapy; it remains unclear whether P-gp overexpression is correlated with resistance to drug treatment or an aggressive tumour phenotype.

Experimental models have shown that, at least *in vitro*, excessive drug efflux mediated through P-gp can readily be reversed by a plethora of drugs, for example: phenothiazines, verapamil and cyclosporin A. This led to a large number of clinical trials with P-gp-modulating agents (e.g. with verapamil and cyclosporin), but these drugs were only effective at toxic doses [18].

PSC-833 (Valspodar), an analogue of cyclosporin, was found to be an P-gp inhibitor 10 times more potent than cyclosporin, and without its side-effects of nephrotoxicity and immunosuppression. Combination phase I and II studies in different tumour types showed that PSC-833 had a profound effect on the pharmacokinetics of the co-administered chemotherapy. This effect was predictable, and could be adjusted for by reducing the dose of chemotherapy given. Dose-limiting toxicities included myelosuppression, hyperbilirubinaemia and ataxia. Many of these combinations had encouraging antitumour efficacy, leading to phase III testing in a number of tumour types.

One such phase III trial compared carboplatin and paclitaxel with or without PSC-833, for the treatment of 762 patients with advanced ovarian cancer. Patients receiving PSC-833 were treated with a reduced dose of paclitaxel 80 mg/m^2 and carboplatin area under the curve (AUC) 6. The results showed that the addition of PSC-833 led to a reduction in response rates and no difference in overall survival. These data suggest that the strategy of tackling P-gp-mediated drug resistance alone is questionable; it may be more important to focus on other targets or a combination of factors together.

2.2.2. Activation of detoxification systems

Since the introduction of cisplatin into the clinic over 30 years ago, preclinical studies have provided considerable insight into the mechanisms of resistance to platinum-based chemotherapy. Cisplatin causes cancer cell death through the formation of DNA adducts which may be on the same strand (intrastrand adducts) or different strands (interstrand adducts). Resistance to cisplatin has been studied in tumour cells that have been repeatedly exposed to the drug *in vitro*. These experiments have shown that *in vitro*, resistance to platinum results from a combination of mechanisms that include: reduced drug uptake, increased inactivation by intracellular thiols, and aberrant DNA repair. In our institution, particular focus was placed on inactivation by thiols, and experimental *in vivo* models of platinum resistance in which this was an important factor were developed as a means to test novel agents [19].

ZD0473 is a platinum analogue which avoids binding to cytoplasmic thiols, hence overcoming thiol-mediated detoxification [20] (Fig. 2). In a phase I study at our institution, escalating doses of single agent ZD0473 were administered as a 1-h infusion every 21 days to 42 patients with advanced solid tumours [21]. Neutropenia and thrombocytopenia were dose-limiting, with the MTD identified as 150 mg/m^2. 2 heavily pretreated patients with ovarian cancer had partial responses, and 5 others had prolonged stable disease. The recommended dose of 120 mg/m^2 was taken on to a phase II trial in platinum-pretreated ovarian cancer. A total of 94 patients were treated, two-thirds of whom had been platinum-resistant, the other one-third, platinum-sensitive. Objective response rates were 8.3 and 32.4%; median time to progression was 57 and 180 days and median survival was 242 and 402 days, for resistant and sensitive patients, respectively [22]. The hypothesis was that, if clinical resistance related to thiol-mediated toxification, the results of treatment with ZD0473 should be superior to those expected with carboplatin, to which clinical resistance had developed. Although no randomised studies have been done, these results do not support this hypothesis.

Our experience with ZD0473 leaves a number of unanswered questions: should we conclude that *in vitro* models are irrelevant?; is it unrealistic to expect significant activity in platinum-refractory patients, where multiple mechanisms for clinical resistance probably apply?; and how do we define a level of clinical activity

in this group of patients which would justify further testing of a novel platinum analogue?

2.2.3. Alteration of drug target(s), specifically tubulin mutations

Data from experimental models suggest that one important factor causing paclitaxel resistance is the development of tubulin mutations which interfere with binding of the drug to its target, thus reducing its efficacy [23]. The paclitaxel analogue, docetaxel, is a more potent inhibitor of microtubule function than paclitaxel, and it differs in its effect on certain classes of microtubules. Docetaxel also does not activate the orphan nuclear receptor SXR (in contrast to paclitaxel) and this may explain its longer intracellular retention [24] and the lack of cross-resistance to paclitaxel in some experimental models. In addition, a number of new microtubule-binding agents that may be non-cross-resistant with paclitaxel are in clinical development, including various members of the epothilone family.

2.2.3.1. Docetaxel. Clinical studies have shown that it has a different toxicity profile to paclitaxel, with a higher incidence of myelosuppression, but lower incidence of peripheral neuropathy. Docetaxel is currently licensed for use in a range of malignancies, including breast and lung cancers [25,26]. Initial phase II trials confirmed activity in relapsed ovarian cancer [27] and, more recently, this was also demonstrated in patients with paclitaxel-resistant ovarian cancer [28].

Trials in first-line treatment have therefore been conducted, and the Scottish Randomised Trial in Ovarian Cancer (SCOTROC1) tested the combination of docetaxel with carboplatin against the standard treatment of paclitaxel with carboplatin. The study randomised 1,077 patients with the International Federation of Gynecology and Obstetrics (FIGO) stage Ic to IV disease to six cycles of carboplatin AUC 5 with either paclitaxel or docetaxel at 75 mg/m^2. The authors found no significant difference in the response rates, progression-free or overall survival, but there were differences in the toxicities experienced by patients in either arm. There was more myelosuppression with docetaxel–carboplatin (without additional mortality), whereas patients receiving paclitaxel–carboplatin had a higher incidence of peripheral neuropathy. These results suggest that although there is no evidence of superior efficacy, docetaxel–carboplatin may be a reasonable first-line option for patients with ovarian cancer in view of its toxicity profile. Recent data from randomised clinical trials suggest that repeat treatment with taxanes may be practised more widely for relapsed patients, and, in this context, the earlier data on the treatment of paclitaxel-resistance may support an additional role for docetaxel.

2.2.3.2. Epothilones. Epothlione B is a naturally-occurring macrolide produced by the myxobacterium *sorangium cellulosum* which has anticancer effects by interfering with tubulin polymerisation in a similar way to the taxanes. Epothilone B is twice as potent as paclitaxel, kills cells at low nanomolar concentrations and retains activity in paclitaxel-resistant ovarian cancer cells which express P-gp and have the *β-tubulin* gene mutation [29].

Two molecules have been taken into clinical development: EPO-906 (Novartis) and BMS 247550. They differed somewhat in their spectrum of toxicity in phase I trials, with diarrhoea being more prominent with EPO-906, and neurotoxicity more prominent with BMS 247550. A phase II study in patients with platinum-refractory ovarian cancer has been conducted with EPO-906, and preliminary results indicate a degree of activity [30]. Further studies, probably with a 3-weekly rather than a weekly schedule, are planned.

2.2.4. DNA repair

Experimental models have, over the last 50 years, dramatically increased our understanding of the response of cells to DNA damage [31]. Very briefly, mispaired bases, usually from mistakes in DNA replication, are excised as single nucleotides during mismatch repair. Damaged bases are normally excised as single free bases in base excision repair or as oligonucleotide fragments during nucleotide excision repair. Double-strand breaks, often the result of exposure to ionising radiation or oxidative damage, are either repaired by non-homologous end joining or by homologous recombination. The response to DNA damage is either DNA repair, damage tolerance (i.e. resistance) or apoptosis (i.e. sensitivity).

This DNA damage response has a profound influence on tumour sensitivity or resistance to chemotherapy and radiotherapy. Resistance can result either from DNA repair or damage tolerance, where 'sloppy' DNA polymerases allow DNA replication to continue past damaged sites. Cells have evolved complex signalling pathways to arrest the cell cycle in the presence of DNA damage to allow time for DNA repair or tolerance mechanisms to operate. If the genomic insult is too large, cells initiate apoptosis, resulting in sensitivity to treatment [32].

2.2.4.1. Mismatch repair deficiency, loss of hMLH1 and microsatellite instability in ovarian cancer. Mismatch repair (MMR) deficiency can cause drug resistance through the failure of tumour cells to recognise DNA damage. Such tumours are often characterised by the instability of microsatellite sequences (microsatellite instability). Experimental data implicate MMR-deficiency in the resistance to a wide range of DNA-damaging agents, including cisplatin and doxorubicin [33]. *In vitro* selection for cisplatin resistance in the

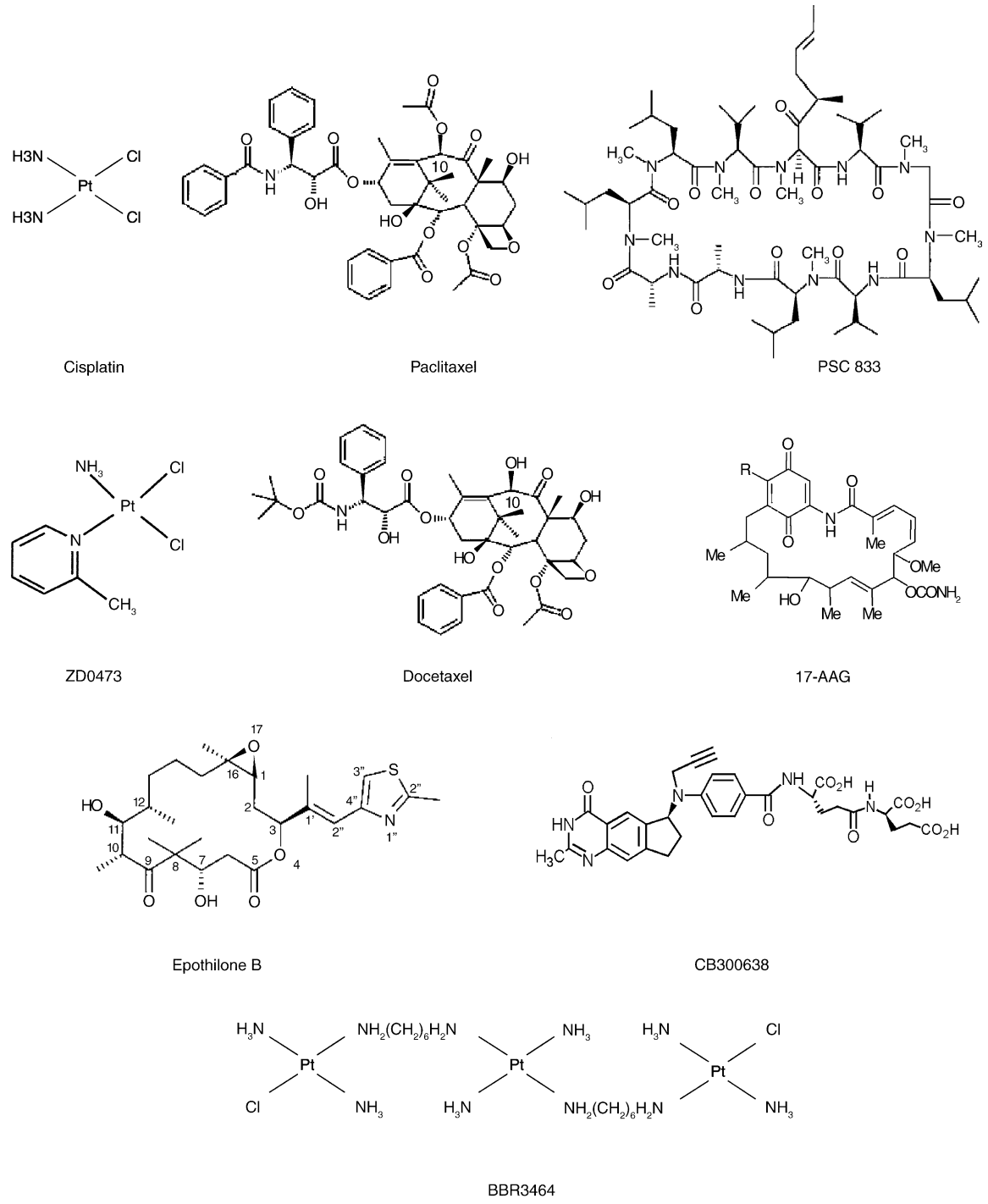

Fig. 2. Selected drug structures.

A2780 ovarian cancer cell line results in the loss of hMLH1 expression in 90% of the resultant cell lines.

One clinical series has compared microsatellite stability with hMSH2 and hMLH1 expression between resected ovarian tumours before and after five or six cycles of cisplatin-based chemotherapy [34]. Of the 24 primary resected tumours, nine (38%) showed microsatellite instability, while 15 (63%) showed microsatellite stability. Interestingly, all of the cases with microsatellite stable primary tumours before chemotherapy exhibited microsatellite instability in the residual tumours after chemotherapy ($P<0.001$). Furthermore, 11 (73%) of these cases which changed from stable to unstable also had a change in the expression of hMLH1 from positive to undetectable ($P<0.001$). The authors concluded that loss of hMLH1 expression is an important factor inducing changes in tumour microsatellite instability during cisplatin-based chemotherapy. More recently, a large-

scale study examined pre- and postchemotherapy blood samples for microsatellite instability (MSI) in tumour DNA. Preliminary data on the first 90 patients do indicate an increase in MSI on relapse, in keeping with the hypothesis that mismatch repair deficiency is relevant to the development of clinical resistance in ovarian cancer [35]. However, there are data to indicate that loss of hMLH1 expression (i.e. MSI) in the initial tumour correlates with an improved progression-free survival in ovarian cancer [35]. This correlation has also been seen in colon cancer. Although this seems contradictory to the notion that MSI relates to drug resistance, in fact the two observations are not mutually exclusive. It has been suggested that MSI may lead to an increased antitumour immune response, and this is a further example of the complexities involved where molecular prediction of resistance overlaps with those relating to biological phenotypes. This complexity may explain the data in other clinical series of patients with advanced ovarian cancer, which found that hMLH1 and hMSH2 staining by immunohistochemistry decreased significantly after platinum-based therapy. However, the authors could not find a correlation between immunohistochemical staining for either hMLH1 or hMSH2 and response or survival [36].

2.2.4.2. 2′-Deoxy-5-azacytidine (Decitabine). Treatment of hMLH1-deficient, platinum-resistant ovarian cell lines with the demethylating agent 2′-deoxy-5-azacytidine (decitabine) results in re-expression of hMLH1 and re-sensitisation of the cells to cisplatin [37]. Futhermore, decitabine has been used *in vivo* to re-sensitise MMR-deficient, drug-resistant ovarian and colon tumour xenografts that are hMLH1-negative because of gene promoter hypermethylation [38]. Decitabine treatment alone had no effect on xenograft growth. However, treatment sensitised the xenografts to cisplatin, carboplatin, temozolamide and epirubicin. Interestingly, decitabine treatment does not appear to sensitise to paclitaxel, nor does it sensitise HCT116 xenografts which have deficient MMR because of hMLH1 mutation (as opposed to gene silencing by hypermethylation).

The increasing evidence that methylation of the *hMLH1* promoter may be a common mechanism for the silencing of hMLH1 expression and platinum resistance has led to clinical trials of decitabine. In our institution, a phase I trial of decitabine and carboplatin asks the question: is it feasible to give decitabine (starting dose 45 mg/m^2) together with carboplatin (AUC 5) in order to achieve hypomethylation (and potential reversal of resistance) without prohibitive myelosuppression? The trial design incorporates biological endpoints to monitor the effects of decitabine on DNA methylation in surrogate tissues and tumour. The initial experience in Glasgow and the Marsden is encouraging, and randomised studies in ovarian cancer are currently under discussion.

2.2.5. Evasion of apoptosis

Chemotherapy and radiotherapy kill proliferating cells mainly by apoptosis. Experimental models indicate that a critical balance exists between cell cycle arrest (which may allow DNA repair and drug resistance) and apoptosis (chemosensitivity). An intricate network of factors has evolved to control this balance. The response to chemotherapy may also be attenuated through the overexpression of other anti-apoptotic proteins, or the reduced activity of pro-apoptotic proteins. Probably the exact mechanisms of chemotherapy-induced cell death depends on the cell type, the drug given, the tumour microenvironment and a host of other factors.

2.2.5.1. Dysfunctional p53. *TP53* is the most frequently mutated gene in human cancers, and has a central role in determining the response of tumour cells to chemotherapy. There is a large body of experimental data which indicates that lack of functional p53 may contribute to drug resistance through the inability of resistant cells to undergo apoptosis in response to DNA damage [39–41]. p53 is activated by DNA damage either directly or indirectly. Active p53 can induce the expression of mitochondrial pro-apoptotic genes (e.g. BAX, NOXA, PUMA and p53AIP1) and those in the death receptor pathway (including CD95, TRAIL-R1 and TRAIL-R2).

The *TP53* gene is frequently altered in ovarian cancer. A number of studies have demonstrated that patients whose ovarian tumours show dysfunctional p53 before chemotherapy have worse clinical outcomes following platinum-based chemotherapy [14,42–44]. Once again, interpretation of these results is difficult because we do not know whether the p53 status predicts poor response to chemotherapy or an inherently aggressive tumour phenotype.

Nevertheless, numerous attempts to circumvent resistance based on dysfunctional p53 have been made. These include the assessment of platinum analogues such as BBR3464, a trinuclear platinum complex with *in vitro* IC_{50} values 20-fold more potent than cisplatin and a different pattern of activity across the National Cancer Institute (NCI60) cell line panel. It forms intrastrand cross-links that are not removed from DNA by nucleotide excision repair, and thus may persist considerably longer in tumour cells [45]. The drug shows little cross-resistance with cisplatin *in vitro* and keeps its potency in cells resistant to other platinum compounds [46]. Furthermore, good *in vivo* activity against xenografts, particularly those with p53 mutations[47] led to clinical trials of this drug. In phase I, 14 patients received BBR3464 on a daily ×5 schedule every 4 weeks. Short-

lasting neutropenia and late-onset diarrhoea were dose-limiting and defined the MTD at 0.12 mg/m^2. Nausea and vomiting were rare, and neither neuro- nor nephrotoxicity were seen [48]. In a phase II trial, BBR3464 has demonstrated activity in platinum-sensitive patients (>6 months interval from prior therapy), but showed little activity in patients with platinum-refractory ovarian cancer [49].

Other relevant clinical trials include those involving intraperitoneal 'genetic' therapy in which a replicating virus, Onyx 015, has been used. This virus should replicate preferentially in p53-mutant cells and a phase I trial indicated that the approach was feasible, although associated with some toxicity. A different IP study has utilised an adenoviral vector in an attempt to transfect tumour cells with wild-type p53; analysis indicated that transfecion had taken place and this led to a subsequent randomised trial incorporating IP delivery of this adenoviral vector into first-line therapy. However, at this stage, neither of these approaches have yielded clearly positive data; nevertheless, it remains possible that p53 plays a central role in the evolution of drug resistance, and further innovative approaches are clearly justified.

2.2.5.2. Expression of other apoptosis-related proteins. Expression levels of a number of apoptosis-related proteins have also been correlated with clinical outcome in ovarian cancer. These include: Bcl-2 [50,51], BAX [52], the cyclin-dependent kinase inhibitors CDK1A (p21, CIP1) [53] and CDK1B (p27, Kip1) [16], survivin [54] and components of the phosphoinositide-3-kinase (PI3K) pathway [55–58].

Survivin overexpression has been associated with resistance to paclitaxel in one series of 124 advanced ovarian carcinomas [54]. Using immunohistochemistry, 90/124 (73%) of these tumours showed high levels of survivin expression. In the 95 patients receiving a paclitaxel/platinum-based regimen, survivin overexpression correlated with a lower complete remission rate than absent or low protein expression (43 versus 75%, $P=0.0058$ by logistic regression adjusted for tumour stage, histological grade and p53 expression). Conversely, in the 29 cases treated with cisplatin-containing regimens not including paclitaxel, survivin expression was unrelated to tumour response.

Components of the anti-apoptotic PTEN/PI3K pathway have also been found to be overexpressed in ovarian cancer. Downregulation of PTEN has been associated with an increase in AKT phosphorylation and activity [59,60]. Lysophosphatidic acid, a growth factor found in ovarian cancer ascites, also promotes cell survival by stimulating the PI3K pathway. Interestingly, ovarian cancer cell lines with an overactive pathway have been shown to be unusually sensitive to the PI3K inhibitor CCI-779. It will be interesting to see if patients whose ovarian tumours reflect the same phenotype are sensitised to chemotherapy with PI3 kinase inhibitors in due course. Indeed, the modulation of a patient's response to chemotherapy by signal transduction inhibitors is one of the most exciting fields in contemporary drug development.

2.2.5.3. The role of signal transduction inhibitors. The production of signal transduction inhibitors is designed to specifically target deregulated pathways driving malignant progression. Following the dramatic success of drugs like imatinib mesylate (Gleevec; Glivec) for chronic myeloid leukaemia and gastro-intestinal stromal tumours [61,62], and trastuzumab (Herceptin) for HER2-positive breast cancer [63], there are a myriad of strategies being pursued. For an overview of recent developments in this area, see Ref. [64]. For more detailed consideration of the progress in particular areas, readers are directed to the following reviews: protein kinase inhibitors in general [65]; CDK inhibitors [66]; inhibitors of PI3 kinase [58]; anti-angiogenic agents [67,68]; farnesyl-transferase inhibitors [69,70]; inhibitors of the RAS-RAF-mitogen-activated protein kinase (MAPK) pathway [71,72]; drugs modulating the p53 pathway [73–75]; drugs targeting telomeres and telomerases [76–78]; inhibitors of integrins [79] and proteosome inhibitors [80].

Some of these signal transduction inhibitors have anti-tumour activity in their own right, but in the short-to-medium term, signal transduction inhibitors will probably find greater use in combination with 'traditional' cytotoxic agents as chemosensitisers. One promising signal transduction approach, in development at our institution and others, is heat shock protein 90 (HSP90) inhibitors. The concept here is that the inhibition of several key signalling proteins together may be more effective than the selective inhibition of a single signal.

HSP90 is a molecular chaperone that functions to stabilise a number of mutated and overexpressed 'client' signalling proteins that promote the proliferation and survival of cancer cells [81,82], including: RAF-1 [83], ErbB2 [84], CDK4 [85], mutant p53 [86], oestrogen and androgen receptors [87–90], AKT and the catalytic component of telomerase, hTERT [81].

Inhibition of HSP90 is an extremely attractive means for tackling multiple deregulated genes and pathways simultaneously, and HSP90 inhibitors could have the potential to tackle all six of the so-called 'hallmark traits' of malignancy [91].

In the early 1990s, geldanamycin and other benzoquinone ansamycins were found to bind to HSP90. These agents compete with ATP for binding at the nucleotide-docking site and prevent the correct assembly of mature HSP90/client protein/co-chaperone complexes. This, in turn, leads to proteasomal degradation by recruitment of an ubiquitin ligase.

Geldanamycin proved too hepatotoxic for clinical use, but one of its derivatives, 17-allylamino, 17-

demethoxygeldanamycin (17AAG), was found to be less hepatotoxic, while retaining the antitumour activity of geldanamycin [92]. 17AAG has consistent antitumour activity in xenografts [93–95], acceptable toxicity, and is currently in phase 1 clinical trials. Through its effects on several key signalling proteins controlling the process of apoptosis, 17AAG may also act as a modulator of resistance to chemotherapy. Indeed, *in vitro* and *in vivo* experiments do demonstrate additive or synergistic effects in combination with doxorubicin, cisplatin and possibly paclitaxel [96]. Various techniques including Western blotting and gene-expression microarray analysis have been used to define a molecular signature of HSP90 inhibition [97], including the depletion of client proteins such as Raf-1, ErbB2, and CDK4 and the simultaneous upregulation of HSP70. Proteomic techniques were used successfully to identify a further marker, AHA1, which represents the first HSP90 co-chaperone to be identified that acts as an activator of the ATPase activity of the chaperone [98].

Of particular interest in the phase 1 trial of 17AAG at our own centre has been the use of this molecular signature to help define the optimal dose and schedule of the drug. This pattern of HSP90 inhibition has been followed and validated through cancer cells in culture and human xenografts to patient samples that include both peripheral blood lymphocytes and tumour biopsies [99]. In addition, 2 patients with previously progressive disease have had stable disease after treatment with 17AAG for several months. These results have stimulated interest in phase II studies, as well as further trials of 17AAG in combination with chemotherapy.

3. Targeted cytotoxics

Thymidylate synthase (TS) is a key enzyme in the synthesis of DNA and an important target in cancer chemotherapy. TS inhibitors in widespread clinical use include 5-fluorouracil and methotrexate, whereas more recently developed TS inhibitors include raltitrexed, pemetrexed and capecitabine [100]. However, the clinical utility of TS inhibitors is limited by normal tissue toxicity.

3.1. Thymidylate synthase inhibitors: ZD9331 and CB300638

ZD9331 is a potent (TS ki = 0.4 nM), non-polyglutamatable inhibitor of TS that is largely transported into cells via the ubiquitously expressed reduced-folate carrier (RFC) [101]. It overcomes resistance to raltitrexed-resistant ovarian cancer cell lines which express low levels of the TS activating enzyme, folylpolyglutamyl synthetase (FPGS) often required to activate TS inhibitors, and inhibits tumour xenograft growth *in vivo*. Phase I studies of ZD9331 have used either intravenous (i.v.) [102] or oral (p.o.) [103] formulations of the drug. However, preliminary phase II data indicate only a modest degree of activity in refractory ovarian cancer, and other avenues in novel TS inhibitors are therefore being explored in this disease.

The alpha-folate receptor (α-FR) is the alpha isoform of the membrane-associated folate binding protein. It is able to bind and mediate the internalisation of folates. α-FR expression is normally restricted to the choroid plexus, placenta, and kidney but is significantly overexpressed in certain human cancers. These include epithelial ovarian carcinoma, and using immunohistochemical assays, studies have indicated that over 90% overexpress the receptor. Moreover a link with clinical platinum resistance has been suggested [104]. It may therefore provide a unique target for the selective uptake of cytotoxic agents.

CB300638, an example of a new generation of TS inhibitors that is selectively transported via the α-FR, is currently being developed at our institution. It has a very low RFC affinity, but high affinity for the α-FR. It is highly selective for α-FR-overexpressing cell lines with normal RFC function, with IC_{50} values in the low nanomolar range for these cell lines. In addition, it is also non-polyglutamated and water-soluble. Mice bearing KB cell xenografts were treated with 100 mg/kg of CB300638 or ZD9331. In contrast to ZD9331 the drug CB300638 was taken up preferentially into KB tumour tissue and TS inhibition occurred specifically in tumour cells, but not normal cells. CB300638 is the first antimetabolite to inhibit TS in tumours, but not in normal tissues. This suggests an improved therapeutic index compared with other TS inhibitors [105].

4. Drug resistance reversal: are we getting closer?

It is clear from the approaches described above that we are getting closer to the reversal of drug resistance. Further progress will require a three-pronged approach, namely: the development of novel cytotoxics which exploit selectively expressed targets; modulation of resistance to conventional agents and, perhaps most importantly of all, a serious attempt to understand resistance mechanisms in tumour samples, both pre- and postchemotherapy.

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