Pergamon

European Journal of Cancer, Vol. 34, No. 10, pp. 1535–1542, 1998

Printed in Great Britain
0959-8049/98/$—see front matter

PII: S0959-8049(98)00227-5

Clinical Oncology Update

Cellular and Molecular Determinants of Cisplatin Resistance

R.P. Perez

Section of Hematology/Oncology, Dartmouth-Hitchcock Medical Center, One Medical Center Drive, Lebanon, NH 03756, U.S.A.

Cisplatin and carboplatin are among the most active and widely used cytotoxic anticancer drugs. However, the acquisition or presence of resistance significantly undermines the curative potential of these drugs against many malignancies. Multiple potential mechanisms of resistance have been identified at the cellular and molecular levels. Alterations in cellular pharmacology, including decreased drug accumulation, increased cellular thiol levels and increased repair of platinum–DNA damage, have been observed in numerous model systems. More recently, it has become apparent that an enhanced capacity to tolerate cisplatin-induced damage may also contribute to resistance. Alterations in proteins that recognise cisplatin–DNA damage (mismatch repair and high-mobility group (HMG) family proteins) and in pathways that determine sensitivity to apoptosis may contribute to damage tolerance. It remains to be determined whether any of these mechanisms contribute significantly to resistance in the clinical setting. Ongoing biochemical modulation and translational correlative trials should clarify which specific mechanisms are most relevant to clinical cisplatin resistance. Such investigations have the potential to improve the ability to predict likelihood of response and should identify potential targets for pharmacological or molecular intervention.

Key words: cisplatin, drug resistance, drug accumulation, glutathione, metallothionein, nucleotide excision repair, apoptosis, mismatch repair, high-mobility group (HMG) proteins, biochemical modulation
Eur J Cancer, Vol. 34, No. 10, pp. 1535–1542, 1998

INTRODUCTION

CISPLATIN AND carboplatin are among the most widely used and broadly active cytotoxic anticancer drugs. Platinum-based chemotherapy is curative for the majority of patients with advanced testis cancer, which was almost uniformly fatal in the pre-cisplatin era [1]. Cisplatin and carboplatin also have major efficacy and are components of standard treatment regimens for ovarian, bladder, cervical, head and neck and small-cell and non-small cell lung cancers (NSCLC) [2, 3]. Unfortunately, many patients with these malignancies eventually relapse and become refractory to chemotherapy. In addition, cisplatin and carboplatin have minimal activity against some common tumour types, such as colorectal or pancreatic carcinoma. Thus, the acquisition or presence of resistance to cisplatin and carboplatin is a major clinical problem that undermines the curative potential of these drugs.

Received 25 Mar. 1998; accepted 1 Apr. 1998.

Considerable effort has been invested in defining the cellular and molecular mechanisms responsible for cisplatin resistance. Most published data are derived from preclinical model systems of varying complexity, where a large number of potential resistance mechanisms have been identified. Such mechanisms can be broadly grouped into two categories: mechanisms that limit the extent of drug-induced damage and mechanisms that alter the cellular response to damage that is induced.

Cellular pharmacology and cellular responses to cisplatin

Cisplatin enters cells by mechanisms that remain incompletely defined. The available data are most consistent with the hypothesis that cisplatin enters cells via transmembrane channels [4], though these data are also consistent with high-capacity facilitated transport. Once inside cells, cisplatin and carboplatin undergo aquation hydrolysis to form identical active species, which is rate-limiting for subsequent interactions with cellular target molecules.

Cisplatin and carboplatin react with many potential target molecules, including genomic DNA, RNA and protein [5]. Approximately 1% of the intracellular cisplatin or carboplatin reacts with genomic DNA yielding a variety of intra- and inter-strand mono-adducts and crosslinks, the most common being an intrastrand crosslink between adjacent guanines [6, 7]. Cisplatin and carboplatin form identical lesions in DNA, although carboplatin reacts with slower kinetics [8]. DNA is clearly an important target for cisplatin. Direct correlations between cisplatin–DNA damage and cytotoxicity or clinical response have been reported and cell lines with nucleotide excision-repair defects are hypersensitive to the drugs [9–11]. In addition, some cisplatin-resistant cell lines have an increased capacity to repair cisplatin–DNA damage and cisplatin cytotoxicity can be potentiated by DNA repair inhibitors in some models [12–17]. At present, the potential contribution of cisplatin-induced RNA or protein damage to cytotoxicity is less clearly defined.

The fate of cells following cisplatin exposure depends both on the extent of damage induced and on the cellular response to damage. One potentially important way that cisplatin may kill cells is by induction of apoptosis. Apoptosis is a ubiquitous, genetically regulated mechanism of active cell death that is conserved in multicellular organisms [18–20]. It has unique morphological and biochemical features, including cell shrinkage, loss of cell–cell contact ('rounding-up'), chromatin condensation and fragmentation and characteristic DNA degradation. These changes are accompanied by loss of vital dye exclusion and loss of mitochondrial membrane potential.

Considerable evidence indicates that cisplatin can kill cells by apoptosis [21–24]. Internucleosomal DNA cleavage and ultrastructural changes characteristic of apoptosis have been observed following cisplatin in murine L1210 leukaemia and Chinese hamster ovary cell lines [21, 22] and in proliferating rat hepatoma cells, but not in proliferating thymocytes [23]. These data suggest that apoptosis may be more readily triggered in rapidly proliferating cells. Apoptosis has also been detected by DNA degradation and 3′ nick-end labelling assays following cisplatin treatment of human bladder and ovarian carcinoma cell lines [25–27]. Whether apoptosis is a clinically significant mechanism of cancer cell death following treatment with cisplatin remains to be established.

The specific mechanism(s) that trigger apoptosis in response to cisplatin have not yet been defined. Logically, such mechanisms must include ways to detect damage as well as to determine whether damage is sufficiently severe to be lethal. Much attention has recently focused on identification and characterisation of proteins that recognise cisplatin-induced DNA damage [28]. At present, at least two such types of proteins have been identified: mismatch repair proteins and high-mobility group (HMG) proteins.

Mismatch repair is a post-replication repair system that corrects unpaired or mispaired nucleotides. Mismatch repair deficiency predisposes cells to genomic instability and also confers tolerance to damage induced by certain alkylating agents in some model systems [29, 30]. Deficiencies of mismatch repair are thought to be aetiologically important in hereditary nonpolyposis colon cancer and have also been identified in a variety of sporadic tumours [29, 30].

Human ovarian carcinoma cell lines selected for cisplatin or doxorubicin resistance *in vitro* acquired mismatch repair defects (deficiency of MutLα complex (a heterodimer of the MLH1 and PMS2 mismatch repair proteins)) and genomic instability [31, 32]. In one of these model systems, loss of mismatch repair was accompanied by loss of p53 function, which could disrupt cell cycle and apoptosis regulation and contribute to further genomic instability [33]. Mismatch repair deficiency was also associated with cisplatin resistance in other models, including HCT116 colon and HEC59 endometrial carcinoma cell lines [34]. In these cell lines, mismatch repair deficiency was not associated with resistance to trans-diamminodichloroplatinum(II), DACH–platinum compounds (tetraplatin, oxaliplatin), or mixed ammine–amine platinum compounds (JM216, JM335), which are incompletely cross-resistant with cisplatin in many preclinical model systems. One recent preliminary report suggests that some cell lines with mismatch repair defects may also be less proficient at nucleotide excision repair, the primary mechanism by which platinum–DNA adducts are repaired [35]. Finally, knockout cell lines deficient in either MSH2 or PMS2 mismatch repair proteins demonstrate similar low-level resistance to cisplatin or carboplatin [36].

The relationship between damage recognition by mismatch repair proteins and cytotoxicity is currently being defined. Mismatch repair proteins recognise but do not remove the cisplatin–DNA adducts. The human mismatch repair complex hMutSα (a heterodimer of the proteins hMSH2 and hMSH6) has been shown to recognise specifically a single cisplatin G-G intrastrand adduct within a synthetic duplex oligonucleotide [37, 38]. It is hypothesised that mismatch repair proteins attempt to insert the 'correct' base on the non-damaged strand opposite to the adduct. Establishment of such a 'futile' repair cycle might then generate a signal triggering apoptosis. According to this hypothesis, loss of mismatch repair proteins confers resistance through failure to recognise and initiate apoptosis in the response to unrepairable DNA damage. Resistant cells thus acquire the ability to tolerate damage that would otherwise be lethal.

The HMG proteins are a multifunctional family of small non-histone chromatin-associated proteins [39]. These proteins are involved in gene regulation and maintenance of chromatin structure. HMG proteins recognise some structural distortions of DNA, and their interactions with distorted DNA regulate transcription either directly or by facilitating interactions of other transcription factors at the same site. Several HMG-family proteins specifically recognise cisplatin–DNA adducts. HMG1 and HMG2 proteins recognise intrastrand guanine–platinum–guanine diadducts [40]. An HMG family protein, called structure specific recognition protein-1 (SSRP-1), specifically binds to cisplatin-DNA intrastrand adducts [41]. Finally, the ribosomal RNA (rRNA) transcription factor hUBF, another HMG-family protein, binds cisplatin G-G intrastrand adducts with equal affinity to its normal target, the rRNA promoter [42].

The immediate potential consequences of the hUBF—cisplatin adduct interaction are relatively straightforward. High levels of damage could sequester hUBF by binding to cisplatin–DNA adducts (termed transcription factor 'hijacking') [42]. Sequestration of an rRNA transcription factor in this manner could decrease the expression of several additional genes. Whether such events trigger apoptosis or otherwise contribute significantly to cisplatin cytotoxicity is unknown.

Several hypotheses have been proposed to explain how interaction of other HMG proteins with cisplatin–DNA adducts might affect cytotoxicity [28]. HMG protein binding

to cisplatin–DNA adducts could trigger apoptosis, modulate cell cycle events subsequent to DNA damage, or protect cisplatin adducts from recognition by DNA repair enzymes. In this latter model, unrepaired adducts would either be repaired very slowly or trigger apoptosis. These hypotheses are not necessarily exclusive. It has recently been reported that Rat HMG1 protein prevents *in vitro* translesion DNA synthesis on a template damaged by cisplatin [43]. Another HMG protein that specifically binds cisplatin-DNA adducts, the product of the Ixr1/ORD1 gene, has been isolated in yeast [44, 45]. Compared with parental strains, Ixr1 deletion mutants contain fewer platinum–DNA adducts and are 2-fold resistant to cisplatin. These data suggest that loss of Ixr1 may facilitate repair, consistent with the hypothesis that this protein protects cisplatin–DNA adducts from repair. Similar studies in human cells have not yet been reported.

Mechanisms that limit damage: altered accumulation, thiols, DNA repair

Since cisplatin cytotoxicity depends at least in part on the extent of drug-induced damage, mechanisms that limit damage are a first line of cellular defence against the drug. Alterations in drug accumulation, cellular thiol levels and DNA repair are consistently seen in cisplatin-resistant cell lines. These mechanisms, which have been reviewed in considerable detail previously [46–48], will be briefly discussed (Figure 1). In most models, resistance at the cellular level is multifactorial.

Decreased accumulation is common in cell lines selected for cisplatin resistance *in vitro* [4, 46, 47]. The methods used in most of these investigations have generally not allowed discrimination between decreased influx and increased efflux as determinants of decreased cisplatin accumulation. However, increased active efflux of cisplatin was recently observed in a cisplatin resistant cell line *in vitro* [49]. Although decreased accumulation has been observed in most cisplatin resistance models, such changes do not generally correlate with the magnitude of resistance observed. Thus, it is probable that additional mechanisms contribute to resistance.

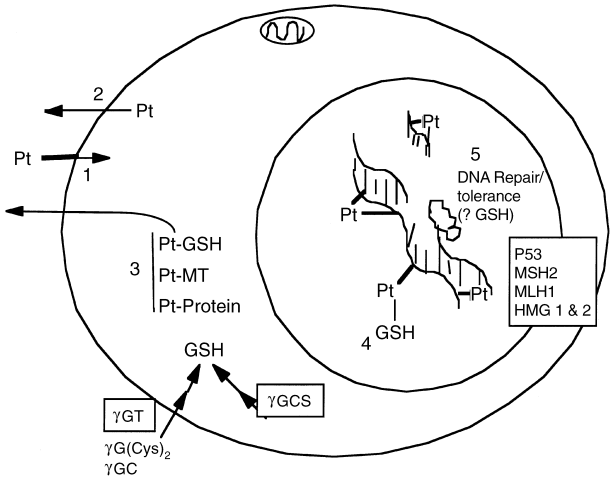

Figure 1. Mechanisms of cisplatin resistance. Drug accumulation may be decreased, either due to decreased influx (1) or increased efflux (2). Cisplatin may be inactivated by cytoplasmic or nuclear molecules (3), such as glutathione (GSH), metallothioneins (MT), or proteins. GSH levels are determined by the synthetic enzyme γ-glutamyl cysteine synthetase (γ-GCS) and the salvage enzyme γ-glutamyl transpeptidase (γ-GT). Export of platinum–GSH conjugates (Pt-GSH) could potentially also contribute to increased efflux. GSH may also bind cisplatin–DNA monoadducts (4), preventing crosslink formation. Finally, resistant cells may have enhanced capacity for repair or tolerance (5) of drug-induced DNA damage. Tolerance may be related to alterations in proteins that recognise damage (mismatch repair (MLH1, MSH2) or HMG proteins) or in pathways that modulate apoptosis.

Cytosolic inactivation is another mechanism that can prevent cisplatin from reacting with intracellular target molecules. Intracellular non-protein (glutathione (GSH)) and protein (metallothionein (MT)) sulphydryl compounds have been proposed to function in this manner. GSH covalently binds cisplatin at physiological concentrations [50, 51] and can inhibit conversion of platinum–DNA monoadducts to potentially cytotoxic crosslinks [52]. Direct interaction between GSH and cisplatin in cells has been reported and cisplatin–GSH conjugates are exported by an ATP-dependent pump [53]. Metallothionein is a low molecular weight, cysteine-rich metalloprotein that can react directly with cisplatin [54]. However, while metallothionein expression has been associated with cisplatin resistance in some models, no association was seen in other models [55–57].

There is considerable evidence linking GSH to cisplatin resistance. Linear correlations between GSH levels and cisplatin resistance have been reported in human renal [58], bladder [58, 59] and ovarian [60–62] cancer cell lines and in human ovarian tumour biopsies [63]. Depletion of GSH by buthionine sulphoximine (BSO), an inhibitor of γ-glutamylcysteine synthetase (γ-GCS; the rate limiting enzyme for GSH synthesis), enhanced cisplatin sensitivity in some cell lines [60, 61] but not others [64–66].

Finally, cisplatin-induced damage can be minimised by removal of the drug from its molecular targets. Increased DNA repair has been observed in several cisplatin-resistant human ovarian cancer cell lines [12–16]. In some model systems, certain types of DNA damage are preferentially repaired in actively transcribed genes relative to the overall genome [67]. Thus, cisplatin-resistant cells might excel at gene-specific repair in order to increase the probability of survival. Preferential cisplatin interstrand crosslink (ICL) removal from actively transcribed genes has been reported in some cisplatin resistant human ovarian cancer cell lines [68] but not in others [16, 69].

Further evidence supporting a role for DNA repair in cisplatin resistance has come from studies with aphidicolin glycinate, an inhibitor of DNA polymerases α, δ and ε. Aphidicolin inhibited DNA repair and enhanced cisplatin cytotoxicity in A2780 and A2780/CP70 cells in a dose-dependent manner [12]. In the 2008/DDP human ovarian cell line, aphidicolin exhibited strong synergism with cisplatin following short-term drug exposure, whilst the parental, cisplatin-sensitive cell line (2008) showed no change [17].

Altered cellular and molecular responses to cisplatin-induced damage

Although DNA is an important cytotoxic target of cisplatin, significantly greater platinum–DNA damage may be required to kill resistant cells compared with sensitive cells [69, 70]. In one model system, equitoxic (IC50) cisplatin treatment yielded 3-fold higher levels of platinum in DNA following completion of repair in a cisplatin-resistant human ovarian carcinoma cell line compared with cisplatin-sensitive parental cells [15] Johnson and colleagues investigated

several potential resistance mechanisms in a series of unrelated human ovarian carcinoma cell lines. In these cell lines, DNA damage tolerance was strongly correlated with cisplatin sensitivity, whereas no correlation was apparent for platinum accumulation, GSH levels, or platinum–DNA adduct repair [71]. These data suggest that tolerance to damage is a common and potentially important determinant of cisplatin sensitivity.

The precise mechanisms that confer tolerance to DNA damage are being defined. Tolerance may result from the capacity of resistant cells to synthesise DNA past adducts. Alternatively, DNA damage in resistant cells may no longer serve as a signal to undergo apoptosis. At present, it is not known whether alterations in mismatch repair or HMG proteins contribute to tolerance, though this would be consistent with known properties of these proteins.

The capacity for DNA synthesis on damaged templates has been clearly shown in some models. Mamenta and colleagues [72] demonstrated a 4.5 and 2.3-fold increased Pt–DNA replicative bypass capacity of cisplatin-resistant C13 and A2780/CP70 human ovarian cancer cell lines, respectively, compared with the parental 2008 and A2780 cell lines. It is interesting to note that mismatch repair defects have been documented in both of these model systems [31–33]. A recent preliminary report has shown correlation between deficient mismatch repair and enhanced replicative bypass capacity in cisplatin-resistant human ovarian carcinoma cell lines [73]. Further investigations should clarify whether mismatch repair defects in these resistant cell lines are mechanistically related to tolerance.

The potential contribution of altered sensitivity to apoptosis to resistance has been increasingly studied as genes regulating apoptosis are defined. One critical regulator of apoptosis in response to irradiation or anticancer drugs is p53. Cisplatin, γ-irradiation, and other DNA damaging agents induce stabilisation and nuclear translocation of p53 [74–76]. p53 is a strong transcriptional activator of the gene encoding $p21^{WAF1/CIP1}$, a protein that mediates cell-cycle arrest [77]. Although best characterised as a cell cycle regulator, $p21^{WAF1/CIP1}$ protein may also protect cells from apoptosis [78, 79].

Potentially compelling evidence implicating p53 in cisplatin resistance comes from recent experiments using p53 genetic suppressor elements (GSE; short gene fragments expressed as either antisense RNA or dominant negative peptides). Gallagher and colleagues isolated six independent p53 DNA binding domain GSEs that conferred up to 8-fold cisplatin resistance to the A2780 human ovarian carcinoma cell line [80]. GSE expression was associated with decreased p53 protein levels and loss of p53 function, including cell cycle arrest and apoptosis. These data provide direct evidence that inactivation of the p53 DNA binding domain can confer resistance to cisplatin.

p53 also directly affects expression of downstream genes that regulate sensitivity to apoptosis (Figure 2), activating transcription of *BAX* (promotes apoptosis) and repressing transcription of *BCL2* (inhibits apoptosis) [81–83]. It has been proposed that the ratio of apoptotic to antiapoptotic proteins (for example, Bax:BCL2) ultimately determines the propensity of a cell to undergo apoptosis [81]. The *BAX* promoter contains consensus binding sites for p53 and its activity is unregulated by wild-type, but not mutant, p53 [84–87]. Transcription of *BCL2* is repressed by wild-type p53 [85–87]. Thus, wild-type p53 can produce reciprocal changes in *BAX* and *BCL2* transcription that favour apoptosis.

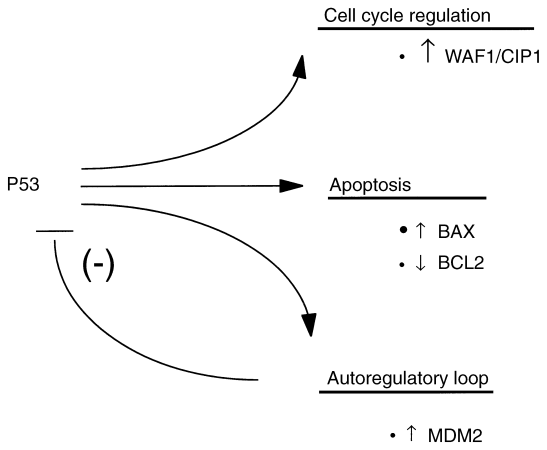

Figure 2. p53 effects on downstream genes that modulate cell cycle and sensitivity to apoptosis. p53 activates transcription of $p21^{WAF1/CIP1}$, which produces cell cycle arrest. p53 also activates transcription of *BAX* and represses transcription of BCL2, changes which favour apoptosis. Finally, p53 potentially autoregulates its own activity, by induction of expression of an inhibitor, MDM2.

Altered expression of the *BCL2*, *BCLx*, and/or *BAX* genes can affect cisplatin sensitivity. Reduced *BAX* expression was seen in association with *p53* mutation and *MDM2* expression, in a cisplatin-resistant subline of the IGROV human ovarian carcinoma cell line [88, 89]. Transfection of *BCL2* or *BCLXL* conferred resistance and inhibited apoptosis following cisplatin and other anticancer drugs in several models [82, 83, 90, 91]. Transfection of the *BCL2* gene into A2780 human ovarian carcinoma cells conferred approximately 3-fold resistance to cisplatin [92]. Thus, apoptosis regulatory genes downstream from p53 clearly can affect sensitivity to cisplatin.

We investigated expression of p53 and downstream genes in the A2780 human ovarian carcinoma cell line and a cisplatin-resistant subline, 2780/CP [25]. Following equimolar cisplatin treatment, nuclear p53 protein levels increased to a greater extent in A2780 cells than in the 2780/CP cell line. However, *BAX* mRNA expression in both cell lines was unchanged from baseline post-cisplatin. In contrast, mRNA for another p53-regulated gene, $p21^{WAF1/CIP1}$, increased dramatically in both cell lines after cisplatin. These data are consistent with specific disruption of the p53-BAX interaction in our model system. The potential significance of these observations remains to be determined in ongoing experiments.

It should be noted that the effects of p53 are complex and difficult to study in isolation. It is estimated that between 200 and 300 human genes are transcriptionally activated by p53 [93]. p53 can also act as a repressor of transcription (as, for example, with *BCL2*) and there is presently no estimate of the number of additional genes that may be repressed by p53. The effects of many of these genes could potentially offset one another. Thus, much additional work remains to be done in order to clarify the precise role of p53 as a determinant of cisplatin sensitivity.

The problem of clinical relevance

It is apparent that preclinical models have facilitated identification of many potentially important resistance mechanisms.

However, the clinical relevance of these models is unclear. For example, most data are from cell lines selected for resistance to cisplatin *in vitro*. It is not clear whether drug concentrations and schedules approximate those achieved in patients, nor is it clear whether tumour cells respond similarly to cisplatin *in vitro* and *in vivo*. There are also relatively few data demonstrating relationships between any of the above mechanisms and clinical outcomes.

The problem of clinical relevance is difficult to address, Some potential resistance mechanisms are not readily studied in patients. For example, methods used to quantitate repair of DNA–platinum adducts *in vitro* analysed serial specimens within 12–24 h post-treatment. Acquisition of similar timed specimens in large numbers of patients is constrained by practical considerations, including accessibility of tumours for biopsy and availability of surgical and tissue procurement staff. In addition, acquisition of serial biopsies may raise ethical issues, including whether tissue procurement diverts tissue needed for diagnosis and whether informed consent is appropriately addressed.

Two main approaches are being used to define the clinical relevance of cisplatin resistance mechanisms identified in preclinical models. First, clinical trials are planned or in progress to test whether biochemical modulation of particular mechanisms can increase the activity of chemotherapy. Several potential biochemical modulators have been identified (Table 1). Second, potential determinants of resistance are being assayed in tumour specimens and analysed for independent prediction of clinical outcomes. One would expect that clinically significant resistance mechanisms would independently predict outcomes.

A detailed discussion of biochemical modulation trials is beyond the scope of this review. However, clinical attempts to modulate GSH will be discussed as one potentially important example of the types of investigations that are underway at various centres.

GSH is a logical target for biochemical modulation because it potentially affects cisplatin sensitivity in several ways. As noted above, GSH can bind cisplatin in the cytoplasm or which is monofunctionally bound to DNA. GSH-platinum complexes are actively transported out of cells, which could contribute to cisplatin efflux. This would be consistent with the inverse correlation between GSH levels and cisplatin accumulation we observed in spontaneously transformed rat ovarian surface epithelial cell lines [94]. In addition, GSH may directly or indirectly participate in DNA repair. Depletion of GSH by buthionine sulphoximine (BSO) inhibits DNA repair (assayed by unscheduled DNA synthesis) in cisplatin-resistant human ovarian cancer cells [95]. Moreover, active site cysteine residues of the HMG-1 and HMG-2 proteins must be in a reduced state in order to recognise cisplatin-damaged DNA [96]. GSH, as the predominant intracellular non-protein thiol, is likely to have a major role in reducing these residues. Finally, GSH may modulate induction of transcription factors that potentially affect DNA repair and apoptosis, such as c-*fos* and c-*jun* [97–99].

Table 1. *Biochemical modulators of cisplatin sensitivity*

Resistance mechanism	Modulator
Accumulation	Dipyridamole
	Hyperthermia
Glutathione	Buthionine sulphoximine (BSO)
	? Ifosfamide
DNA repair	
Damage recognition/	Novobiocin
DNA packaging/	Nalidixic acid
Repackaging	Topoisomerase inhibitors
Damage incision	Calmodulin inhibitors
Gap filling	Aphidicolin
	Azidothymidine (AZT)
	Cytarabine (Ara-C)
	Dideoxythymidine triphosphate
	Fludarabine
	Gemcitabine
	Hydroxyurea
	PALA
Ligation	3-Aminobenzamide

Cellular GSH can be depleted by BSO, a specific inhibitor of the GSH-synthetic enzyme γ-GCS. Depletion of GSH with BSO enhanced the cytotoxicity of cisplatin in several *in vitro* and *in vivo* preclinical models [100, 101]. BSO has been administered in combination with the alkylating agent melphalan in phase I trials [101–103]. Depletion of GSH was apparent in tumour samples and in peripheral lymphocytes from the majority of BSO-treated patients. Increased γ-GCS mRNA expression was detected in peripheral lymphocytes of BSO-treated patients, consistent with a compensatory response to pharmacological inhibition of γ-GCS [104]. Trials of BSO plus carboplatin are also planned. However, the availability of BSO is limited, which may impede attempts to define its utility as a modulator of clinical resistance. Investigations of novel strategies to modulate GSH levels are therefore warranted.

At Roswell Park, a phase I trial has been initiated to determine whether the alkylating agent ifosfamide is an effective clinical modulator of GSH levels [105]. This trial is based on preclinical reports showing GSH depletion in cultured cell lines by ifosfamide and its metabolites, as well as data reporting GSH depletion in peripheral leucocytes, of some ifosfamide-treated patients [106–108]. Data from the initial dose levels of this trial ($\leq 4\,g/m^2$ ifosfamide by 24 h infusion followed by carboplatin) demonstrate $\geq 80\%$ depletion of cysteine, a precursor for GSH synthesis, in peripheral blood, in the majority of patients [105]. Depletion of GSH in peripheral leucocytes has not yet been seen. However, dose escalation is ongoing and no conclusions regarding the efficacy of this approach can presently be drawn.

CONCLUSIONS

In recent years, a large number of potential determinants of cisplatin resistance have been identified in preclinical models. In addition to 'classical' resistance mechanisms related to altered cellular pharmacology, several additional mechanisms are now being characterised at the molecular level. In particular, alterations in mismatch repair and apoptosis appear to be potentially important. At present the clinical significance of many of these mechanisms is unknown. However, the pace at which determinants of cytotoxicity are being characterised is quite rapid.

Improved understanding of cisplatin resistance mechanisms may facilitate prediction of clinical response to therapy. Continued efforts to understand the cellular and molecular mechanisms of cisplatin resistance may also identify novel targets for pharmacological or molecular intervention. Such efforts have the potential to enhance the efficacy of cisplatin

and carboplatin against many types of cancer, which may allow the curative potential of these drugs to be more fully realised.

1. Ozols RF, Williams SD. Testicular cancer. *Curr Prob Cancer* 1989, **13**, 287–335.
2. Loehrer PJ, Einhorn LH. Cisplatin. *Ann Intern Med* 1984, **100**, 704–713.
3. Ozols RF. Ovarian cancer, part II: treatment. *Curr Prob Cancer* 1992, **16**, 63–126.
4. Gately DP, Howell SB. Cellular accumulation of the anticancer agent cisplatin: a review. *Br J Cancer* 1993, **67**, 1171–1176.
5. Douple EB. Cis-diamminedichloroplatinum(II): effects of a representative metal coordination complex on mammalian cells. *Pharmac Ther* 1984, **25**, 297–326.
6. Eastman A. Re-evaluation of the interaction of cis-dichloro (ethylenediamine)platinum(II) with DNA. *Biochemistry* 1986, **25**, 3912–3915.
7. Eastman A. Characterization of the adducts produced in DNA by cis-diamminedichloroplatinum(II) and cis-dichloro(ethylenediamine)platinum(II). *Biochemistry* 1983, **22**, 3927–3933.
8. Knox RJ, Friedlos F, Lydall DA, Roberts JJ. Mechanism of cytotoxicity of anticancer platinum drugs: evidence that cis-diamminedichloroplatinum(II) and cis-diammine(1,1-cyclobutanedicarboxylato)platinum(II) differ only in the kinetics of their interaction with DNA. *Cancer Res* 1986, **46**, 1972–1979.
9. Fraval H, Rawlings C, Roberts J. Increased sensitivity of UV-repair-deficient human cells to DNA bound platinum products which unlike thymidine dimers are not recognized by an endonuclease extracted from *Micrococcus luteus*. *Mutat Res* 1978, **51**, 121–132.
10. Hoy C, Thompson L, Mooney C, Salazar E. Defective DNA crosslink removal in Chinese hamster cell mutants hypersensitive to bifunctional alkylating agents. *Cancer Res* 1985, **45**, 1737–1743.
11. Dijt F, Fitchinger-Schepman A, Berends F, Reedijk J. Formation and repair of cisplatin-induced adducts to DNA in cultured normal and repair-deficient human fibroblasts. *Cancer Res* 1988, **48**, 6058–6062.
12. Masuda H, Ozols RF, Lai G-M, Fojo A, Rothenberg M, Hamilton TC. Increased DNA repair as a mechanism of acquired resistance to cis-diamminedichloroplatinum (II) in human ovarian cancer cell lines. *Cancer Res* 1988, **48**, 5713–5716.
13. Lai G-M, Ozols RF, Smyth JF, Young RC, Hamilton TC. Enhanced DNA repair and resistance to cisplatin in human ovarian cancer. *Biochem Pharmacol* 1988, **37**, 4597–4600.
14. Masuda M, Tanaka T, Matsuda H, Kusaba I. Increased removal of DNA-bound platinum in a human ovarian cancer cell line resistant to cis-diamminedichloroplatinum (II). *Cancer Res* 1990, **50**, 1863–1866.
15. Parker RJ, Eastman A, Bostick-Burton F, Reed E. Acquired cisplatin resistance in human ovarian cancer cells is associated with enhanced repair of cisplatin–DNA lesions and reduced drug accumulation. *J Clin Invest* 1991, **87**, 772–777.
16. Johnson SW, Perez RP, Godwin AK, *et al.* Role of platinum–DNA adduct formation and removal in cisplatin resistance in human ovarian cancer cell lines. *Biochem Pharmacol* 1994, **47**, 689–697.
17. Katz E, Andrews P, Howell S. The effect of DNA polymerase inhibitors on the cytotoxicity of cisplatin in human ovarian carcinoma cells. *Cancer Communicat* 1990, **2**, 159–164.
18. Searle J, Kerr JFR, Bishop CL. Necrosis and apoptosis: distinct modes of cell death with fundamentally different significance. *Pathol Annu* 1982, **17**, 229–259.
19. Searle J, Lawson TA, Harmon AB, Kerr JFR. An electron microscope study of the mode of cell death induced by cancer chemotherapeutic agents in populations of proliferating normal and neoplastic cells. *J Pathol* 1975, **116**, 129–138.
20. Wyllie AH, Kerr JFR, Currie AR. Cell death: the significance of apoptosis. *Int Rev Cytol* 1980, **68**, 251–307.
21. Sorenson CM, Barry MA, Eastman A. Analysis of events associated with cell cycle arrest at G_2 phase and cell death induced by cisplatin. *J Natl Cancer Inst* 1990, **82**, 749–755.
22. Barry MA, Behnke CA, Eastman A. Activation of programmed cell death (apoptosis) by cisplatin, other anticancer drugs, toxins, and hyperthermia. *Biochem Pharmacol* 1990, **40**, 2353–2362.
23. Evans DE, Dive C. Effects of cisplatin on the induction of apoptosis in proliferating hepatoma cells and nonproliferating immature thymocytes. *Cancer Res* 1993, **53**, 2133–2139.
24. Eastman A. Activation of programmed cell death by anticancer agents: cisplatin as a model system. *Cancer Cells* 1990, **2**, 275–280.
25. Bollenbacher J, Glaves D, Pendyala L, Perez R. Altered mRNA expression of P53 and BAX in a cisplatin-resistant human ovarian carcinoma cell line. *Proc Amer Assoc Cancer Res* 1997, **38**, 136.
26. Russell PJ, Glaves D. Experimental models of bladder cancer. In Raghavan D, Scher HI, Leibel SA, Lange PH, eds. *Principles and Practice of Genitourinary Oncology*. Philadelphia, Lippincott-Raven, 1997, 195–206.
27. Havrilesky LJ, Elbendary A, Hurteau JA, Whitaker RS, Rodriguez GC, Berchuck A. Chemotherapy-induced apoptosis in epithelial ovarian cancers. *Gynecol Oncol* 1995, **85**, 1007–1010.
28. Chu G. Cellular responses to cisplatin: the roles of DNA binding proteins and DNA repair. *J Biol Chem* 1994, **269**, 787–790.
29. Karran P, Bignami M. DNA damage tolerance, mismatch repair, and genome instability. *BioEssays* 1994, **16**, 833–839.
30. Fishel R, Kolodner RD. Identification of mismatch repair genes and their role in the development of cancer. *Curr Opin Genet Dev* 1995, **5**, 382–395.
31. Drummond JT, Anthoney A, Brown R, Modrich P. Cisplatin and adriamycin are associated with MutLα and mismatch repair deficiency in an ovarian tumor cell line. *J Biol Chem* 1996, **271**, 19645–19648.
32. Aebi S, Kurdi-Haidar B, Gordon R, *et al.* Loss of DNA mismatch repair in acquired resistance to cisplatin. *Cancer Res* 1996, **56**, 3087–3090.
33. Anthoney DA, McIlwrath AJ, Gallagher WM, Edlin ARM, Brown R. Microsatellite instability, apoptosis, and loss of P53 function in drug-resistant tumor cells. *Cancer Res* 1996, **56**, 1374–1381.
34. Fink D, Nebel Aebi S, Zheng H, *et al.* The role of DNA mismatch repair in platinum drug resistance. *Cancer Res* 1996, **56**, 4881–4886.
35. Ferry KV, Fink D, Johnson SW, Hamilton TC, Howell SB. Quantitation of platinum–DNA adduct repair in mismatch repair deficient and proficient human colorectal cancer cell lines using an *in vitro* DNA repair assay. *Proc Am Assoc Cancer Res* 1997, **38** (abstract), 359.
36. Fink D, Nebel S, Aebi S, Nehme A, Howell SB. Loss of DNA mismatch repair due to knockout of MSH2 or PMS2 results in resistance to cisplatin and carboplatin. *Int J Oncol* 1997, **11**, 539–542.
37. Yamada M, O'Regan E, Brown R, Karran P. Selective recognition of a cisplatin–DNA adduct by human mismatch repair proteins. *Nucl Acids Res* 1997, **25**, 491–495.
38. Duckett DR, Drummond JT, Murchie AIH, *et al.* Human MutSα recognizes damaged DNA base pairs containing O6-methylguanine, O4-methylmine, or the cisplatin-d(GpG) adduct. *Proc Natl Acad Sci* 1996, **93**, 6443–6447.
39. Wunderlich V, Bottger M. High-mobility-group proteins and cancer—an emerging link. *J Cancer Res Clin Oncol* 1997, **123**, 133–140.
40. Hughes EN, Engelsberg BN, Billings PC. Purification of nuclear proteins that bind to cisplatin-damaged DNA: identity with high mobility group proteins 1 and 2. *J Biol Chem* 1992, **267**, 13520–13527.
41. Slamon DJ, Godolphin W, Jones LA, *et al.* Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. *Science* 1989, **244**, 707–712.
42. Treiber DK, Zhai X, Jantzen H-M, Essigmann JM. Cisplatin–DNA adducts are molecular decoys for the ribosomal RNA transcription factor hUBF (human upstream binding factor). *Proc Natl Acad Sci* 1994, **91**, 5672–5676.
43. Hoffman J-S, Locker D, Villani G, Leng M. HMG1 protein inhibits the translesion synthesis of the major DNA cisplatin adduct by cell extracts. *J Mol Biol* 1997, **270**, 539–543.

44. Brown SJ, Kellet PJ, Lippard SJ. Ixr1, a yeast protein that binds to platinated DNA and confers sensitivity to cisplatin. *Science* 1993, **261**, 603–605.
45. Lambert JR, Bilanchone VW, Cumsky MG. The ORD1 gene encodes a transcription factor involved in oxygen regulation and is identical to IXR1, a gene that confers cisplatin sensitivity to *Saccharomyces cerevisiae*. *Proc Natl Acad Sci* 1994, **91**, 7345–7349.
46. Andrews P, Howell S. Cellular pharmacology of cisplatin: perspectives on mechanisms of acquired resistance. *Cancer Cells* 1990, **2**, 35–43.
47. Perez R, Hamilton T, Ozols R. Resistance to alkylating agents and cisplatin: insights from ovarian carcinoma model systems. *Pharmacol Ther* 1990, **48**, 19–27.
48. Perez RP, Johnson SW, Hamilton TC. New strategies of systemic therapy: mechanisms and modulation of resistance to platinum-containing anticancer drugs. In Raghavan D, Scher H, Liebel S, Lange P, eds. *Principles and Practice of Genitourinary Oncology*. Philadelphia, Lippincott-Raven, 1997, 111–119.
49. Fujii R, Mutoh M, Niwa K, *et al.* Active efflux system for cisplatin in cisplatin-resistant human KB cells. *Jpn J Cancer Res* 1994, **85**, 426–433.
50. Dedon PC, Borch RF. Characterization of the reactions of platinum antitumor agents with biologic and nonbiologic sulfur-containing nucleophiles. *Biochem Pharmacol* 1987, **36**, 1955–1964.
51. Mistry P, Loh SY, Kelland LR. Effect of buthionine sulfoximine on PtII and PtIV drug accumulation and the formation of glutathione conjugates in human ovarian-carcinoma cell lines. *Int J Cancer* 1993, **55**, 849–856.
52. Eastman A. Cross-linking of glutathione to DNA by cancer chemotherapeutic platinum coordination complexes. *Chem Biol Interact* 1987, **61**, 241–248.
53. Ishikawa T, Ali-Osman F. Glutathione-associated cis-diamminedichloroplatinum (II) metabolism and ATP-dependent efflux from leukemia cells: molecular characterization of glutathione–platinum complex and its biological significance. *J Biol Chem* 1993, **268**, 20116–20125.
54. Pattanaik A, Bachowski G, Laib J. Properties of the reaction of cis-dichlorodiammineplatinum (II) with metallothionein. *J Biol Chem* 1992, **267**, 16121–16128.
55. Kelley SL, Basu A, Teicher BA, Hacker MP, Hamer DH, Lazo JS. Overexpression of metallothionein confers resistance to anticancer drugs. *Science* 1988, **241**, 1813–1815.
56. Andrews PA, Murphy MP, Howell SB. Metallothionein-mediated cisplatin resistance in human ovarian carcinoma cells. *Cancer Chemother Pharmacol* 1987, **19**, 149–154.
57. Schilder RJ, Hall L, Monks A. Metallothionein gene expression and resistance to cisplatin in human ovarian cancer. *Int J Cancer* 1990, **45**, 416–422.
58. Ahn H, Lee E, Kim K, Lee C. Effect of glutathione and its related enzymes on chemosensitivity of renal cell carcinoma and bladder carcinoma cell lines. *J Urol* 1994, **151**, 263–267.
59. Pendyala L, Velagapudi S, Toth K, *et al.* Translational studies of glutathione in bladder cancer. *Clin Cancer Res* 1997, **3**, 793–798.
60. Behrens BC, Hamilton TC, Masuda H. Characterization of a cis-diamminedichloroplatinum (II)-resistant human ovarian cancer cell line and its use in evaluation of platinum analogues. *Cancer Res* 1987, **47**, 414–418.
61. Mistry P, Kelland LR, Abel G, Sidhar S, Harrap KR. The relationships between glutathione, glutathione-S-transferase and cytotoxicity of platinum drugs and melphalan in eight human ovarian cancer cell lines. *Br J Cancer* 1991, **64**, 215–220.
62. Godwin AK, Meister A, O'Dwyer PJ, *et al.* High resistance to cisplatin in human ovarian cancer cell lines is associated with marked increase of glutathione synthesis. *Proc Nat Acad Sci USA* 1992, **89**, 3070–3074.
63. Britten RA, Green JA, Warenius HM. Cellular glutathione (GSH) and glutathione S-transferase (GST) activity in human ovarian tumor biopsies following exposure to alkylating agents. *Int J Radiation Oncology Biol Phys* 1992, **24**, 527–331.
64. Teicher BA, Holden SA, Kelley MJ. Characterization of a human squamous-carcinoma cell line resistant to cisp-diamminedichloroplatinum (II). *Cancer Res* 1987, **47**, 388–393.
65. Andrews PA, Murphy MP, Howell SB. Differential potentiation of alkylating and platinating agent cytotoxicity in human ovarian-carcinoma cells by glutathione depletion. *Cancer Res* 1985, **45**, 6250–6253.
66. Richon VM, Schulte N, Eastman A. Multiple mechanisms of resistance to cis-diamminedichloroplatinum (II) in murine leukemia L1210 cells. *Cancer Res* 1987, **47**, 2056–2061.
67. Bohr VA. Gene specific DNA repair. *Carcinogenesis* 1991, **87**, 1983–1992.
68. Zhen W, Link CJJ, O'Connor PM, *et al.* Increased gene-specific repair of cisplatin interstrand cross-links in cisplatin-resistant human ovarian cancer cell lines. *Mol Cell Biol* 1992, **12**, 3689–3698.
69. Johnson SW, Swiggard PA, Handel LM, *et al.* Relationship between platinum–DNA adduct formation, removal, and cytotoxicity in cisplatin sensitive and resistant human ovarian cancer cells. *Cancer Res* 1994, **54**, 5911–3916.
70. Hill BT, Shellard SA, Hosking LK, Fitchinger-Shepman AMJ. Enhanced DNA repair and tolerance of DNA damage associated with resistance to cis-diamminedichloroplatinum (II) after *in vitro* exposure of a human teratoma cell line to fractionated X-irradiation. *Int J Radiat Oncol Biol Phys* 1990, **19**, 75–83.
71. Johnson SW, Laub PB, Beesley JS, Ozols RF, Hamilton TC. Increased platinum–DNA damage tolerance is associated with cisplatin resistance and cross-resistance to various chemotherapeutic agents in unrelated human ovarian carcinoma cell lines. *Cancer Res* 1997, **57**, 850–856.
72. Mamenta EL, Poma EE, Kaufmann WK, Delmastro DA, Grady HL, Chaney SG. Enhanced replicative bypass of platinum–DNA adducts in cisplatin-resistant human ovarian cancer cell lines. *Cancer Res* 1994, **54**, 3500–3505.
73. Vaisman A, Varchenko M, Chaney SG. Correlation between mismatch repair and increased replicative bypass in cisplatin resistant cell lines. *Proc Am Assoc Cancer Res* 1997, **38** (abstract), 312.
74. Kastan MB, Onyekwere O, Sidrasky D, Vogelstein B, Craig RW. Participation of p53 protein in the cellular response to DNA damage. *Cancer Res* 1991, **51**, 6304–6311.
75. Fritsche M, Haessler C, Brandner G. Induction of nuclear accumulation of the tumor-suppressor protein p53 by DNA-damaging agents. *Oncogene* 1993, **8**, 307–318.
76. Kuerbitz SJ, Plunkett BS, Walsh WV, Kastan MB. Wild-type p53 is a cell cycle checkpoint determinant following irradiation. *Proc Natl Acad Sci* 1992, **89**, 7491–7495.
77. El-Diery W, Tokino T, Velculescu VE, *et al.* WAF1, a potential mediator of p53 tumor suppression. *Cell* 1993, **75**, 817–825.
78. Kawasaki T, Tomita T, Bilim V, Takeda M, Takahashi K, Kumanishi T. Abrogation of apoptosis induced by DNA-damaging agents in human bladder cancer cell lines with p21/WAF1/CIP1 and/or P53 gene alterations. *Int J Cancer* 1996, **68**, 501–505.
79. Gorospe M, Cirielli C, Wang X, Seth P, Capogrossi MC, Holbrook NJ. p21Waf1/Cip1 protects against p53-mediated apoptosis of human melanoma cells. *Oncogene* 1997, **14**, 9229–9935.
80. Gallagher WM, Cairney M, Schott B, Roninson IB, Brown R. Identification of p53 genetic suppressor elements which confer resistance to cisplatin. *Oncogene* 1997, **14**, 185–193.
81. Oltvai ZN, Milliman CL, Korsmeyer SJ. Bcl-2 heterodimerizes *in vivo* with a conserved homolog, Bax, that accelerates programmed cell death. *Cell* 1993, **74**, 609–619.
82. Reed X. Bcl-2 and the regulation of programmed cell death. *J Cell Biol* 1994, **124**, 1–6.
83. Fisher D. Apoptosis in cancer therapy: crossing the threshold. *Cell* 1994, **78**, 539–542.
84. Miyashita T, Reed X. Tumor suppressor p53 is a direct transcriptional activator of the human bax gene. *Cell* 1995, **80**, 293–299.
85. Miyashita T, Krajewski S, Krajewska M, *et al.* Tumor suppressor p53 is a regulator of bcl-2 and bax gene expression *in vitro* and *in vivo*. *Oncogene* 1994, **9**, 1799–1805.
86. Selvakumaran M, Lin H-K, Miyashita T, *et al.* Immediate up-regulation of bax expression by p53 but not TFGβ1: a paradigm for distinct apoptotic pathways. *Oncogene* 1994. **9**, 1791–1798.
87. Zhan Q, Fan S, Bae I, *et al.* Induction of bax by genotoxic stress in human cells correlates with normal p53 status and apoptosis. *Oncogene* 1994, **9**, 3743–3751.

88. Perego P, Giola M, Righetti SC, *et al.* Association between cisplatin resistance and mutation of p53 Rene and reduced Bax expression in ovarian carcinoma cell systems. *Cancer Res* 1996, **56**, 556–562.
89. Fajac A, DaSilva J, Ahomadegbe J-C, *et al.* Cisplatin-induced apoptosis and P53 gene status in a cisplatin-resistance human ovarian carcinoma cell line. *Int J Cancer* 1996, **68**, 67–74.
90. Dole M, Nunez G, Merchant AK, *et al.* Bcl-2 inhibits chemotherapy-induced apoptosis in neuroblastoma. *Cancer Res* 1994, **54**, 3253–3259.
91. Miyashita T, Reed JC. Bcl-2 oncoprotein blocks chemotherapy-induced apoptosis in a human leukemia cell line. *Blood* 1993, **81**, 151–157.
92. Eliopoulos AG, Kerr DJ, Herod J, *et al.* The control of apoptosis and drug resistance in ovarian cancer: influence of P53 and Bel-2. *Oncogene* 1995, **11**, 1217–1228.
93. Tokino T, Thaagalingam S, El-Deiry W, Waldman T, Kinzler KW, Vogelstein B. p53 tagged sites from human genomic DNA. *Hum Mol Genet* 1994, **3**, 1537–1542.
94. Perez R, Johnson S, Handel L, O'Dwyer P, Hamilton T. Determinants of cisplatin sensitivity in normal versus spontaneously transformed rat ovarian surface epithelial cells. *Gynecol Oncol* 1995, **58**, 312–318.
95. Lai G-M, Ozols RF, Young RC, Hamilton TC. Effect of glutathione on DNA repair in cisplatin-resistant human ovarian cancer cell lines. *Biochem Pharmacol* 1989, **37**, 4597–4600.
96. Billings PC, Davis RJ, Engelsberg BN, Skov KA, Hughes EN. Characterization of high mobility group protein binding to cisplatin-damaged DNA. *Biochem Biophys Res Comm* 1992, **188**, 1286–1294.
97. Bergelson S, Pinkus R, Daniel V. Intracellular glutathione levels regulate Fos/Jun induction and activation of glutathione s-transferase gene expression. *Cancer Res* 1994, **54**, 36–40.
98. Potapova O, Haghighi A, Bost F, *et al.* The Jun kinase/stress-activated protein kinase pathway functions to regulate DNA repair and inhibition of the pathway sensitizes tumor cells to cisplatin. *J Biol Chem* 1997, **272**, 14041–14044.
99. Xia Z, Dickens M, Raingeaud J, Davis RJ, Greenberg ME. Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. *Science* 1995, **270**, 1326–1331.
100. Chen G, Zeller W. Augmentation of cisplatin (DDP) cytotoxicity *in vivo* by D,1-buthionine sulfoximine (BSO) in DDP-sensitive and -resistant rat ovarian tumors and its relation to DNA interstrand crosslinks. *Anticancer Res* 1991, **11**, 2231–2238.
101. O'Dwyer P, Hamilton T, Young R, *et al.* Depletion of glutathione in normal and malignant human cells *in vivo* by buthionine sulfoximine: clinical and biochemical results. *J Natl Cancer Inst* 1992, **84**, 264–267.
102. Bailey HH, Mulcahy RT, Tutsch KD, *et al.* Phase I clinical trial of intravenous L-buthionine sulfoximine and melphalan: an attempt at modulation of glutathione. *J Clin Oncol* 1994, **12**, 194–205.
103. Bailey HH, Wilding G, Tutsch KD, *et al.* A phase I trial of L-*S,R*-buthionine sulfoximine (BSO) given as a 24- to 48-hour continuous infusion (CI) with iv melphalan (L-PAM). *Proc Amer Soc Clin Oncol* 1994, **13** (abstract), 334–330.
104. Yao K-S, Godwin AK, Ozols RF, Hamilton TC, O'Dwyer PJ. Variable baseline γ-glutamylcysteine synthetase messenger RNA expression in peripheral mononuclear cells of cancer patients, and its induction by buthionine sulfoximine treatment. *Cancer Res* 1993, **53**, 3662–3666.
105. Pendyala L, Creaven PJ, Raghavan D, *et al.* Thiols in patients (pts) receiving ifosfamide (IF) with mesna and carboplatin (CB). *Proc Am Assoc Cancer Res* 1997, **38** (abstract), 390.
106. Lind L, McGown A, Hadfield J, Thatcher N, Crowther D, Fox B. The effect of ifosfamide and its metabolites on intracellular glutathione levels *in vitro* and *in vivo*. *Biochemical Pharmacol* 1989, **38**, 1835–1840.
107. Meier T, Allenbacher A, Mueller E, *et al.* Ifosfamide induced depletion of glutathione in human peripheral blood lymphocytes and protection by mesna. *Anti-cancer Drugs* 1994, **5**, 403–409.
108. Malik IA, Mehboobali N, Iqbal MP. Effect of ifosfamide on intracellular glutathione levels in peripheral blood lymphocytes and its correlation with therapeutic response in patients with advanced ovarian cancer. *Cancer Chemother Pharmacol* 1997, **39**, 561–565.