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The Role of Cellular Accumulation in Determining Sensitivity to Platinum-Based Chemotherapy
Matthew D. Hall et al

Supplemental material

Speciation of platinum drugs

Unlike cisplatin, carboplatin contains a bidentate cyclobutane-1,2-dicarboxylate (CBDCA) leaving group that occupies the two cis leaving group sites through two carboxylate oxygens, creating a highly stable complex. Tobe and co-workers demonstrated that the rate of aquation is slow at 37ºC ($t_{1/2}$ = 16 days in water according to a the rate constant of 5.09×10^{-7} s^{-1} reported by (1)), which suggests that intact drug is probably the only major species present in the extracellullar matrix (and therefore the major pool of platinum that enters the cell). To determine how carboplatin may be activated *in vivo*, displacement of the CBDCA ligand by various nucleophiles has been investigated. For example, the reaction of carboplatin with the thioether L-methionine is rapid and produces a stable ring-opened species, *cis*-[Pt(*O*-CBDCA)$(NH_3)_2$(*S*-L-HMet)] (L-Met concentration in RPMI: 1 mM, blood plasma: ~30 mM (2)) (3). Dabrowiak and co-workers have again demonstrated that carboplatin can form stable ring-opened species with carbonate, and measured the relative rates of disappearance of carboplatin in a range of solutions and media, showing that the rate of carboplatin disappearance in 23.8 mM carbonate was an order of magnitude greater than in water (water: $t_{1/2}$ = 16 days, $k = 5.09 \times 10^{-7}$ s^{-1} vs. carbonate: $t_{1/2}$ = 3.9 days, $k = 2.04 \times 10^{-6}$ s^{-1}) and a similar rate of loss was shown in growth medium ($t_{1/2}$ = 2.6 days, $k = 3.08 \times 10^{-6}$) (1). This stability is in keeping with clinical pharmacokinetics data that shows carboplatin to be ten times more stable than cisplatin (4).

A number of octahedral platinum(IV) compounds have also entered clinical trials, the main compounds being tetraplatin, iproplatin and satrapltin (5). They can enter the cell intact, after reduction to platinum(II). (6) Reduction by thiols or ascorbate (7) generally occurs more rapidly than aquation or reactions with biological molecules due to the inherent inertness of the complexes (8). As such,

the main consideration when examining platinum(IV) drug accumulation is an understanding of how readily the compound can be reduced in media.

Changes in plasma membrane composition and biophysics

Taylor *et al.* showed by infrared spectroscopy of the phospholipid phosphatidylserine (PS) that both cisplatin and an aquated cisplatin formulation interacted non-covalently with the carboxylate group, and the aquated form also interacted with the phosphate head-group, probably by hydrogen bonding (9). In a series of papers, De Kruijff and co-workers showed that under extracellular conditions (100 mM Cl^-, pH 7.4) cisplatin associated electrostatically with unilamellar vesicles of DOPA (1,2-dioleoyl-*sn*-glycero-phosphatidic acid) but not other phospholipids (PLs) including phosphatidylinositol (PI) and exhibited minimal binding to PS (10). A complex between cisplatin and DOPS (1,2-dioleoyl-*sn*-glycero-phosphoserine) has been isolated from model membranes (Supplemental Figure 2) (11), though as PS is exclusively located in the inner leaflet of lipid bilayers, it is probably not involved in cisplatin accumulation, and has been shown not to form in a number of human tumor cell lines or play a role in cytotoxicity (12). Assessment of cisplatin interaction with erythrocytes and their bilayer components confirmed the disruption of PS in the inner leaflet; however, no evidence of disruption of the outer leaflet was found, nor interaction with any zwitterionic PLs (13), though it has been proposed that a non-specific action on lipid bilayers may be responsible for the neurotoxic side effects of cisplatin (14).

Some newer drugs have shown differing interactions with PLs; the family of multi-nuclear drugs developed by Farrell, such as BBR3464, are in clinical trials, and it was recently shown that the more strongly charged the complex, the greater the degree of cellular accumulation (15). This may be related to the observation that BBR3464 binds strongly (electrostatically) to negatively charged lipids, even in high (100 mM) chloride (16). A ‘phosphate shuttle’ across lipid membranes is proposed (rather than passive diffusion) given the strength of interaction and

positive charge on the drugs, and it is of interest to note that a cell line resistant to BBR3464 derived from A2780 ovarian cells does not display reduced accumulation (17).

Given the observations that membrane disrupting agents enhance cisplatin accumulation, surprisingly few studies have examined differences in sensitive and resistant cell lines. Mann *et al.* assessed changes in cell surface membrane phosphoplipid content and fluidity between 2008 and 2008/DDP ovarian cancer cell lines to determine whether reduced accumulation (about 50% in this cell line pair) could be attributed to impaired passive diffusion through the membrane bilayer (18). Only small changes in PL content, and no alteration in cholesterol content (cholesterol rigidifies membranes) were found, and there were no differences in the fluorescence polarization of the trimethylammonium diphenylhexatriene (TMA-DPH) fluorescent probe in membranes of whole cells, which gives a direct measure of membrane fluidity (18). The accumulation profile for both cell lines across a range of temperatures from 4 – 40ºC was similar, suggesting little difference in membrane composition and fluidity overall (18).

Liang *et al*. also found no significant changes in PL content in the resistant cell line KCP-20 derived from the KB-3-1 epidermoid carcinoma cell line (19). The TMA-DPH probe was employed to report that the resistant cell membranes were more fluid, though the difference in values is so small (0.3668 vs 0.3612 at 37ºC) that they are almost negligible (19). The ESR probes 5-doxyl-SA and T-SASL both demonstrated that the resistant KCP-20 cells were more fluid than sensitive cells, and Liang *et al.* also used the fluorescent Merocyanine 540 (MRC 540) dye to probe lipid packing, showing reduced incorporation of MRC 540 in resistant membranes – this was interpreted as being due to more tightly packed membranes of the parental cell line KB-3-1 intercalating MRC-540 more tightly (19), though Huang and co-workers observed exactly the same relationship between human lung adenocarcinoma A_{549} and A_{549}/DDP cisplatin resistant cells

and made the reverse conclusion, that PL packing is tighter in resistant cells, occluding MRC-540 insertion (20). While the MRC 540 dye is sensitive to lipid packing, a number of factors contribute to its photoproperties, including lipid phase changes, temperature, and apoptosis. The fluorescence maxima shifts from 594 nm (tight packing) to 624 nm (loose packing), not due to overall dye incorporation (as assayed above), but because of a reorientation of the dye in the membrane (21). As such, the reduced fluorescence intensity in resistant cells observed at 575 nm by both reports is probably due to tighter lipid packing causing the peak to shift towards 624 nm and lowering signal intensity at 575 nm, though a more sophisticated analysis is required to confirm this. Huang also showed that translational diffusion of the fluorescent NBD-PE probe (*N*-7-nitro-2,1,3-benzoxadiazol-4-yl)-1,2-hexadeanoyl-Sn-glycero-3-phosphatodyl-ethanolamine) inserted into membranes slower in resistant cells (20).

The studies described above show generally small alterations (if any) in plasma membrane biophysical properties, such that there is some conflict in the data. Given the marked reduction in cisplatin accumulation in each of the cell lines examined: 2008/DDP, 50% (18); A_{549}/DDP, 46%[1] (20); and KCP-20, 5% (22) compared to their respective parental cell lines, it is difficult to see how the small changes in plasma membrane properties could account for retarded passive diffusion of drugs into the cell.

Membrane disrupting agents

A number of membrane disrupting agents have been shown to enhance cisplatin accumulation. The membrane-specific detergent digitonin has been shown to enhance accumulation and modulate cytotoxicity of cisplatin, probably by altering

[1] Calculated from data provided in reference, average of reduced accumulation at 10 min (58%), 40 min (36%) and 60 min (44%).20. Liang X, Huang Y. 2002. Physical state changes of membrane lipids in human lung adenocarcinoma A549 cells and their resistance to cisplatin, *The International Journal of Biochemistry & Cell Biology* 34:1248-55

membrane permeability (23). Carboplatin accumulation in mouse liver tumors is enhanced by co-administration of a non-toxic dose of digitonin (24). Bergstrom *et al*. assessed the effects of a range of antimicrobial drugs and noted that amphotericin B (AmB), an antifungal that forms non-specific barrel-shaped pores in cell membranes (25), increased cisplatin cytotoxicity (26). This effect was shown to be due to increased platinum drug accumulation in a range of cell lines (27, (28), though interestingly some researchers have observed that AmB increases cisplatin uptake only in resistant cell lines that display an accumulation defect. Sharp *et al*. demonstrated this with the human ovarian carcinoma pairs 41M and 41McisR6, (29) and Morikage *et al*. in the PC-9, PC-9/CDDP and PC-14 (30). PC-14/CDDP NSCLC cell lines, and while researchers concluded that AmB only sensitizes resistant lines, no direct mechanism was given. AmB binds specifically with cholesterol in the cell membrane, and though cholesterol has not generally been assessed in resistant cells, it may be the feature that facilitiates AmB efficacy. AmB's effects appear not to be clinically useful, as effective doses are not tolerable *in vivo* (25, (29).

Spermine (but not spermidine) has been shown to stimulate drug accumulation (31), though the precise nature of biochemical disruption makes it difficult to assign specific causes to this enhanced accumulation. Collectively, membrane disrupting agents appear to sensitize resistant cells to cisplatin by facilitating passage of drug across the cell membrane, increasing accumulation.

Organic cation transporters (The SLC22 family)

Pan *et al.* showed that the uptake of TEA was inhibited competitively by cisplatin in rat OCT2 (rOct2)-transfected NIH3T3 mouse fibroblast cells (32). Kolb *et al.* then showed that the transepithelial transport of cisplatin in rabbit proximal tubules (and its conjugate with *N*-acetylcysteine) was reduced by TEA (33). Yonezawa *et al.* investigated platinum accumulation by HEK293 cells expressing

rOct1 or rOct2 (34). When treated with 500 mM cisplatin for 1 hour, platinum accumulation was elevated in rOct2-expressing cells, but not in rOct1-expressing cells, in comparison to the mock vector-transfected cells (34).

In the case of human OCTs (hOCTs), Briz *et al.* reported that cisplatin uptake by *Xenopus laevis* oocytes expressing hOCT1 or hOCT2 was not increased compared to control oocytes (35). They also investigated the uptake by oocytes expressing human organic anion transporting polypeptide A (OATP-A, new nomenclature: OATP1A2), OATP-C (new nomenclature: OATP1B1), or Na^+-taurocholate cotransporting polypeptide (NTCP), which are encoded by the *SLCO1A2*, *SLCO1B1*, and *SLC10A1* genes, respectively, finding no role for these transporters in cisplatin uptake (35). Interestingly, two bile acid-conjugated cisplatin complexes (Bamet-R2, *cis*-[PtCl(cholylglycinate)$(NH_3)_2$] and Bamet-UD2, *cis*-[Pt(ursodeoxycholate)$_2(NH_3)_2$]) designed to be recognized by carrier transporters (36, (37) showed increased, saturable accumulation in oocytes expressing all five transporters. Bile acid is a good substrate for some OATP family members.

In contrast to the results of Briz and colleagues, Ciarimboli *et al.* reported that hOCT2 mediates cisplatin uptake. Uptake of the fluorescent cation 4-[4-(dimethylamino)styryl]-*N*-methylpyridinium (ASP) in HEK293 human embryonic kidney cortex cells stably expressing hOCT2 was inhibited in a dose-dependent manner by cisplatin, but no inhibition was seen in HEK293 hOCT1 cells (38). Inhibition of ASP uptake by carboplatin and oxaliplatin was not seen in either hOCT1 or hOCT2-expressing cells. An uptake assay of cisplatin with one concentration (100 µM) of the drug at one time point (10 min) at 37 and 4^{o}C was performed, and platinum accumulation was elevated at 37^{o}C but not at 4^{o}C in hOCT2-expressing cells compared to parental HEK293 cells without empty vector (38).

Yonezawa *et al.* reported differing results concerning the interaction of hOCTs with the uptake of platinum compounds. HEK293 cells transiently expressing hOCT1, hOCT2, or hOCT3 were incubated with various concentrations of cisplatin, carboplatin, oxaliplatin, and nedaplatin ([Pt(glycolato)(NH_3)$_2$], a highly soluble cisplatin derivative approved in Japan for a number of malignancies (39, (40)) for 1 hr (41). After treatment with cisplatin, platinum accumulation was significantly elevated in a dose–dependent manner in hOCT2-expressing cells but only slightly elevated in hOCT1-expressing cells. In hOCT2-expressing cells, cisplatin uptake appeared saturable. Oxaliplatin accumulation was significantly elevated in a dose–dependent but not saturable manner in hOCT2-expressing cells and slightly elevated in hOCT3-expressing cells. Increased accumulation of carboplatin or nedaplatin did not occur in any of these OCT-expressing cells. The uptake of 50 µM TEA by hOCT1 and hOCT2-expressing cells was partially inhibited by cisplatin over a wide concentration range (50 - 5000 µM). The uptake of TEA by hOCT3-expressing cells was slightly inhibited by oxaliplatin. These findings suggest that cisplatin is a good substrate for hOCT2 and a poor substrate for hOCT1, and oxaliplatin is a substrate for both hOCT2 and hOCT3 (41). Yonezawa *et al.* also showed that cisplatin uptake can be mediated by the human apical multidrug and toxin extrusion 1 (hMATE1) transporter, and oxaliplatin was transported by hMATE1 and hMATE2-K (41). The MATE transporters are localized to the luminal membranes of urinary tubules and bile cannaliculi and are believed to be involved in the final export of organic cations into the bile and urine (42).

Some contradiction on substrate-specificity of hOCTs was found by Zhang *et al.*, who investigated the uptake of a range of complexes including cisplatin, carboplatin, and oxaliplatin by Madin-Darby canine kidney (MDCK) cells stably transfected with hOCT1, hOCT2, or hOCT3 (43). Cells were incubated at a single dose point, revealing a slight increase in cisplatin uptake in hOCT1-expressing cells but not hOCT2- or 3-expressing cells compared to control cells. Carboplatin

uptake was also seen in hOCT1-expressing cells. In the case of oxaliplatin, a significant increase in uptake was demonstrated in hOCT1- and hOCT2-expressing cells, which was competitively inhibited by disopyramide (against hOCT1) and cimetidine (against hOCT2) (43). Using cytotoxicity to infer accumulation, the authors measure response of OCT transfected cells to a broader range of platinum drugs (44) and propose that drugs with alkylamine non-leaving ligands (such as oxaliplatin or tetraplatin) are OCT1 substrates. The authors then examine 'aquated' oxaliplatin by two methods: replacing the oxalate leaving group with aqua ligands at pH 7.4, and in 'high chloride' PBS (104 mM) assume that the displaced oxalate in chloride solution would result in a positively charged aquachloro species, which accounts for the increased uptake in OCT1 cells, however Figure 2 shows that at pH 7.4 in high chloride, most drug would be present as neutral dichloro or (to a lesser extent) aquachloro drug. The pH dependence of uptake in OCT1-expressing cells, which would perhaps provide a more reliable uptake study, is yet to be reported (44). Although IC_{50} values of the same drug against the same transfected cells are quite different from table to table in their paper, it seems that hOCT1- or 2-expressing cells were more sensitive to oxaliplatin compared to the mock vector-transfected cells. The authors concluded that hOCT1 and 2 determine the sensitivity of cells to oxaliplatin by mediating uptake of the drug.

Arguments for active efflux

When Mann *et al.* exposed 2008 and 2008/DDP cells to 500 mM cisplatin for 10 minutes (a relatively high dose achieved in some intraperitoneal treatments) biphasic elimination was observed with rapid efflux for the first few minutes in drug-free media followed by slow drug elimination (45). The initial rate loss (first 10 minutes) was 53% greater in resistant cells, and the authors attributed the overall difference in drug accumulation in the cell pair to be due to this efflux. No

energy-depletion assays were incorporated into this study to determine whether the efflux was energy-dependent, or simply passive efflux of drug from cells into the drug-free media due to the high doses used, and interestingly when cells were exposed to lower doses (2, 5 and 10 mM) of drug, the authors did not observe increased efflux (46).

Fujii *et al*. also used a high dose (500 mM) of cisplatin to compare efflux in KB-3-1 and KCP-4 (resistant) cells and observed similar biphasic elimination in drug-free media that could be reduced by pre-incubation with 2,4-dinitrophenol (a decoupler of oxidative phosphorylation that depletes cellular ATP) (47). The authors did note that ATP-depletion may have prevented the ATP-dependent glutathione-conjugate pump GS-X from effluxing Pt-glutathione adducts (47), and subsequently demonstrated that ATP-dependent transport of leukotriene C_4 (LTC_4), a high-affinity GS-X substrate, was transported into membrane vesicles generated from KCP-4 cells (*vide infra*), suggesting the species effluxed is not cisplatin, but a glutathione-conjugated metabolite (48).

Finally, Chau and Stewart used a HTB56 human lung adenocarcinoma line and a line selected from it named E-8/0.7, to monitor efflux in the first minute after exposure to 509 mM cisplatin (49). To achieve 10 s time-points over the first minute, cells were held in suspension rather than as an adhered monolayer, and efflux rate was found to be greater in the first 10 s, but comparable after that. As such, efflux differences observed were not large enough to account for the accumulation defect in E-8/0.7 cells, and metabolic inhibitors did not prevent efflux, suggesting efflux was passive (49). Most of this effluxed platinum could pass through a 500 Da cut-off filter (mw cisplatin = 300 $gmol^{-1}$), suggesting it is intact drug or metabolites that are leaving the cell. The authors show that the intracellular pH (pH_i) of both the E-8/0.7 (pH_i 7.65) and the resistant OV2008/C13 (pH_i 7.38) lines is higher than their parental lines (HTB56 pH_i 7.51, OV2008 pH_i 6.98) (49). As outlined in the section on Speciation, an increased pH in resistant

cells would shift the equilibrium of aquated cisplatin towards the neutral, unreactive hydroxo species, **4**, that can potentially diffuse out of the cell. This would account for the increased passive efflux observed in resistant cells, and not be in conflict with the increased accumulation observed at lower pH (50).

ATP7A/B as markers for chemoresistance

Unlike other putative transporters described already, there is a significant amount of clinical evidence for ATP7B expression relating to outcome prognosis in a range of human solid carcinomas (51). Takebayashi and co-workers have examined the prognostic value of ATP7B in primary ovarian carcinomas (52, (53), human gastric carcinoma (54), human oral squamous cell carcinoma (55), human esophageal carcinoma (56), human hepatocellular carcinoma (57), and human endometrial carcinoma (58) using quantitative PCR and immunohistochemical analysis. The results of these analyses have been summarized briefly elsewhere (51) and warrant their own review, though some observations will be recapitulated here in the context of cisplatin resistance.

Cisplatin is regularly used in ovarian cancer chemotherapy; 104 ovarian carcininomas removed from patients who had received ‘cisplatin-based’ chemotherapy (no specifics on cisplatin vs. carboplatin treatment are given) were examined for p53 and ATP7B levels (59). ATP7B staining was observed in 35% of samples (commonly undifferentiated carcinomas that are more refractory), and never observed in adjacent non-neoplastic tissue, and patients with APT7B-positive tumors demonstrated an inferior response to chemotherapy compared to ATP7B-negative patients (median survival of 33 months for ATP-7B positive vs. 66 months for ATP-7N negative) (59). No mutations were observed in a copper-binding domain, or the ATP-binding domain in clinical samples, and no association between p53-positive and ATP-7B positive samples was found. A separate study of 83 patients came to similar conclusions, but also demonstrated that the expression levels of MDR1 (encoding P-glycoprotein), MRP1, MRP2,

LRP and BCRP were not prognostic indicators, collectively leading the authors to suggest that ATP7B is an independent marker of prognosis (52).

ATP7B-positive expression was also unfavorably associated with response to chemotherapy in gastric (54), oral squamous cell (55), esophageal (56), and endometrial (58) carcinomas, and no mutations in ATP7B were detected in the key copper binding and ATP binding domains in breast, gastric or oral squamous carcinoma samples (60). While there was not a strong correlation in hepatocellular carcinoma, strong ATP7B expression was observed in bile duct tissue (possibly related to copper secretion into the bile) (57). ATP7B was found to be expressed in breast carcinoma cells, and to be up-regulated in adjacent normal tissue, demonstrating that ATP7B is generally expressed in breast tissue (cisplatin is used in combination with gemcitabine in advanced breast cancer), though no correlation with prognosis was reported (61).

Howell and co-workers have reported the presence of ATP7A by immunohistochemistry in normal endometrium, prostate, testes, kidney (but not other organ samples) and a range of primary tumor samples, including tumors such as breast, colon and ovarian whose tissue of origin does not express detectable ATP7A (62). There was no correlation between pre-treatment ATP7A levels and clinical outcome, though there was some evidence that poor survival was related to high ATP7A expression before treatment commenced (though the authors note this may be an artifact) (62). Owatari *et al*. have shown that ATP7A-expressing colon cancer specimens were less responsive to chemotherapy than ATP7A-negative samples (63).

Cross-resistance to metal ions

Cisplatin-resistant cell lines have been shown to be cross-resistant to a range of metal ions, whether they be described as ‘heavy’ metals, transition metals, or trace metals, though not all resistance is due specifically to accumulation defects

(64). Naredi *et al*. tested whether the known conserved prokaryotic and lower eukaryotic metal ion transporters responsible for heavy metal tolerance played a role in cisplatin resistance (65, (66). The 2008/C13*5.25 ovarian carcinoma cell line (15-fold resistant to cisplatin) was shown to be 4-fold cross-resistant to antimony potassium tartrate[2] ($K_2[Sb_2(C_4H_4O_6)_2]$), and in a reciprocal manner cells selected for resistance to Sb (7-fold resistant) were 17-fold resistant to cisplatin (65). The radiolabeled cisplatin analogue [$PtCl_2$(en)] showed ~50% reduction in accumulation in both lines (Sb accumulation was not assessed) but the pattern of cross-resistance did not correlate with known metal transporters (65).

Naredi *et al.* subsequently showed that both the Sb and cisplatin-resistant lines were cross-resistant to arsenite (Na_3AsO_3), which demonstrates lowered accumulation in both cell lines, and no alteration in efflux is apparent for [$PtCl_2$(en)] or $^{73}AsO_4$ (66). Cisplatin cross-resistance with arsenite has also been shown in a rat liver epithelial cell line selected by chronic exposure to arsenite (69), and arsenate and arsenite resistance was shown in 7404-CP20 and KB-CP20 cells (70).

Akiyama and co-workers assessed the cross-resistance of the KCP-4 (63-fold resistance to cisplatin) cell lines derived from KB-3-1 cells, which demonstrated cross-resistance to Sb (43-fold), As(III) (10-fold) and As(V) (13-fold) (71). Efflux of Sb was observed in the KCP-4 cell line to a greater degree than the 3-1 cell line (49% vs. 22% Sb after 40 mins), and this efflux was reduced by approximately half in the KCP-4 line when incubated with ATP-depleting agents.

[2] Originally known as 'tartar emetic', the Sb(III) salt was originally used as a treatment for leishmaniasis, though use was discontinued because of variable toxicity. It is now being examined as a potential treatment for acute promyelocytic leukemia (APL).67. Wyllie S, Fairlamb AH. 2006. Differential toxicity of antimonial compounds and their effects on glutathione homeostasis in a human leukaemia monocyte cell line, *Biochem Pharmacol* 71:257-67 While little was known of the aqueous chemisty of antimony potassium tartrate when these reports were published, it has subsequently been shown to rapidly exchange ligands at physiological pH, readily enter erythrocytes, and form complexes with ligands such as glutathione,68. Sun H, Yan SC, Cheng WS. 2000. Interaction of antimony tartrate with the tripeptide glutathione implication for its mode of action, *Eur J Biochem* 267:5450-7 meaning the metal salt and a range of small complexes may be present in media.

Given that the KB-derived family of cisplatin-resistant lines do not demonstrate enhanced cisplatin or carboplatin efflux, but do show reduced accumulation/uptake (72), it seems that Sb and As are effluxed by an ATPase that may play a role in glutathione-conjugated efflux, while the cross-resistance with platinum drugs is due to diminished uptake. Cole *et al*. have shown that cells transfected with MRP1 are resistant to Sb and As as well as natural product drugs such as taxol and vincristine, but not cisplatin (73), and Akiyama has shown MRP1 levels do not contribute to lowered cisplatin accumulation in KCP-4 (68), but that the KCP-4 cells do rapidly efflux natural product drugs such as camptothecin that are substrates for MRP1 (74).

It is likely that cross-resistance to Sb and As is due in part to a generic pleiotropic mechanism for response to soluble cytotoxic compounds that subserves a normal physiological function either in development or in response to environmental adversity. The increased efflux of Sb and As observed in resistant cells may be due to the energy-dependent extrusion of glutathione-conjugated metals from the cell, and as such is an observable phenotype of the GS-X pump expression described in this review.

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Supplementary Table 1. Various names that exist for each of the GS-X transporter ATP-Binding Cassette (ABC) proteins[a]

MRP name	ABC name	Other common names	Tissue expression
MRP1	ABCC1	MRP	Widely expressed, lung, liver
MRP2	ABCC2	cMOAT	Liver, duodenum
MRP3	ABCC3	cMOAT2	Liver, colon, adrenal gland
MRP4	ABCC4		Low expression
MRP5	ABCC5		Widely expressed, brain, skeletal
MRP6	ABCC6	MLP-1	Kidney, liver
MRP7	ABCC10		Skin, colon, testis
MRP8	ABCC11		Breast, testis

[a]Names were derived from the original observation that ABCC1 was a multidrug resistance protein. Sequence analysis culminated in the systematic ABC names; however, the transporter names are used interchangeably in the literature.

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