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Resistance to Chemotherapy in Cancer: A Complex and Integrated Cellular Response

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Abstract
Inherent and acquired resistance pathways account for the high rate of failure in cancer chemotherapy. The mechanisms or pathways mediating resistance may be classified as pharmacokinetic (i.e. alter intratumour drug exposue) or pharmacodynamic (i.e. failure to elicit cytotoxicity). More often than not, the resistant phenotype is characterised by alterations in multiple pathways. Consequently, the pathways may act synergistically or generate a broad spectrum of resistance to anticancer drugs. There has been a great deal of systematic characterisation of drug resistance in vitro. However, translating this greater understanding into clinical efficacy has rarely been achieved. This review explores the phenomenon of drug resistance in cancer and highlights the gap between in vitro and in vivo observations. This gap presents a major obstacle in overcoming drug resistance and restoring sensitivity to chemotherapy.

Introduction

Chemotherapy remains one of the major therapeutic avenues in oncology and is used for primary treatment, adjuvant therapy and palliation. It is of particular benefit for disseminated disease which is notoriously difficult to treat with radiation and surgery. The previous 50 years have seen numerous advances in the properties of chemotherapeutic agents; however, the primary mechanism of action remains genotoxicity. Unfortunately, a significant proportion of cancers are inherently unaffected by the administration of anticancer drugs. Furthermore, another considerable proportion of patients undergoing chemotherapy display an initial reduction in tumour size only to relapse with a marked insensitivity to a variety of drugs. Both scenarios are brought about by a resistant phenotype, which presents perhaps the single greatest barrier to successful chemotherapy. As outlined in this review, the resistant phenotype is an adaptive response of cancer cells and comprises multiple pathways. Moreover, cancer cells often display multiple different pathways, which interact synergistically to confound the cytotoxicity of chemotherapeutic agents.

Pharmacodynamic Resistance Pathways

Chemotherapeutic drugs primarily target proliferating cells, mainly through inhibition of specific steps of the DNA replication process. This may result from inhibition of nucleoside biosynthesis, direct interaction with the DNA or preventing the cell entering mitosis following drug-induced damage. Chemoresistance arises as a result of changes in the biology of cancer cells, often as a consequence of prior chemotherapy or in response to the micro-environment found within solid tumours.

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p53 Status and Drug Efficacy

The transcription factor p53 is a critical regulator of the cellular stress response. p53 is activated by a diverse range of stimuli including DNA damage, inappropriate oncogene activation, hypoxia and loss of cell-cell contact [1–3]. The mechanism of activation is attributed to increased stability of p53, which is normally a short-lived protein due to the presence of an endogenous inhibitor *(Hdm2)* that promotes its degradation. Cellular stress results in stabilisation of p53 by posttranslational modification, leading to its interaction with DNA and co-operating factors. Activated p53 initiates a chain of events that minimise the adverse effects of damage, primarily by suspension of the cell cycle through up-regulation of numerous genes including p21 [2]. Suspension of the cell cycle permits repair of cellular damage, particularly to DNA, thereby maintaining cell viability and promoting survival. However, if the magnitude of the DNA damage is too great or is irreparable, p53 can induce the expression of apoptosis inducers (e.g. PUMA, Fas and Bax), which ensures that DNA mutations are not replicated in daughter cells. This duality of p53 function renders it a central role in cell biology, particularly by acting as a tumour suppressor gene.

Evidence of its tumour suppressor activity is reflected by the high degree of mutations (approx. 53%) observed in cancer. In fact, the p53 gene is one of the most highly mutated in biology. Many of these mutations compromise the ability of p53 to effect cell cycle arrest and promote apoptosis in response to cellular damage. A prominent role in dictating the effectiveness of genotoxic chemotherapeutic agents in treating cancer would therefore be anticipated. For example, loss of p53 function due to mutation could prevent the initiation of apoptosis following chemotherapeutic insult, thereby conferring resistance. Indeed this has been demonstrated for a number of anticancer drugs (e.g. 5-fluorouracil, 5-FU, in colorectal cancer). Consequently, restoration of functional p53 to cancer cells through gene therapy strategies is being investigated as a means to restore chemotherapeutic efficacy [4, 5]. However, a number of studies have failed to demonstrate a link between dysfunctional p53 and efficacy of certain anticancer drugs [for a review, see 6]. Clearly, the complex cellular role of p53 engenders unpredictability when attempting to correlate p53 defects with the drug-resistant phenotype in cancer.

DNA Repair Pathways and Drug Efficacy

Cancer cells employ a number of endogenous DNA repair pathways including base excision, nucleotide excision, mismatch or direct repair of damage introduced by exposure to radiation or chemicals [7, 8]. The precise pathway chosen to repair damage introduced by chemotherapeutic agents depends on the nature of the drug-DNA adduct formed. In addition, there are a number of tissue differences in the prevalence or fidelity of repair pathways, thereby making predictions of chemotherapeutic drug efficacy based on this parameter a difficult task.

Nucleotide excision repair (NER) comprises a large number of proteins, with the ERCC1 (excision repair cross-complementing) protein a crucial player in dictating success or failure of chemotherapy. For example, elevated mRNA levels of ERCC1 are observed in drug-resistant cell lines, and the reduction in levels with inhibitory RNA improved drug cytotoxicity. Moreover, there appears to be a correlation between the levels of ERCC1 mRNA and chemotherapy using platinum compounds in both ovarian and non-small-cell lung cancer (NSCLC) in the clinic [9, 10].

DNA mismatch repair (MMR) [11] deficiency has been reported to occur in many cancer cell types and is also considered a causative factor in several tumour types including endometrial and hereditary non-polyposis colon cancer [8]. A deficiency in MMR has been widely attributed as a key determinant in the efficacy of chemotherapy, particularly for alkylating and platinating drugs. The aetiology of MMR deficiency has two primary causes: genetic mutation [12] or hypermethylation of the promoter [13] (see also below). Whilst both produce resistance to chemotherapy, there is potential to overcome the latter through inhibition of the methylation process.

The impact of base excision repair (BER) on the efficacy of chemotherapeutic drugs is less well established. The methylating drug temozolamide produces a variety of covalent adducts, and at least two pathways are involved in repair [14]. The O^6-methylguanine and 7-methylguanine adducts are repaired by NER or methylguanine-DNA methyltransferases, whilst the predominant 7-methyladenine adducts require BER [15]. It has been observed in cancer cells in vitro that disruption of BER enhances the toxicity of the methyladenine adducts [15]. However, the impact of BER on drug cytotoxicity in the clinical setting has not been tested, nor has the premise that disrupting this pathway may provide a therapeutic option.

The activity of the DNA repair enzyme O^6-methylguanine-DNA methyltransferase (MGMT) has been widely investigated in cancer and a correlation with chemotherapeutic efficacy established. Details on this correlation and its role in drug resistance are described later.

Reduced Sensitivity to Apoptosis

Apoptosis occurs via two main routes, namely the extrinsic or intrinsic pathways depending on the initial signal [16–18]. The extrinsic pathway involves the recruitment of death receptors belonging to the tumour necrosis factor receptor family leading to a cascade of events that leads to the activation of caspases 3 and 8. These proteases dismantle the cell and produce the morphological changes that characterise apoptosis (i.e. DNA condensation and degradation). The intrinsic 'mitochondrial' death pathway, initiated in response to DNA damage and many forms of cellular stress, also involves the activation of caspases. However, this pathway is characterised by release of cytochrome c following mitochondrial membrane depolarisation. The intrinsic pathway is the major route for chemotherapy-induced apoptosis, and perturbation of this may lead to considerable alterations in the response to chemotherapy.

Mitochondrial membrane integrity is governed by the balance of pro- and anti-apoptotic proteins, such as the Bcl-2 family. It is well established that overexpression of the anti-apoptotic Bcl-2/Bcl-x_L proteins correlates with chemotherapy resistance, whilst down-regulation with RNA interference enhances the drug response. Clinical data in acute myeloid leukaemia, advanced breast cancer and non-Hodgkins lymphoma concur with this relationship [19, 20]. However, Bcl-2/Bcl-x_L overexpression is also correlated with slow proliferation and high steroid receptor levels, both of which confer a positive prognosis. Similarly, correlating Bax (pro-apoptotic) expression levels or mutation with response to chemotherapy in clinical samples has generated conflicting reports [21–23]. Clearly, disrupting the fine balance between pro-/anti-apoptotic factors will impact on cell survival during chemotherapy in a drug- and tissue-specific manner. Extensive microarray and proteomic data will be required from clinical material to elucidate the correlation between apoptotic markers and chemotherapy response.

Alteration of Drug Targets in Resistant Tumours

Resistant cancer cells are frequently associated with altered targets for chemotherapeutic agents, thereby strengthening the cellular defence mechanism.

DNA Methylation

A frequent cellular target for chemotherapeutic agents is DNA, and its methylation status is a strong determinant of outcome [24, 25]. Approximately 1% of DNA bases are modified by the addition of a methyl group to the 5′-carbon group on cytosine, and in cancer cells this often occurs at cytosine-guanine dinucleotides. DNA methylation is generated by one of the three DNA methyltransferase iso-enzymes and is often associated with transcriptional inhibition. Furthermore, in cancer cells tumour suppressor genes are a frequent methylation target. A commonly observed site of methylation is the caspase 8 promoter, and this ultimately reduces the execution and extent of apoptosis, as observed in neuroblastoma [26]. Consequently, inhibiting or reversing the process is an attractive therapeutic approach [27]. The dinucleoside analogue, 5-aza-2′-deoxycytidine has been used to inhibit DNA methyltransferases thereby preventing hypermethylation of the caspase 8 promoter and thus enhancing drug-mediated apoptosis [28].

Unfortunately, due to the diverse range of genes silenced through methylation, inhibition of DNA methyltransferases does not universally result in enhanced drug efficacy. For example, the DNA repair enzyme MGMT promoter is also hypermethylated in a number of tumours. These tumours had an enhanced sensitivity to DNA alkylating agents, and the use of a methylation inhibitor would in fact compromise chemotherapy by promoting increased DNA repair through MGMT [29].

DNA Topoisomerases

Topoisomerase I is a key enzyme in the DNA replication process by virtue of its ability to introduce single-strand breaks in supercoiled DNA. Consequently, a number of chemotherapeutic drugs (e.g. camptothecins) have been developed to target this enzyme. Camptothecins stabilise the topoisomerase-DNA interaction and thereby prevent re-ligation of the nucleotide strands, which promotes apoptosis [30–32]. The topoisomerase II enzyme works in a similar manner but induces a double-strand break in DNA and is a primary target for the anthracycline (e.g. doxorubicin) and epipodophyllotoxin (e.g. etoposide) chemotherapeutic agents [33, 34].

Unfortunately, topoisomerase I or II inhibition also appears to be associated with drug resistance, through two main routes. Firstly, many cancer cell lines display reduced expression of the enzyme(s), and secondly, there are a number of mutations to topoisomerase I/II that prevent or reduce the affinity of drug binding. However, the vast majority of data has been compiled from in vitro studies using cancer cell lines, and the importance or prevalence of this resistance mechanism and its effect on prognosis in vivo remains to be established.

Cellular Metabolic Pathways

The 'antimetabolite' compounds are a widely utilised class of chemotherapeutic drug, for example the pyrimidine analogue 5-FU. 5-FU is processed by the cell in the pyrimidine synthesis pathway, ultimately resulting in the formation of an inhibitor (5-fluoro-2′-deoxyuridine-5′-monophosphate, FdUMP) of thymidylate synthase (TS). The inhibition of TS causes depletion of thymidine and halts cell replication in the S phase of the cell cycle. 5-FU efficacy inversely correlates with the expression levels of TS, and induction causes resistance to the chemotherapeutic agent by restoration of the nucleoside synthetic pathway.

Environmental Influences on Efficacy: pH, Quiescence and Hypoxia

The rate of growth of solid tumours in vivo outstrips the ability to provide sufficient nutrients (e.g. metabolic fuels), oxygenation and clearance of metabolic by-products. Consequently, the local intratumour environment, particularly in avascular regions, is characterised by low oxygen (hypoxia), acidic extracellular pH and populations of quiescent cells. Moreover, each of these characteristics may impact on the success of chemotherapy in solid tumours.

Acidic pH

The rapid rate of tumour growth necessitates increased levels of energy production through cellular metabolism. However, inadequate vascularisation precludes efficient and homogeneous oxygen delivery to cells, which manifests as hypoxia that increases in severity in proportion to the distance of cells from vessels. The hypoxic environment requires that cellular energy requirements are met through glycolytic pathways rather than the more efficient oxidative phosphorylation route. Additionally, the high interstitial pressure prevents clearance of metabolic end-products such as lactic acid [35]. A consequence of this scenario is acidification of the extracellular space [36–38] and eventually the cytosol, which has catastrophic implications for cell viability. Therefore, cancer cells adapt to promote acid extrusion from the cytosol and maintenance of a neutral pH_i.

Increased expression of an arsenal of H^+ pumps is the primary mechanism by which the cells ensure pH homeostasis, and the acidic extracellular environment has important consequences for chemotherapy. In particular, a number of clinically important chemotherapeutic agents are either weakly acidic (e.g. camptothecins) or weakly basic (e.g. mitoxantrone and doxorubicin). According to the 'ion-trapping' hypothesis, weakly basic drugs would be ionised in the acidic interstitial compartment and thereby display reduced permeability across the plasma membrane of cancer cells [39]. The resultant reduction in steady-state accumulation would manifest as resistance, and this has been observed for mitoxantrone and doxorubicin. Conversely, the weakly acidic camptothecins would display increased accumulation in cancer cells due to the relatively higher intracellular pH compared to the interstitium.

The pH homeostatic balance in cancer cells may therefore offer therapeutic possibilities. For example, the presence of weak acid moieties will ensure high partition. Perturbation of the homeostatic mechanism by inhibition of plasma membrane H^+ pumps may (i) enhance intracellular accumulation of weakly basic drugs or (ii) cause cell death directly through the acidification of the cytosolic compartment. Proof of principle for the latter has been demonstrated by Na^+/H^+ antiporter and Cl^-/HCO_3^- exchanger inhibition using cariporide and S3705, respectively [40, 41]. The inhibition was associated with significant growth inhibition of cultured breast cancer cells.

Quiescent Cells

Although tumours are characterised by rapid growth, their inherent heterogeneity includes a significant proportion of cells not undergoing proliferation. A number of studies have used immunohistochemistry or FACS analysis to quantify the number of quiescent cells; a gross average from multiple different tumour types estimated a tumour growth fraction of 20 $\pm$ 15% [42]. However, inspection of individual cancer types revealed considerable variation; for example tumour growth fraction values were 17% colorectal, 10% lung cancer, 3.5% ovarian and <3% prostatic [43–45]. The aetiology of this reversibly quiescent cell population in solid tumours has not been fully elucidated and may be tissue dependent. However, it is clear that a number of stress stimuli including hypoxia and acidic pH contribute. In particular, hypoxia has been demonstrated to induce cell cycle arrest via induction of the cyclin-dependent kinase inhibitor $p27^{kip1}$. This large population of quiescent cells in solid tumours is a potent barrier to chemotherapy since the vast majority of conventional or genotoxic anticancer drugs target some aspect of the replication process. Consequently, a large proportion of tumour cells are inherently resistant.

Cells in the quiescent state are characterised by high levels of the marker $p27^{kip1}$ and low expression of the pro-

liferation marker Ki-67 [46–49]. The impact of quiescence on chemotherapy is demonstrated by the ability of these two cell cycle proteins to act as prognostic markers. Chemotherapy is therefore likely to kill the drug-sensitive proliferating cells at the periphery [50] or perivascular regions in solid tumours. Crucially, when reperfused, the quiescent cells in a solid tumour may resume growth and act as stem cells, partially or fully repopulating the tumour. Quiescent cells that inherently express a resistance mediator will resume growth even in the presence of the anticancer drug.

Hypoxia

Widespread hypoxia is an inevitable occurrence in solid tumours due to their inherently poor blood supply and the high energy demands to power replicative processes. The tissue response to hypoxia is dramatic and involves an adaptive survival response orchestrated predominantly by the transcription factor hypoxia-inducible factor (HIF) 1, and the reader is directed to a number of reviews [51–54]. Hypoxia and the associated HIF-1 response have significant ramifications for the efficacy of chemotherapeutic agents in tumours [55]. The molecular mechanism of bleomycin-induced strand breaks in DNA is contingent on the presence of oxygen, and therefore, hypoxic cells are resistant to its cytotoxic effects. In contrast, hypoxic cells display enhanced bioreductive activation of mitomycin C and stabilisation of the cytotoxic forms of tirapazamine. Consequently, there is considerable interest in bioreductively activated anticancer drugs as a means to produce selective cytotoxicity in hypoxic tumour cells.

Hypoxia increases the presence of nucleophiles such as glutathione (GSH) and the metal binding protein metallothionein (MT) [56–58]. Their presence greatly reduces the efficacy of alkylating and platinating compounds by scavenging reactive species or competing with DNA for interaction with the drugs. HIF-1 is known to directly induce the expression of specific resistance mediators including the multidrug efflux pump P-glycoprotein (P-gp), thereby reducing the intracellular accumulation of a vast array of anticancer drugs. Amplification of the enzyme dihydrofolate reductase (DHFR) overcomes the ability of methotrexate to inhibit the regeneration of tetrahydrofolate (THF), which is an essential step in nucleoside synthesis. Hypoxic cells also display reduced rates of cell cycle progression, characterised by S phase arrest due to metabolic restrictions. This occurs through HIF-1-induced expression of the cyclin-dependent kinase inhibitor $p27^{kip1}$ and renders cells considerably less sensitive to the majority of conventional anticancer drugs, which target proliferating cells. Moreover, hypoxic stress exerts a selection pressure on tumour cells that leads to a decreased propensity to undergo apoptosis (e.g. through p53 mutation), which results in broad chemoresistance.

Pharmacokinetic Resistance Pathways

In addition to affecting aspects of anticancer drug activity in the cell, resistance mechanisms also compromise a number of pharmacokinetic properties of drugs. By ensuring reduced exposure of cellular targets to the active drug species, the tumour is afforded a pharmacokinetic resistance to chemotherapy.

Intratumour Drug Distribution

Solid tumour architecture reveals that a significant proportion of cancer cells lie at a distance of >100 μm from the nearest blood vessel, which is in complete contrast to non-malignant tissue. This factor plays an important role in generating a local micro-environment characterised by acidic pH and hypoxia. The aberrant structural organisation also impacts on the intratumour pharmacokinetics of drugs and therapeutic macromolecules [59]. In particular, the impact manifests as a poor drug distribution within the tumour mass and a failure to expose the most distal regions to chemotherapy.

The low density of functional vessels found in many tumours prevents many drugs and macromolecules from achieving a uniform or complete distribution. Numerous studies using model tumour systems (e.g. multicellular spheroids, multicell layers and xenografts) and a variety of imaging approaches have demonstrated heterogeneity in the tissue distribution profile of drugs, antibodies and other novel therapeutics [60–62].

An additional factor expected to contribute to poor drug distribution across regions of high cell density and inadequate vascularity is the lower hydrostatic fluid pressure difference between the vessel lumen and the tumour interstitium [63]. Consequently, compared with the situation in normal tissues, the extravasation at the tumour site would not be favoured. However, the low hydrostatic fluid pressure difference is often counterbalanced by the high permeability of microvessels within solid tumours. The structural organisation of endothelial cells in intratumour vessels formed during angiogenesis is incomplete, and thus overall, the extravasation of drugs and plasma constituents is favoured.

Once a drug molecule has reached the interstitial region, its movement is controlled by diffusion and convection [64]. The diffusion component is dictated by the concentration differences across the intervessel region, which favours homogeneous distribution in the tumour. For many drugs this gradient is maintained, even over extended periods as even high peripheral accumulation in cells is possible due to the avid sequestration of drugs in intracellular sites. Convection of drug molecules through the tissue depends on the interstitial tissue fluid velocity, which is in turn affected by the interstitial fluid pressure. The latter is high at the centre of intervessel regions due to the high cell density and presence of debris from regions of necrosis. The pressure gradient favours drug distribution at the periphery and retards penetration. Convection of drug through the tissue is also retarded by binding to cell surfaces or the accumulation at intracellular sites.

In summary, despite the potential for angiogenesis to improve distribution of drugs within solid tumour masses, there are a number of characteristics that prevent a homogeneous exposure of cancer cells to chemotherapeutic agents. The greatest limitation appears to be the fluid dynamics within the interstitium.

Drug Efflux Pumps and 'Multidrug Resistance'

Once chemotherapeutic agents have reached the cells comprising the tumour, passage across the plasma membrane represents the first possible impediment to their efficacy. It is just over 30 years since the seminal discovery that a plasma membrane glycoprotein is responsible for conferring drug resistance to cultured cancer cells [65]. The protein was named the permeability or P-gp and is now known to be a member of the ATP binding cassette (ABC) superfamily of transporters. The presence of P-gp was rapidly observed as a negative prognostic factor in cancer and impacted on the efficacy of a multitude of chemotherapeutic agents. The ability of P-gp to confer resistance to a large number of structurally and functionally unrelated compounds is referred to as 'multidrug resistance'. The protein is able to confer resistance by reducing the intracellular accumulation of anticancer drugs to levels below their therapeutic threshold. The ability of P-gp to act as a multidrug efflux pump has yet to be elucidated at a molecular level but the transporter provides a robust mechanism of resistance [66].

Two further members of the ABC superfamily of transporters have been implicated as multidrug efflux pumps capable of conferring resistance to chemotherapy. The multidrug-resistance-associated protein (MRP1), also an ABC transporter, was discovered in resistant lung cancer cells [67] and confers resistance to a subset of anticancer drugs distinct from those transported by P-gp. Moreover, two iso-enzymes of MRP (i.e. MRP1 and MRP2) have been demonstrated to transport drug metabolites (e.g. glucuronide or GSH conjugates), and their expression patterns do not completely overlap with P-gp. More recently, the breast cancer resistance protein (BCRP), or the mitoxantrone resistance protein, has been isolated from cell lines selected for resistance to mitoxantrone [68, 69]. Although BCRP is also an ABC transporter, in contrast to P-gp or MRP it is a half-transporter, the functional unit being a homodimer. Like P-gp, BCRP translocates unmetabolised drugs. Moreover, it confers resistance to a subset of drugs, distinct to those transported by P-gp and MRP. Overall, these three transporters provide protection to cancer cells from an extraordinarily large number of diverse anticancer agents and their expression in tumours is widespread, thereby ensuring a prominent role in the phenotype. The three transporters have now been assigned systematic nomenclature (P-gp = ABC^{B1}, MRP1 = ABC^{C1} and BCRP = ABC^{G2}), and the reader is directed to a website outlining the classification of the ABC superfamily (http://www.genome.ad.jp/dbget-bin/show_pathway?hsa02010+6833). This systematic nomenclature will be used for the remainder of the review.

The three multidrug ABC transporters are expressed in a variety of normal tissues, with highest levels observed in tissues providing a secretory/excretory function (e.g. gastro-intestinal tract and the liver) or those forming barriers to sensitive organs (e.g. blood-brain barrier). Malignancies arising from sites of endogenous expression display significant expression of these transporters and are inherently resistant to chemotherapy. Expression of these proteins is often elevated in response to chemotherapy, most likely due to the selection pressure that treatment exerts on the tumour cells. Cell damage and the stress response are associated with increased expression of multidrug transporters; hence, the levels of transporters are paradoxically elevated in response to the early success of chemotherapy.

Metabolic Biotransformation and/or Inactivation

Metabolism or biotransformation of anticancer drugs is an important factor in establishing the plasma levels of the active form of the molecule and, in turn, the duration and level of drug exposure to the tumour. Factors influencing drug metabolism include hepatic viability (i.e metabolic capacity) and hepatic blood flow. Meta-

bolic capacity is primarily governed by phase I or oxidative pathways (e.g. cytochrome P450 mediated) and phase II conjugation (e.g. UDP-glucuronosyltransferase and glutathione-S-transferase, GST). The focus of this review is the intratumour resistance, and thus the hepatic metabolism of anticancer drugs will not be discussed. There are a number of metabolic activities associated with tumours per se that will influence drug activity at a local level.

GST and MT

Platinum drugs, due to their high inherent reactivity, are readily inactivated by conjugation with the peptide GSH, which is catalysed predominantly by the GST-π isoform [70]. This fate has been demonstrated for a number of platinum complexes in cancer cell lines that display elevated levels of the substrate GSH and/or the enzyme GST-π [71]. Moreover, depletion of cellular GSH levels with L-buthionine-S,R-sulfoximine enhances the cytotoxicity of dinuclear platinum drugs in resistant human ovarian cells [71].

MT is known to bind and sequester high amounts of heavy metals (e.g. Zn, Cu, Se, Cd, Hg and Ag) by virtue of the high proportion of cysteine residues within their structure. A number of in vitro and clinical studies have shown that MT is able to inactivate several types of platinum complexes, and elevated expression of MT is associated with poor response to platinum compounds [72–75].

However, a definitive correlation with platinum drug efficacy and inactivation by conjugation with GSH or sequestration by MT is yet to be unequivocally demonstrated in the clinical setting, and a role in drug resistance has not been established.

Cytochrome P450 and UDP-Glucuronosyltransferase

There are numerous reports detailing the expression of various cytochrome P450 (CYP) isoforms in tumour tissue [76–78]. However, the contribution of the proteins to drug activation or metabolism to inactive species at the tumour site has not been elucidated. The CYP2C8 and CYP3A4, which are known to metabolise anticancer drugs, were amongst those expressed at tumour sites, and in vitro testing has confirmed a role in predicting drug cytotoxicity [76]. Furthermore, low expression of CYP3A4 was associated with improved response to docetaxol in breast tumours [79] and the high expression of this isoform in osteosarcoma tumours [76] from a small group of patients correlated with poor prognosis. However, other CYP isoforms, known to be expressed at tumour sites (1B1, 2J2, 2W1 and 4Z1), are not utilised in drug metabolism. The therapeutic expression of these isoforms is being utilised to activate novel prodrugs specifically at the tumour site.

Carboxy-Esterases

The widely used topoisomerase I inhibitor irinotecan is in fact a prodrug that requires activation by the ubiquitous carboxy-esterases (human liver carboxy-esterase, hCE) to the active species SN-38. Cell lines rendered drug resistant by prolonged exposure to irinotecan were associated with reduced activation by hCEs. Moreover, transfection of hCEs into cell lines increased sensitivity to irinotecan [80], and adenoviral delivery of hCE to a resistant in vivo adenocarcinoma model restored sensitivity to the drug [81, 82].

Polyglutamation

The antifolate drug methotrexate enters cancer cells via the reduced folate carrier [83]. To ensure that the concentration gradient into the cell is maintained, methotrexate is sequestered by conjugation with several glutamate residues (i.e. polyglutamation). The reaction is catalysed by folypolyglutamate synthase (FPGS), and reductions in the activity or expression level of this enzyme appear to provide a significant contribution to the complex resistance profile for methotrexate in a variety of cultured cell lines.

Resistance Pathways in Summary

The inherent features or adaptive responses of solid tumours present a significant barrier to the success of chemotherapy. Resistance pathways are interdependent or interconnected and affect the delivery, stability and function of anticancer drugs. It is however worth noting that the precise contribution of specific resistance pathways to anticancer drug efficacy in specific cancers remains to be fully elucidated. It is likely that in many cases, resistance may arise through multiple mechanisms that develop in parallel. We have generated a solid understanding of many of the main pathways and the respective relevance of these in the resistant phenotype in vivo continues to engender lively debate. These issues urgently need to be addressed to shape or prioritise future strategies designed to overcome this considerable impediment to a major form of cancer treatment.

Resistance to Alkylating Agents

Compounds that produce DNA alkylation are amongst the oldest established anticancer agents, having been in clinical use for over 50 years [84, 85]. These drugs are highly reactive, producing covalent modification of macromolecules, the predominant cellular lesion being towards O or N atoms in nucleobases [86]. The main classes of alkylating agents include nitrogen mustards/oxazaphosphorines (e.g. cyclophosphamide, CPA), nitrosoureas (e.g. carmustine), triazenes (e.g. temozolomide) and alkyl sulfonates (e.g. busulfan). The nature of the alkylation generated depends on the physicochemical properties of the drug and the localisation of the DNA lesion [for a review, see 86]. The two main sites of alkylation are (i) the O^6 position in guanine or (ii) the N^7 position of purine bases. The type of modification produced by alkylating agents also falls into two distinct categories: (i) methylation (e.g. temozolamide) or (ii) chlorethylation (e.g. carmustine).

Approximately 20,000 lesions/day arise in DNA, and these are rapidly removed by repair pathways. Defects in DNA repair may lead to the prolonged presence of adducts that may facilitate or initiate carcinogenesis. Similarly, DNA lesions evoked by anticancer drugs may be reversed by the same repair pathways. There are numerous DNA repair mechanisms; for simple mono-adduct formation the major ones involved are: (i) direct base repair by methyltransferases [86], (ii) BER by DNA glycosylases [87] and (iii) NER [88]. Failure to repair drug-induced DNA lesions usually results in apoptosis induction. The fidelity of DNA repair pathways and the ability of this machinery to reverse DNA damage largely dictate the efficacy of alkylating chemotherapy.

Resistance Pathways against O^6-Guanine Alkylation

The primary resistance mechanism for monofunctional alkylating drugs (i.e. adduct at a single site) is dealkylation by the enzyme MGMT. MGMT is classified as a suicide enzyme as it is used up in the reaction that transfers alkyl moieties (e.g. methyl, ethyl, benzyl) from the O^6-guanine of DNA to cysteine 145 in the protein-active site [89]. Expression levels of MGMT vary considerably within normal tissues, with the highest observed in the liver. Tumour levels have been extensively examined, and significant expression is observed in melanoma, glioma and colon, pancreatic and lung cancers [90–94]. A number of anticancer drugs are known to generate adducts at the O^6-guanine position and they include (i) carmustine, which is used in myeloma, brain tumours and lymphoma, (ii) temozolamide, which is used in brain tumours, and (iii) procarbazine, which is used in Hodgkins and non-Hodgkins lymphoma. The role of MGMT in modulating the tumour response to chemotherapeutics is supported by clinical studies correlating its expression with patient prognosis and survival characteristics. For example, a link has been shown between MGMT levels and survival following carmustine treatment of brain tumours [95] or temozolamide chemotherapy in glioblastoma [96]. Moreover, the methylation status of the MGMT promoter in glioblastoma patients was attributed as the critical factor in predicting efficacy of chemotherapy by alkylating agents [96–98]. Increased methylation of the MGMT promoter results in reduced expression of the protein and, therefore, lower capacity to repair alkylating-agent-induced DNA damage.

The clinical importance of MGMT activity in dictating response to numerous alkylating anticancer drugs resulted in several attempts to exploit its presence in resistant tumours. One such strategy has led to the development of guanine analogues to inactivate MGMT. The first compound developed was O^6-methylguanine (O^6-MG), which acted as a competitive inhibitor of MGMT and reduced enzyme activity in cultured cancer cells [99]. Moreover, the addition of O^6-MG could enhance the cytotoxicity of alkylating chemotherapeutic drugs [99, 100]. Unfortunately, the concentrations of O^6-MG required to effect inhibition of MGMT in whole animal studies precluded clinical use [101]. Further preclinical investigations ascertained that the O^6-benzyl guanine (O^6-BG) analogue was considerably more potent than O^6-MG and displayed improved pharmacokinetic properties [102]. O^6-BG binds within the active site of MGMT similarly to the natural substrate and covalently binds to a cysteine residue, thereby producing irreversible inhibition [103]. Numerous subsequent studies demonstrated the efficacy and improved potency of O^6-BG, relative to O^6-MG, in cultured cells and animal models. In clinical trials, MGMT inhibition was achieved, albeit at varying extents [104]. Unfortunately, although O^6-BG did not produce toxicity per se when used in combination with alkylating drugs (e.g. carmustine); the development of significant myelosuppression necessitated dose reduction of the alkylating agent. The most recently developed MGMT inhibitor, lomeguatrib, has demonstrated great success in enhancing the growth-inhibitory activity of alkylating drugs such as temozolamide in xenografts [105, 106]. Lomeguatrib is considerably more potent than O^6-BG and sensitises numerous tissue types (melanoma, breast and prostate) to alkylating agents and, moreover, does

not add to the non-specific toxicity of the anticancer drug. A recent early-stage clinical trial of lomeguatrib in combintion with temozolamide for advanced solid tumours has led to the development of a suitable dosing regime for the two compounds for future studies [107].

Resistance Pathways against N^7-Alkylation

The nitrogen mustard class of anticancer drugs produces a complex array of DNA modifications; the major types involve adduct formation at N^7-positions in purine bases. Two of the most widely employed nitrogen mustards, CPA and ifosfamide (IFO), have been used to treat numerous malignancies including lymphomas, myeloma, breast, lung, prostate and ovarian cancer [108]. Both compounds require metabolic activation (primarily hepatic) to produce highly reactive bifunctional species, one 'arm' of which will modify purine bases flanked by guanines, and the second 'arm' of the drug may bind to guanines on either the same or opposite strand. The nitrogen mustards can also be rendered ineffective by cellular resistance pathways, although unlike drugs such as temozolamide, there is no single stand-out factor involved.

CPA and IFO undergo complex oxidative activation by the CYP2B6 (primarily), CYP3A4 and CYP2C9 isoforms of cytochrome P450 [109]. These isoforms are most abundantly found in the liver, although extrahepatic and intratumoral metabolism may also play a significant role. Polymorphic variations in these isoforms alter the *activation* characteristics and hence the efficacy of compounds such as CPA and IFO [for a review, see 108], but this remains to be validated in the clinic.

The 4-OH metabolites of CPA and IFO readily enter tumour cells by passive diffusion before undergoing further metabolism to nitrogen mustards via a number of intermediates. There have been reports that isoforms of the drug efflux pumps ABC^{C1}, ABC^{C2}, ABC^{C4} and ABC^{G2} may mediate the efflux of some of these compounds, particularly the GSH conjugates [110, 111]. Although the GSH conjugates are inactive, their transport out of tumour cells may alter the dynamic equilibrium between metabolic intermediates of CPA and IFO. Clinical studies have attempted to correlate the presence of multidrug transporters with resistance to CPA or IFO [112, 113]. However, the evidence has been largely obtained from patients undergoing combination chemotherapy; therefore, direct attribution to a single chemical species has not been demonstrated unequivocally.

Conjugation of CPA metabolites involves several GST isoforms, some of which display increased levels in tumours. Enhanced detoxification of CPA metabolites would conceivably reduce the efficacy of chemotherapy; however, the clinical data available thus far do not provide supporting evidence of a correlation [114, 115]. Aldehyde dehydrogenase isoforms are also involved in the detoxification of CPA and IFO metabolites [108], and their overexpression in cultured cells decreases the sensitivity of cells to CPA and the related mafosamide [116, 117]. In addition, metastatic tumours with high aldehyde dehydrogenase 1A1 levels responded poorly to CPA [116]; however, this correlation was not observed in ovarian tumours [114].

Tumour cells employ a network of repair pathways to remove the DNA adducts formed by nitrogen mustards. In general, the primary adducts are repaired by MGMT, whereas the critical secondary adducts require nucleotide excision factors (e.g. ERCC1 and ERCC4) and homologous recombination [86]. The complex nature of the DNA repair response may account for the contradictory clinical reports regarding the response to CPA and MGMT expression [118, 119].

As discussed, a great deal of information exists on in vitro resistance mechanisms associated with CPA or IFO chemotherapy. However, the importance of these factors in mediating clinical resistance remains unresolved, thereby preventing the development of strategies to restore the efficacy of alkylating chemotherapy.

Resistance to Platinating Agents

Cisplatin was discovered serendipitously by Rosenberg in the mid-1960s and rapidly ascended to a prominent role in cancer therapy. It was originally used in the treatment of lung, oesophageal, urothelial and ovarian cancers. Unfortunately, despite an initial reduction in size, tumours frequently returned in a highly resistant form. Today, cisplatin remains an important drug in the treatment of a number of solid tumours. The clinical efficacy of cisplatin has resulted in many years of development of literally thousands of analogues and chemical derivatives. Rather surprisingly, only two compounds (carboplatin and oxaliplatin) have entered widespread clinical use. Cisplatin and carboplatin are currently used predominantly in ovarian, bladder and testicular cancers, and the primary clinical use for oxaliplatin is for colorectal cancer. The cytotoxicity of cisplatin in cancer cells is based on the formation of intrastrand DNA crosslinks. A wealth of data exists on the molecular mechanisms of cisplatin-induced DNA damage, and the reader is directed to a thorough review by Jamieson and Lippard

[120] on the topic. Cisplatin resistance is a complex and multifactorial process; the main mechanisms are discussed below.

Repair of DNA-Platinum Adducts

When cisplatin-resistant cancer cells are selected through culture in the presence of the drug, a common feature of surviving cells is elevation in DNA repair capacity. Moreover, cisplatin-resistant tumour samples display increased expression of the ERCC1 protein, which is involved in the NER pathway. The efficiency of NER in removing platinum-DNA adducts suggests that this is the major repair pathway for this type of lesion. The key role of NER in the removal of platinum adducts is demonstrated by the correlation between NER activity and resistance to cisplatin in ovarian cancers [121–123].

There is also a considerable amount of preclinical data on the ability of MMR to recognise and repair DNA adducts generated by oxaliplatin and cisplatin. Several clinical studies have confirmed the importance of MMR-based repair through correlation of its capacity with the cisplatin response in NSCLC [124]. Elevated MMR status in colorectal and ovarian cancer has also been associated with increased patient survival times and clinical outcome [125, 126].

Transport Systems and Cisplatin Resistance

The three transporters consistently associated with drug resistance in cancer are ABC^{B1}, ABC^{C1} and ABC^{G2}. However, there is no clear evidence to suggest that any of these transporters are capable of mediating the efflux of cisplatin from cancer cells. There are no unequivocal correlations between ABC transporter expression with either outcome or prognosis [127, 128] for patients on platinum chemotherapy. ABC^{C2} (a.k.a. MRP2) was proposed a potential mechanism of resistance [129]; however, in ovarian cancer its expression was only observed in a subset of primary tumours and was not correlated with clinical response.

There does appear to be a definite role for transport systems in the resistance of cancer cells to platinum-based chemotherapeutics. The protein responsible is not a drug transporter, but the endogenous copper transport protein ATP7B. The link between ATP7B expression and cisplatin efficacy was first observed in a prostate cancer cell line selected for cisplatin resistance [130]. Increased expression of ATP7B reduced the cytotoxic potency of cisplatin, and transfection of 'null' cells with the protein conferred resistance. The mechanism of ATP7B-mediated resistance in cancer cells is via cisplatin efflux [131, 132]. Clinical investigations demonstrated elevated mRNA levels of ATP7B in ovarian [133], gastric [134] and breast cancer [135] tissue samples. Recent investigations suggest that the level of ATP7B expression is a prognostic marker in human endometrial [136] and ovarian [133] carcinoma.

Detoxification of Platinum Complexes

Platinum complexes, particularly in the Pt^{2+} ionic state, are notoriously reactive species and are rapidly inactivated via formation of adducts with cellular macromolecules.

MT is a low-molecular-weight protein containing multiple cysteine residues that chelate a number of metals. The protein functions to control cytosolic concentrations of trace elements (e.g. Zn and Cu) and to sequester toxic metal species (e.g. Cd and Hg). Every MT monomer can react with up to 5 molecules of cisplatin [72], and it is therefore a potent mechanism of reducing the cellular pool of free cisplatin. The relationship between MT expression and cisplatin sensitivity has been clearly demonstrated in cultured cells [137], and there is evidence in support of this association from clinical studies. MT expression is elevated in oesophageal, ovarian, ductal mammary tumours and adenocarcinoma of the large intestine [73, 138–140]; and moreover, correlations between expression and the resistant phenotype have been observed in both oesophageal [73] and urothelial [128] cancers. The ovarian cancer study indicates that increased expression of MT is associated with a poor prognosis. Further, the subcellular localisation of MT in ovarian tissue is an important factor in this association. There was no relationship between clinical parameters and cytosolic MT expression, but nuclear localisation generates a clear protective effect against cisplatin chemotherapy [75].

GST is a cytosolic enzyme that mediates deactivation of highly reactive electrophiles (e.g. drugs and lipid peroxidation products) by conjugation with GSH. The GST enzyme, in particular the π-isoform, mediates general cellular detoxification and has been proposed as a cisplatin resistance mechanism [141]. GST-π amplification has been observed in ovarian and squamous neck carcinomas, and increased expression appears to correlate with cisplatin resistance in vivo, but the magnitude and prognostic significance remain controversial [127, 141–143].

Apoptotic Signalling of Cisplatin-Induced DNA Damage

Following the production and detection of cisplatin-DNA adducts, inadequate or unsuccessful DNA repair

leads ultimately to apoptosis. As already discussed, the transcription factor p53 has a key role in monitoring DNA integrity, co-ordinating repair and mediating stress-induced apoptosis. p53 is mutated in greater than 50% of late-stage ovarian tumours [144], a disease that is managed by cisplatin-based chemotherapy. Despite numerous studies, no clear relationship between p53 status and cisplatin resistance has emerged for ovarian cancer. The inability to establish a clear relationship may result from the heterogeneity and cell type specificity of p53 mutations and the contributions of other resistance mechanisms, therefore rendering p53 status alone a poor predictor of cisplatin efficacy in ovarian tumours.

Alterations in the expression or activities of apoptotic mediators (e.g. Bcl-2, AKT, Fas-L) has been shown to influence cisplatin sensitivity [145–148]. Moreover, manipulating the expression levels of these proteins represents a potential chemosensitisation approach. For example, chemical inhibition of X-linked inhibitor of apoptosis overcomes cisplatin resistance in several cancer cell lines [149]. The precise role individual apoptotic mediators play in cisplatin resistance requires further examination, particularly in the clinical setting. Moreover, a greater understanding of the molecular pathways involved in mediating cisplatin resistance in tumours is likely to reveal novel therapeutic avenues.

Resistance to Mitotic Inhibitors

Taxanes

The taxane class of chemotherapeutic drugs is derived from extracts of the European yew tree. The two most clinically important compounds in this group are paclitaxel and the second-generation derivative docetaxel. Both of these compounds are classified as antimitotic agents due to their ability to inhibit replication by perturbing mitotic spindle formation [150, 151]. Mitotic spindles are formed by the polymerisation of heterodimers of α- and β-tubulin. Paclitaxel and docetaxel bind to β-tubulin subunits, which results in microtubule stabilisation and extension of the microtubule polymer [152, 153]. This prevents depolymerisation of the spindles and results in cell cycle arrest and, ultimately, apoptosis.

Since their inception as anticancer drugs, the taxanes have been widely used in the treatment of solid tumours of the ovary, prostate, head and neck, lung, breast and malignant melanoma [154]. The use of taxanes in breast cancer, either as a single agent or in combination with other anticancer drugs, has been a mainstay of treatment since the 1980s [155, 156]. In particular, taxanes are useful as adjuvant therapeutics for operable metastatic breast cancer [157, 158]. Docetaxel steadily assumed a greater prominence and is now preferred over taxol for breast cancer treatment [159]. Taxanes have been incorporated into numerous combination regimes, with the most efficacious partner being CPA. The docetaxel-CPA combination has been widely compared to the doxorubicin-CPA regime and is now associated with improved disease-free survival and considerably reduced cardiotoxicity, an inherent complication associated with anthracycline treatment [159].

Despite the clear benefits associated with taxane chemotherapy, the problem of clinical resistance is significant. For example, response rates to docetaxel are in the region of 30–50% in metastatic breast cancer [160], and more than 75% of women with ovarian cancer undergo relapse from remission following chemotherapy. The acquired and inherent taxane resistance exhibited by cancer cells has been extensively investigated in vitro, with the two most prominent mechanisms being reduced cell accumulation and target alteration. Reduced cellular accumulation has been observed for both paclitaxel and docetaxel in cultured cancer cells and the transporters ABC^{B1}, ABC^{B11} (bile salt export pump) and ABC^{C1} have been implicated [66, 161, 162]. A relationship between ABC^{B1} expression and reduced docetaxel accumulation/sensitivity has been established for numerous cell lines and is supported by the ability of inhibitors such as verapamil and cyclosporin A to restore efficacy [163, 164]. Based on these in vitro observations, a number of clinical trials have focused on the restoration of docetaxel sensitivity through inhibition of ABC^{B1}. First- and second-generation ABC^{B1} inhibitors generated either significant toxicity or pharmacokinetic interactions that resulted in dose reduction of the chemotherapeutic agent [165, 166]. Unfortunately, the most promising of the third-generation inhibitors also yielded poor results at the clinical trial stage. For example, the tariquidar-docetaxel combination failed to increase docetaxel efficacy. A further ABC^{B1} inhibitor, elacridar, developed to improve CNS penetration of docetaxel as a means of treating brain tumours, unfortunately increased the systemic levels of docetaxel and reduced clearance of the drug in phase I trials [167]. These disappointing results have highlighted our lack of understanding concerning the precise contribution of ABC^{B1} to clinical drug resistance. It has also cast doubt over the validity of targeting this and other cancer-associated ABC transporters as an approach to improve chemotherapeutic efficacy.

Alterations in the expression levels or activity of a particular drug target are a major factor contributing to chemoresistance. In the case of taxane resistance, the primary alteration involves mutation or altered expression of the various β-tubulin protein isotypes [168–170]. X-ray crystallographic data have revealed the impact of β-tubulin mutations on taxane binding and the stability of tubulin polymers [169, 171]. The relevance of mutations or altered expression levels of β-tubulin to resistance in the clinical setting remain the subject of controversy. The β-tubulin gene exhibits remarkable evolutionary conservation, and very few polymorphic variations are observed in humans [172]. The lack of β-tubulin mutations in tissue obtained from paclitaxel-resistant lung, ovarian and breast tumours [173, 174] fails to support any involvement of β-tubulin mutations in the taxane-resistant phenotype.

Clearly, the question of causative factors in clinical resistance to taxane chemotherapy remains unresolved. ABC^{B1} does seem to be a significant factor but circumventing its actions appears problematic. With these issues in mind, the development of a new taxane derivative that was not a substrate for ABC^{B1} was greeted with considerable optimism. DJ-927 (tesetaxel) displayed increased solubility and bio-availability, affording the advantage of oral delivery [175]. Critically, this drug was as effective in cells that expressed ABC^{B1} as in the drug-sensitive parental lines. Unfortunately, development of the compound has ceased due to an inability to demonstrate a clear benefit over existing taxanes in the treatment of colorectal and gastric cancer. Despite this, the principle of designing anticancer drugs that evade multidrug efflux pumps remains a plausible alternative to transport inhibition strategies.

Vinca Alkaloids

Four vinca alkaloids (vinblastine, vincristine, vinleurosine and vinrosidine) were originally extracted from the periwinkle plant *Catharanthus roseus,* and their potential for anticancer activity was demonstrated in 1958 [176]. Vinblastine and vincristine were fast tracked into clinical studies and remain important chemotherapeutic compounds to this day. In contrast to the actions of taxanes, vinca alkaloids work by altering tubulin dynamic interactions to increase the rate of microtubule depolymerisation [177]. Microtubule disassembly leads to cell cycle arrest in metaphase and to apoptosis. Vincristine is primarily used in acute lymphocytic leukaemia (ALL), Hodgkins and non-Hodgkins lymphoma, but has also been included in combination chemotherapy for multiple myeloma, breast cancer, small-cell lung cancer (SCLC) and several childhood solid tumours [176]. Vinblastine is mainly used to treat advanced Hodgkins lymphoma and testicular, bladder and breast cancers. These two vinca alkaloids differ structurally by only a single methyl substitution in the vindoline ring, yet they display remarkably different activities and toxicity profiles [178, 179].

The natural vinca alkaloids and two synthetic derivatives, vindesine and vinepidine, have been thoroughly investigated for effects on microtubule dynamics and proliferation in vitro using cultured cancer cell lines. Vinepidine and vincristine are the most potent inhibitors of tubulin assembly, whereas vindesine and vinblastine share a similar, lower potency [180]. Animal studies revealed that vinepidine was less rapidly metabolised than vinblastine or vincristine and that this property contributed to low clearance of the drug [181]. In the late 1980s, clinical development of vinepidine was discontinued, primarily due to an unacceptable toxicity profile. In contrast, development of vindesine progressed at pace, and the compound is considered one of the most active compounds in the treatment of locally advanced NSCLC [182–184]. Application of vindesine in combination therapy with either cisplatin or mitomycin C demonstrates the greatest response in NSCLC [182].

Further drug development programmes resulted in the generation of two highly promising second-generation vinca alkaloids, vinorelbine and vinflunine. Vinorelbine was synthesised by modification of the catharanthine nucleus, and it demonstrated antiproliferative activity in cultured cells [185, 186]. The clinical development of vinorelbine established the drug's utility both in single-agent therapy and as a component of combination regimes, for NSCLC, lymphoma, breast and ovarian cancer [187–189]. For example, in advanced NSCLC, vinorelbine produces an approximately 30% response with a median survival of 33 weeks [190], and combinations with either cisplatin [191] or docetaxel [192] are more efficacious than when these drugs are used without the vinca alkaloid. Vinorelbine has been applied successfully in the first-line treatment of metastic breast cancer since the early 1990s [193], although its main use is as salvage therapy following the failure of anthracyclines and taxanes, particularly in the elderly [194, 195]. In combination therapy, the best results are achieved when combined with anthracyclines, where response rates of 38–77% have been observed [194, 195].

Vinflunine is a bifluorinated derivative of vinorelbine [196] and is the latest vinca alkaloid to reach the clinic. Although vinflunine initiates apoptosis by inhibiting mi-

totic spindle formation [197], its effects differ subtly from those of established vinca alkaloids [198, 199]. Interestingly, the strength of the interaction between vinflunine and β-tubulin is weaker than for several established vinca alkaloids, yet it demonstrates the greatest intracellular accumulation and cytotoxic potency [for a review, see 197]. The high efficacy of vinflunine in a breast tumour xenograft model [176] prompted its evaluation in phase I studies for metastatic breast cancer, wherein it produced encouraging results [200]. A recent phase II trial supported the use of vinflunine in metastatic breast cancer, particularly as a second-line treatment following anthracycline/taxane chemotherapy [196].

A major mechanism of resistance to vinca alkaloids appears to be the presence of one or more multidrug efflux pumps. All of the vinca alkaloids examined to date have been demonstrated to interact directly with ABC^{B1}. In addition, the related transporter ABC^{C1} is able to mediate the translocation of vincristine [201–203]. The evidence for a role of multidrug transporters in resistance in vitro is unequivocal; however, the situation in the clinical setting is rather less clear.

One possible means of overcoming resistance to vinca alkaloids is to generate compounds that are not recognised by the transporters. During pre-clinical evaluation of vinflunine it was reported that resistance to the drug in cell lines was conferred specifically by ABC^{B1}, but that the degree of resistance was considerably lower compared to other vinca alkaloids [204]. Long-term exposure of cancer cells to vinca alkaloids has been used to generate drug-resistant cell lines that express multidrug efflux pumps. This long-term selection was also undertaken in the presence of vinflunine and the process compared to that for vinorelbine [205]. The investigation demonstrated that the time required for drug resistance to emerge was significantly longer in the presence of vinflunine than for vinorelbine in both P388 leukaemia and A549 lung cancer cells. These findings have considerable clinical implications with respect to the efficacy of vinflunine; however, there are no data available on the time course for the initiation of drug resistance following exposure to the drug in vivo.

Resistance to Antimetabolites

The high proliferation rate in cancer exerts a significant demand on the cellular biosynthetic pathways, in particular those related to production of nucleotides. One of the key steps in pyrimidine biosynthesis is the reductive methylation of deoxyuridine-5′-monophosphate (dUMP) to deoxythymidine-5′-monophosphate (dTMP). The reaction is catalysed by TS and requires the cofactor THF to donate a formyl group to dUMP and releasing dihydrofolate (DHF). The reaction requires a constant supply of THF, which is generated from DHF in a reaction catalysed by DHFR. Unsurprisingly, both TS and DHFR have been considered attractive targets in the design of chemotherapeutic agents. Methotrexate and 5-FU are widely used in numerous clinical applications but unfortunately their usage has been compromised by resistance pathways.

Methotrexate

The chemotherapeutic agent methotrexate is a structural analogue of THF. Methotrexate is able to inhibit the DHFR enzyme by a high-affinity, but reversible, competitive mechanism. This in turn results in elevated levels of DHF and, more significantly, inhibition of TS and reduced nucleotide biosynthesis. The primary clinical utilisation of methotrexate is in the treatment of lymphoma, choriocarcinoma and ALL. The success of this compound is best illustrated by a 70% cure rate in childhood ALL [206]. In addition, methotrexate is an important component of many combination chemotherapy regimes used in metastic breast, advanced bladder and gastric cancer. It also plays a role in the adjuvant regime with CPA, methotrexate and 5-FU for operable breast cancer.

Methotrexate therapy is associated with drug resistance, and in vitro studies have revealed that numerous mechanisms may be responsible for the phenotype. Folates are relatively hydrophilic molecules, thereby requiring specific *membrane translocation systems* to maintain sufficiently high intracellular concentrations. The first and most important mechanism is via an energy-dependent bidirectional membrane transporter known as the reduced folate carrier (RFC). The RFC (i) displays higher affinity for reduced folate cofactors compared to folic acid per se [83], (ii) is ubiquitously expressed in normal and cancer cells and (iii) expression is regulated in these tissues by the cellular folate status. Moreover, the affinity of methotrexate for transport by the RFC is similar to that reported for folate cofactors. The levels or activity of this transporter are linked to the efficacy of methotrexate and other antifolates. Folates or antifolate chemotherapeutics bind to the folate receptor (FRα or FRβ) at the plasma membrane and can also enter cells via an endocytotic process that involves clathrin- or caveolin-linked vesicles [207].

Once inside the cell, folate and its analogues are rapidly trapped as polyglutamates in order to maintain a high intracellular pool of these critical molecules.

Polyglutamation

Polyglutamation involves the sequential addition of glutamate residues to a chain length of 5–8 residues, thereby increasing polarity of the folate to prevent diffusion out of the cell. The polymerisation is catalysed by the cytosolic enzyme FPGS [208] and the terminal glutamates are removed by γ-glutamylhydrolase (GGH) [209], which is a lysosomally located enzyme. Methotrexate is also a low-affinity substrate for polyglutamation by FPGS and is therefore a competitive inhibitor to endogenous folates for this enzyme. Moreover, the polyglutamated methotrexate derivatives display higher affinity for inhibition of the DHFR enzyme, and the decreased polymerisation by FPGS may therefore contribute to cellular drug resistance. Similarly, increased levels of GGH would also be expected to contribute to resistance against methotrexate, and a correlation has been observed for sarcoma cell lines [210]. However, one of the consequences of elevated GGH expression/activity is a concomitant reduction in the cellular pool of reduced folate cofactors, which would reduce the rate of proliferation in cancer cells, thereby augmenting chemotherapy. Thus, the role of this enzyme in a resistant phenotype is complex. This complexity is highlighted in the results of in vitro studies. For example, transfection of MCF7 cells with GGH caused significantly elevated expression but had no impact on the resistance to methotrexate [211]. In contrast, rat hepatoma cell lines selected for resistance to lometrexol displayed increased GGH activity and a large drop in methotrexate polyglutamates [212, 213]. However, there was no impact of the increased GGH activity on the total cellular folate pool.

Target Alteration

The primary intracellular target for methotrexate is the DHFR enzyme, and gene amplification for this protein was first reported 30 years ago [214]. The gene amplification ultimately results in elevated expression of DHFR, and this is frequently observed in cells selected for drug resistance in the presence of methotrexate. Increased expression of the enzyme will necessitate administration of higher methotrexate doses to ensure that the concentration of THF is maintained at a sufficiently low level. In addition to gene amplification, resistance to methotrexate in tumour cell lines has also been linked to altered affinity of DHFR for the drug [214]. The reduced activity is caused by a number of mutations to DHFR (e.g. L22R, G15W, F31W) [215–217]. However, resistance-inducing mutations in DHFR rarely affect normal function of the protein. Indeed, the L22R DHFR mutation results in a 270-fold lower binding of methotrexate but only a 3-fold change in the production of THF from endogenous DHF [218].

Other potential mechanisms of resistance to the antifolate methotrexate including (i) active efflux of polyglutamated derivatives by the ABC transporters $ABC^{C1–C5}$ and ABC^{G2} or (ii) an expanded cellular folate pool [for a review, see 219]. However, the contributions of the RFC, DHFR and FPGS dominate the preclinical observations, but do they also translate into clinical resistance?

Clinical Observations

Clinical observations suggest that both intrinsic and acquired resistant phenotypes operate against antifolates [220]. Resistance pathways have been most extensively investigated in acute myelogenous leukaemia, which presents as intrinsically resistant, and ALL, which is usually initially sensitive to methotrexate treatment. Comparison of patient blast cell samples from the two sets of patients indicates that the predominant resistance mechanism is defective polyglutamation of methotrexate [221, 222]. The ability to form methotrexate polyglutamates has also been identified as a predictor of outcome in specific subsets of childhood leukaemia [223]. Similar data from solid tumours are scarce. Nonetheless, in cells obtained from soft tissue sarcoma and grown as primary cultures, it has been shown that reduced polyglutamation of antifolates is responsible for 12- to 15-fold resistance [224].

Acquired methotrexate resistance (i.e. after chemotherapy) displays a more multifactorial resistance profile, particularly involving altered transport and DHFR expression or activity [225]. A study of patients with ALL relapse indicated low-level DHFR amplification, but the major resistance mechanism was impaired accumulation of methotrexate [226]. The molecular basis for the latter mechanism appears to be mutation in the DNA encoding the RFC, at least in osteosarcoma [227]. Several clinical investigations focusing on ALL [228] and osteosarcoma [229] correlate the resistance to methotrexate with reduced expression of RFC rather than specific mutations. The importance of altered DHFR expression or activity has been demonstrated in numerous cancer cell lines and one study comparing childhood leukaemia types indicates higher levels of the enzyme in ALL [230]. However, this study also demonstrated that levels of DHFR expres-

sion in ALL samples displayed considerable heterogeneity, which may account for the difficulty in attributing a clear role for this protein in clinical resistance.

5-Fluorouracil

5-FU is a fluorinated pyrimidine nucleotide base analogue that is used primarily in metastatic colorectal cancer. More specifically, the drug is utilised in adjuvant therapy for the approximately 50% of patients that relapse following surgical removal of the primary tumour [231]. The recurrence rate is significantly reduced in the presence of 5-FU; however, 30% of patients receiving the drug relapse within 5 years of treatment.

5-FU inhibits the cytosolic enzyme TS, which catalyses the rate-limiting step in pyrimidine deoxynucleotide biosynthesis. The precise step catalysed is the reductive methylation of dUMP to dTMP, and the TS enzyme requires the cofactor THF. The latter forms a ternary complex between dUMP and TS during the reaction sequence. 5-FU is in effect a prodrug that requires conversion to the active metabolite FdUMP. The FdUMP also forms a ternary complex with THF and TS, in direct competition with the endogenous substrate dUMP. However, the complex formed by FdUMP cannot dissociate due to the inability to transfer the methyl group of THF, and the TS enzyme is in effect inhibited. Ultimately this leads to a build-up of dUMP with a resultant depletion of dTMP/deoxythymidine triphosphate, thereby interfering with the process of DNA replication. In addition, 5-FU may lead to cytotoxicity by direct incorporation of the metabolite fluorodeoxyuridine triphosphate into DNA, which results in miscoding and cell death.

The clinical efficacy of 5-FU is often compromised due to inherent or acquired resistance pathways. Preclinical investigations using cultured colon cancer cell lines demonstrated that long-term exposure of cells to 5-FU rapidly led to the emergence of a resistant phenotype [232–234]. The mechanism of resistance was attributed to gene amplification and overexpression of the TS enzyme. Increased cellular TS levels require higher concentrations of 5-FU to perturb nucleoside synthesis and generate cytotoxicity. This mechanism of resistance has been widely examined in the clinical setting. When surgically resected colorectal cancer tissue was examined for TS mRNA, a correlation was noted between expression levels and 5-FU sensitivity [235]. In addition, the expression of TS (protein and/or mRNA levels) provides a strong prognostic and predictive marker for 5-FU chemotherapy in colorectal cancer patients [236, 237].

Gemcitabine

Gemcitabine, or 2,2-difluorodeoxycytidine (dFdC), is a difluorinated synthetic analogue of deoxycytidine, thereby also belonging to the antimetabolite class of anticancer drug. dFdC is a prodrug that requires sequential phosphorylation by deoxycytidine kinase (dCK) to generate the active species dFdC triphosphate. The active phosphorylated derivatives of dFdC interact with numerous cellular targets, and the mechanism of cytotoxicity is complex. dFdC triphosphate is incorporated into DNA causing single-strand damage, inhibition of ribonucleotide metabolism, hindered DNA processing, intrastrand adducts and interstrand cross-links. This culminates in G_1 phase growth arrest and the incorporated base, dFdC monophosphate, cannot be excised by DNA exonuclease. There are a number of intracellular inactivation mechanisms including deoxycytidine deaminase (dFdC monophosphate → difluorodeoxyuridine) or deoxycytidine monophosphate deaminase (dFdC monophosphate → dFdUMP → difluorodeoxyuridine), both of which generate difluorodeoxyuridine, which is then excreted. The partially phosphorylated metabolite dFdC diphosphate inhibits the enzyme ribonucleotide reductase, which generates deoxyribonucleotides (nucleoside diphosphate → deoxynucleoside diphosphate) and thereby controls the cellular deoxynucleoside triphosphate pool. Inhibition of the enzyme causes elevated deoxycytidine triphosphate concentrations, thereby reducing the feedback inhibition of dCK and increasing the rate of dFdC phosphorylation.

The primary clinical usage of dFdC is in locally advanced or metastasized NSCLC, bladder cancer and ovarian cancer. These cancers are treated with combination therapy, usually involving platinum drugs or taxanes, whereas single-agent efficacy is reported for adenocarcinoma of pancreatic cancer. Ovarian cancer displays a 60–80% response rate to standard platinum/taxane regimes; however, this success is tempered by the development of chemoresistance in greater than 80% of cases. However, dFdC has shown considerable efficacy in platinum/taxane-resistant ovarian cancers, and a number of phase II clinical studies demonstrate 13–30% response in salvage therapy. In addition, the single-agent efficacy in metastatic breast cancer is reflected by 37% response in first-line, 26% in second-line and 18% in third-line therapy. Moreover, combination therapy with taxanes, vinca alkaloids, platinum drugs or triple therapies (dFdC/anthracycline/paclitaxel) display 58–92% response. The reported clinical efficacy, combined with a relatively mild toxicity profile has led to the increased use of dFdC in cancer treatment.

The most commonly observed dFdC resistance mechanism in cultured cell lines is a deficiency in dCK, thereby preventing activation of the drug. dFdC entry to cells occurs via nucleoside transporters, and reduced membrane influx has been implicated based on studies using tissues derived from pancreatic cancers [238]. Various studies have failed to correlate dFdC resistance with dCK activity or expression levels. Based on expression array analyses, the primary resistance marker in these systems is the expression of ribonucleotide reductase, in particular the M_1 subunit [239–242]. Interestingly, multidrug efflux pumps are not associated with resistance, and, in fact, cells expressing these proteins actually display increased sensitivity (collateral sensitivity) to dFdC.

Resistance to Topoisomerase Inhibitors

Camptothecins

Topoisomerase I plays a major role in the replication process by virtue of relaxing DNA supercoiling through induction of single-strand breaks. The protein is located at supercoiled regions of DNA in association with transcription and replication complexes [243]. The process does not require energy and topoisomerase I religates the strand breaks following relaxation [243]. Whilst topoisomerase II inhibitors have a long history in chemotherapy, the first topoisomerase I inhibitor did not enter clinical trials until the mid 1970s [244]. It was not until the mid 1980s [245] that topoisomerase I was identified as the target of the lead compound camptothecin, derived from an extract of the plant *Camptotheca acuminata* [246]. Camptothecin generated excessive non-specific toxicity and therefore did not progress significantly in clinical trials [247]. Further mechanistic, pharmacokinetic and chemical data led to the development of two clinically useful drugs, topotecan (Hycamptin) and irinotecan (Camptosar). These camptothecins do not disrupt the interaction of topoisomerase I with DNA or the subsequent strand breakage [246, 248]. Their primary effect is to stabilise the DNA-topoisomerase-I complex, which results in the accumulation of single-strand breaks. These lesions are not intrinsically toxic and are readily reversed upon removal of the topoisomerase I inhibitor [249]. The mechanism of cell cytotoxicity is thought to be due to 'collision' of the replicating DNA fork with the DNA-topoisomerase-I complex [250]. This ultimately produces double-strand breaks leading to the initiation of apoptosis.

A major limitation to the efficacy of camptothecins is their relatively low affinity interaction with the topoisomerase-I-DNA complex. The drugs rapidly dissociate as the local drug concentration decreases, and therefore, prolonged exposure such as a continuous infusion is required to attain sufficient cytotoxicity [249]. Camptothecins also generate unwanted side-effects [251, 252], the primary being leucopenia where a dose reduction is indicated. A final limitation to camptothecin chemotherapy is the propensity of the lactone ring to be chemically modified to a carboxylate. The carboxylate derivatives do not interact with topoisomerase I and bind tightly to serum albumin, thereby reducing the biodistribution of parent compounds. As a result, synthetic derivatives are being developed with greater stability in this moiety [253].

The major clinical indication of topotecan is in ovarian cancer, particularly in patients displaying resistance to standard chemotherapy [254]. First-line therapy of ovarian cancer is dominated by platinum and taxanes; therefore topotecan is primarily used in salvage therapy [255]. Topotecan is also beneficial in recurrent SCLC with similar efficacy to the CPA-doxorubicin-vinblastine combination [256]. Haematological cancers also respond well to topotecan, and complete response rates have been reported in 27–37% of chronic myelomonocytic leukaemia and myelodysplastic syndromes [257]. In contrast, the drug displays limited efficacy (i.e. <10% response) in CNS, breast and colorectal cancers [258–260].

The major clinical indication of irinotecan is colorectal cancer with single-drug response rates of 10–35%, which includes those tumours displaying resistance to 5-FU [261]. Irinotecan is being increasingly used in SCLC, NSCLC, ovarian and cervical cancers both with and without prior chemotherapy. The combination of irinotecan and 5-FU in colorectal cancer produces greater response rates, progression-free survival and overall survival [262].

Numerous investigations have been undertaken on resistance mechanisms against topoisomerase I inhibitors, but almost exclusively these studies are based on in vitro data. The dominant mechanism of camptothecin resistance is through ABC transporter expression. Camptothecins do not appear to be substrates of ABC^{B1} [263]; however, both ABC^{C1} and the half-transporter ABC^{G2} mediate resistance to these compounds in cultured cells [for a review, see 255]. The latter protein has been correlated with resistance to topoisomerase I/II inhibitors in a number of cell lines, and given their clinical indications; the most noteworthy findings have been reported in

ovarian cell lines resistant to topotecan and in lung cancer cells resistant to irinotecan. Human lung cancer cell lines resistant to irinotecan also display reduced expression of carboxylesterase, which is required to activate irinotecan to SN-38 [264]. However, hepatic carboxylesterase is likely to dominate in vivo activation, and low intratumoral enzyme levels are unlikely to contribute significantly to the phenotype [265]. Reduced topoisomerase I expression also correlates with camptothecin efficacy in cell lines [266], and this is thought to arise due to hypermethylation of the promoter [267]. However, the relationship between topoisomerase I levels and chemotherapy outcome has not been confirmed in clinical samples to date. Similarly, mutations in topoisomerase I that lead to reduced activity or affinity of camptothecin binding have been observed in cell lines, but not reported in patient samples.

There is very little available evidence or information on the primary clinical resistance mechanisms. Thus, the proposed pathways in cell lines should be cautiously viewed in terms of clinical significance.

Anthracyclines

The anthracyclines were initially developed as antibiotics; however, their high levels of inherent toxicity precluded their clinical use in this context. Compared to other classes of chemotherapeutic agents, anthracyclines perhaps display the widest spectrum of activity in the clinical setting. In 30 years of use in oncology, very few types of cancer (e.g. colon) have been demonstrated to be unresponsive to anthracycline drugs. The two most prevalent compounds in this class are doxorubicin and daunomycin, which differ by a single hydroxyl moiety. Daunorubicin is used primarily in haematological malignancies, in particular ALL. Doxorubicin is primarily used to treat solid tumours, primarily breast, NSCLC, uterine and ovarian cancer. The use of doxorubicin in NSCLC is targeted towards inoperable or locally advanced tumours. The overall response rates are in the range of 20–40%, with the remainder appearing to display an inherent resistance. Doxorubicin use in breast cancer is primarily directed towards hormonally insensitive tumours and was first used in this capacity in the 1970s. The drug is usually administered in conjunction with alkylating agents, antimetabolites and taxanes. When used as a single agent for first-line therapy, the response to doxorubicin varies in the range of 35–50%, and in combination with alkylating drugs, the response improves to 50–80%, with a median survival of 17–25%.

The extensive use and wide clinical applicability of anthracyclines has sparked enormous interest in developing novel synthetic analogues. To date, more than 300 biosynthetic compounds have been generated from chemical modification studies on established anthracyclines. However, few of these have reached worldwide clinical usage with the two main exceptions being epirubicin and idarubicin. Many of the derivatives display similar activity to doxorubicin or daunomycin but are frequently associated with increased non-specific toxicity. In other cases, the novel analogues display lower toxicity than the established anthracyclines but with a concomitant reduction in anticancer activity. As a consequence, doxorubicin and daunomycin remain the mainstays of anthracycline-based chemotherapy regimes.

The accepted main mechanism of cytotoxicity of anthracyclines is via inhibition of the topisomerase II enzyme. The topoisomerase II enzyme plays a crucial role in the replication process by reducing the twisting and supercoiling of DNA through the introduction of a local double-stranded DNA break. Once the duplex has been 'untangled', the topoisomerase II dissociates to facilitate repair of the strand break. Anthracyclines avidly intercalate into DNA and can produce a stable tertiary complex with the DNA and topoisomerase II enzyme. The stability of this complex results in a slower dissociation and prevents strand religation. The persistence of the double-strand break initiates a cascade of events that triggers cell death via apoptosis. Anthracyclines are also capable of generating highly reactive free radicals via an enzymatic route through mitochondrial oxidation or non-enzymatically by iron [268]. These free radical species can cause extensive cell damage, which is beneficial if directed at the tumour but also results in side-effects. Particularly sensitive to the anthracycline radicals are cardiac myocytes, damage to which can result in debilitating cardiotoxicity.

Resistance to anthracyclines in cultured cell lines was initially attributed to the expression of the multidrug efflux pump ABC^{B1}. As investigations proceeded to more cell types it became clear that whilst accumulation deficits played a major role in the resistant phenotype, they could not always be attributed to ABC^{B1}. This facilitated the discovery of ABC^{C1} and, more recently, ABC^{G2} as drug efflux pumps contributing to anthracycline resistance. However, the controversy of the applicability of in vitro observations to the clinical situation hindered the elucidation of a precise mechanism(s) of resistance to anthracyclines. Trock et al. [269] analysed 31 distinct clini-

cal studies on this issue in breast cancer and observed that (i) ABC^{B1} expression is associated with chemotherapy in breast cancer, (ii) ABC^{B1} expression is linked to poor response to chemotherapy and (iii) the protein contributes differently to the resistance profile, depending on the type of breast cancer. A similar correlation between outcome and ABC^{B1} expression was observed in acute myelogenous leukaemia patients [270]. In contrast, in osteosarcoma no relationship between ABC^{B1} expression and resistance to neoadjuvant chemotherapy was found [271].

It is clear from clinical trials that either ABC transporter expression is not a universal mechanism of anthracycline resistance or that alternative resistance pathways are often predominant in cancer cells. Low topoisomerase II expression is associated with anthracycline resistance in lung cancer cell lines [272]. Moreover, the altered expression of topoisomerase II can be inherent or arise as a consequence of drug exposure. Point mutations in topoisomerase II have been described in resistant cancer cell lines, in particular mutations within the ATP binding motif and a critical tyrosine at position 804 [273]. A systematic study in several cell lines reveals the possibility of a co-ordinated response between topoisomerase II and efflux pump mechanisms. Reduced topoisomerase II expression often occurs early in the selection process, and expression of ABC^{C1} appears to confer a high level of resistance. Moreover, the elevation in ABC^{C1} levels enables some recovery in topoisomerase II activity [274].

Despite few clinical trials relating topoisomerase II expression to anthracycline sensitivity, a definite correlation has been shown in both ovarian and endometrial carcinomas [275, 276]. In NSCLC the expression of topoisomerase II was not correlated with response to chemotherapy and mutations are rarely observed [277], although a small study in SCLC found point mutations in 1 of 13 subjects following prior exposure to topoisomerase II inhibitors [278]. Breast tumour cells exhibiting increased topoisomerase II expression following chemotherapy may be associated with relapse and resistance [279]. However, despite this finding and the extensive in vitro evidence of a correlation, topoisomerase IIα expression alone is unlikely to predict response to chemotherapy in advanced breast cancer [280]. Similarly, a review of the available data by Di Leo and Isola [281] indicates that clinical data have been variable and although an association between anthracycline efficacy and topoisomerase II expression exists, it is not at present firm enough to countenance its use as a predictive marker.

Brief Perspectives in Anticancer Resistance

As outlined in this review, the presence of a resistant phenotype in cancer cells provides a highly efficient, interconnected and synergistic set of pathways to negate the efficacy of chemotherapy. Clearly the oncologist requires some means to overcome drug resistance in order to achieve treatment or palliation of cancer. Data from preclinical or in vitro studies provide a wealth of information on the properties and types of resistant pathways, in addition to the specific effects of defined pathways on specific anticancer drugs. Moreover, a number of strategies have been implemented to overcome the resistant phenotype for selected pathways. However, few of these strategies have made the leap to successful clinical applicability. Based on the information presented in this review, it is apparent that one of the obstacles is determining, for a given class of anticancer drug, which resistance pathway contributes most significantly to human tumours in vivo. In general, this is not available for the majority of drugs, and previous attempts at clinical reversal of drug resistance may not have employed the correct strategy. More exhaustive and systematic attempts to provide this information are essential if chemotherapy is to be fully exploited in cancer patients.

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