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TUMOR AND HOST FACTORS THAT MAY LIMIT EFFICACY OF CHEMOTHERAPY IN NON-SMALL CELL AND SMALL CELL LUNG CANCER

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Abstract

While chemotherapy provides useful palliation, advanced lung cancer remains incurable since those tumors that are initially sensitive to therapy rapidly develop acquired resistance. Resistance may arise from impaired drug delivery, extracellular factors, decreased drug uptake into tumor cells, increased drug efflux, drug inactivation by detoxifying factors, decreased drug activation or binding to target, altered target, increased damage repair, tolerance of damage, decreased proapoptotic factors, increased antiapoptotic factors, or altered cell cycling or transcription factors. Factors for which there is now substantial clinical evidence of a link to small cell lung cancer (SCLC) resistance to chemotherapy include MRP (for platinum-based combination chemotherapy) and MDR1/P-gp (for non-platinum agents). SPECT MIBI and Tc-TF scanning appears to predict chemotherapy benefit in SCLC. In non-small cell lung cancer (NSCLC), the strongest clinical evidence is for taxane resistance with elevated expression or mutation of class III β -tubulin (and possibly α tubulin), platinum resistance and expression of ERCC1 or BCRP, gemcitabine resistance and RRM1 expression, and resistance to several agents and COX-2 expression (although COX-2 inhibitors have had minimal impact on drug efficacy clinically). Tumors expressing high BRCA1 may have increased resistance to platinum but increased sensitivity to taxanes. Limited early clinical data suggest that chemotherapy resistance in NSCLC may also be increased with decreased expression of cyclin B1 or of Eg5, or with increased expression of ICAM, matrilysin, osteopontin, DDH, survivin, PCDGF, caveolin-1, p21WAF1/CIP1, or 14-3-3sigma, and that IGF-1R inhibitors may increase efficacy of chemotherapy, particularly in squamous cell carcinomas. Equivocal data (with some positive studies but other negative studies) suggest that NSCLC tumors with some EGFR mutations may have increased sensitivity to chemotherapy, while K-ras mutations and expression of GST-pi, RB or p27kip1 may possibly confer resistance. While limited clinical data suggest that p53 mutations are associated with resistance to platinum-based therapies in NSCLC, data on p53 IHC positivity are equivocal. To date, resistance-modulating strategies have generally not proven clinically useful in lung cancer, although small randomized trials suggest a modest benefit of verapamil and related agents in NSCLC.

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Keywords

lung cancer; chemotherapy; resistance

1.0 BACKGROUND

1.1 Lung cancer and resistance

Lung cancer is the leading cause of cancer death in the United States and has a 5-year relative survival rate of only 16%¹. Chemotherapy yields response rates of 20–50% in advanced non-small cell lung cancer (NSCLC) and 60–80% in extensive small cell lung cancer (SCLC), but almost all tumors that are not intrinsically resistant rapidly develop acquired resistance, often with broad cross-resistance to other unrelated chemotherapy agents. Alternating chemotherapy agents with differing mechanisms of action does not overcome this resistance².

1.2 Types of resistance

Resistance may be classified in several ways (Table 1). In addition to being intrinsic vs acquired, it may also be classified pharmacodynamically as “active” (due to excess of a resistance factor, analogous to competitive inhibition of drug effect) vs “non-saturable passive” (due to mutation or alteration of a factor, analogous to decreased affinity of a drug for its receptor) vs “saturable passive” (due to deficiency or saturation of a factor required for drug efficacy, analogous to non-competitive inhibition of drug effect)³. Just as dose-response curve shapes reflect whether drug inhibition is competitive vs non-competitive, they may also reflect the predominant type of resistance (Fig. 1)³. Dose response curve flattening at higher drug doses in NSCLC and the failure of bone marrow transplant approaches to impact other epithelial tumors suggests that the major reason for not being able to cure metastatic NSCLC and other epithelial tumors is due to saturable passive resistance (deficiency or saturation of factors required for drug efficacy)⁴.

Resistance may also be classified as “quiescent” (due to lack of cell cycling through a sensitive cell cycle phase) vs “accelerated” (with treatment failure due to rapid repopulation after initial tumor cell killing). Resistance may be classified as genetic (due to selection of tumor cell subpopulations with mutations that render the cells resistant) or it may arise epigenetically (with rapid up-regulation or down-regulation of relevant genes). In genetic resistance, mutations could involve simple point mutations or could be associated with extensive chromosomal changes⁵ including aneuploidy⁶ or with amplification of resistance factors as a result of repeated chromosomal breakage-fusion-bridge cycles⁷. Epigenetic changes could arise due to alterations of DNA methylation⁸.

Resistance may also be classified as arising due to tumor cell characteristics or due to factors in the tumor environment. With few exceptions⁹, in vitro sensitivity testing in lung cancers has done well at predicting resistance with very few false negatives (ie, there have been few patients predicted to be resistant but who responded clinically), while false positives have been relatively common (predicted sensitive but failed clinically)^{10–15}. Cases that are resistant clinically despite in vitro testing predicting sensitivity are potential examples of resistance arising from tumor environmental factors despite intrinsic sensitivity of the tumor cells.

More than one of these classifications may be relevant for a single resistance mechanism. For example, the broad down-regulation of membrane transporters reported previously in cisplatin-resistant hepatoma and cervical carcinoma cell lines¹⁶ would be an example of resistance that is both epigenetic and saturable passive. The development of reversible

senescence¹⁷ and autophagy¹⁸ in response to chemotherapy exposure has characteristics of each of acquired, epigenetic, quiescent and saturable passive resistance. Clinically, quiescent tumor cells may survive the first cycle of chemotherapy, and surviving tumor cells may then undergo rapid epigenetic changes leading to enhanced resistance due to upregulation of active resistance mechanisms and to accelerated repopulation⁴. Alternatively, the epigenetic changes may lead to down-regulation of growth factors, transporters, etc, and saturable passive resistance associated with reversible quiescence⁴.

In preclinical models, a sensitive tumor may also be rendered resistant if a resistant tumor is implanted on the opposite flank¹⁹. While the mechanism of this effect is unknown, one might speculate that it is mediated through cytokines produced by the resistant tumor. Such cytokines could potentially directly impact characteristics of tumors located in other sites, or, alternatively they could have an indirect effect (eg, mediated by their stimulation of release of mesenchymal stem cells from the bone marrow²⁰).

1.3 Resistance factors and normal tissues

Many putative cancer resistance factors are the same factors that normal cells use to protect themselves from oxygen radicals, ingested toxins, radiation, temperature extremes, and other components of a hostile environment. The dependence of normal tissues on these may explain in part our limited ability to successfully and safely counter them sufficiently with resistance modulators to augment sensitivity of tumors to therapy. Adding to the problem is the fact that multiple different resistance factors probably enter into play simultaneously^{21–23}.

1.4 Cross-resistance

The problem is also magnified by the reality that factors that render a tumor resistant to one drug may also simultaneously increase resistance to several other agents. As a result, alternating up to 4 regimens² or combining 6 chemotherapy agents²⁴ with differing mechanisms of action in patients with NSCLC did not appear to improve outcome. In preclinical studies in lung cancer cell lines and xenografts, cross-resistance is seen frequently, but is not universal^{25–33}. While there are no completely consistent patterns, there may be a higher probability of lack of lung cancer cross-resistance for platinum vs taxanes^{25, 27–29} or vs pemetrexed³⁴, or for gemcitabine vs other agents^{26, 30, 33, 35, 36}.

1.5 Implications of correlations of resistance with the host genotype

In addition to tumor cell characteristics being important, the observed relationship between chemotherapy-induced leukopenia and tumor response in SCLC³⁷ and in NSCLC³⁸ supports a role for host factors in treatment outcome. For example, patient genotype for various drug metabolism pathways could alter both drug efficacy and drug toxicity. We will not discuss these further in this paper, but several examples of these have been described in lung cancer patients^{39–41}. However, we will discuss in later sections how host genotype for specific resistance factors impacts therapy outcome, and examples are presented in Table 2. Resistance to chemotherapy may correlate with a resistance factor's gene copy number, gene mutation status, mRNA expression, or protein expression within a tumor, or with host genotype polymorphisms. Little work has been done to correlate host genotype polymorphisms with tumor expression of a gene, but genotype variations may lead to alteration of a factor's enzymatic activity or protein stability or half-life. Hence, altered (increased or decreased) expression of a resistance or sensitivity factor in a tumor could result from increased or decreased production related to gene copy number or due to up-regulation or down-regulation in response to cellular factors, or it could be due to prolonged or shortened protein half-life due either to tumor gene mutations or due to the tumor inheriting from the host a specific gene factor polymorphism that prolongs or shortens the

half-life of the gene product. Similarly, enzymatic activity of the protein could be increased or decreased due to tumor gene mutations or due to polymorphisms inherited from the host. Hence, for a specific factor that may be important in resistance, there could be lack of correlations of outcome with mRNA expression or protein expression because of mutations or polymorphisms that alter gene product half-life or activity. During tumorigenesis, oncogene mutations often drive tumor growth, and are selected for, but one might not expect to see selection for specific resistance factor mutations until after initiation of therapy; hence, host genotype polymorphisms might hypothetically be particularly likely to play a major role in intrinsic resistance, with a possibility of resistance factor mutation or gene amplification or deletion playing a bigger role in acquired resistance.

1.6 Chemotherapy as “targeted” therapy

There is now substantial interest in “targeted therapies” for cancer, but chemotherapy agents may also be “targeted”. The fact that there is any selectivity of effect, with marked shrinkage of some tumors in response to chemotherapy, suggests the possibility that those tumors not only are deficient in resistance factors but that they also possess the target required for drug effect. We have traditionally thought of chemotherapy as simply targeting DNA, tubulin, topoisomerases, etc, but all normal cells also possess these, and the ability to selectively kill some (but not other) tumor cells suggests that the sensitive tumor cells may possess an additional target (or activating system, etc) required for sensitivity, and hence, resistance may arise from the absence or saturation of a required target, etc, rather than from the presence of a resistance factor. Dose-response curve flattening at higher drug doses would be in keeping with this. On the whole, we have not done a good job of searching for these hypothetical unique chemotherapy targets. In the past, the sensitivity of cells to chemotherapy has been attributed to rapid cell growth, but this does not explain things well. For example, cisplatin is active against many types of tumors and is toxic to cochlear hair cells, renal convoluted tubule cells, dorsal root ganglion neurons and gastrointestinal enterochromaffin cells but is not toxic to most rapidly dividing normal tissues.

While tumor type may be used to guide choice of agents (eg, pemetrexed was more effective in lung adenocarcinomas than was gemcitabine, while gemcitabine was more effective than pemetrexed against squamous cell carcinomas⁴²), molecular characteristics are only infrequently used in choosing patients for clinical trials. We feel that it is important to raise the efficacy bar by putting substantially more effort into defining molecular determinants of sensitivity vs resistance⁴³. Resistance modulators have generally had equivocal or negative effects in solid tumors. Modulators aimed at resistance factors such as efflux pumps might not be expected to make much of a difference if the tumor lacks a target, activating system, etc required for chemotherapy action.

Below we will discuss resistance to a variety of chemotherapy agents in lung cancer. Resistance mechanisms, agents affected and presence or absence of supporting clinical evidence is summarized in Table 3.

2.0 Drug and oxygen delivery (Fig. 2)

Drug concentrations achieved in tumor may conform to either a “flow-limited model” (proportional to tumor blood flow)⁴⁴ or to a “membrane-limited model” (not proportional to blood flow and presumably limited by ability of the drug to cross cell membranes)^{45, 46}. In a SCLC model, uptake of gemcitabine appeared to be flow-limited⁴⁷, but correlation with tumor blood flow has not been adequately assessed in lung cancer for most drugs. A variety of observations suggest that delivery of cisplatin to tissues may be primarily membrane-limited⁴⁸. As recently reviewed⁴⁸, several factors associated with tumors may reduce tumor blood flow, including high tissue pressure, high blood viscosity (eg, due to fibrinogen), and

decreased red blood cell membrane deformability. Due to impaired vascular autoregulation, tumor blood flow is more susceptible to blood pressure fluctuations than is blood flow to normal tissues⁴⁹. As reviewed⁴⁸, delivery of drugs such as cisplatin could also be reduced by rapid, irreversible binding to blood and extracellular proteins.

Factors limiting blood flow to tumor also secondarily reduce delivery of oxygen, and hypoxia has a variety of effects on therapy efficacy⁵⁰. Some agents that are important in the treatment of lung cancer (eg, etoposide⁵¹ and paclitaxel⁵²) are less effective in hypoxic tumor cells, while others (eg, topotecan⁵³) are more effective in hypoxic tissues, and still others (eg, cisplatin⁵³⁻⁵⁴) are equally effective in aerobic and hypoxic conditions. While hypoxia itself may not alter efficacy of some agents, cell exposure to hypoxia may activate anti-apoptotic signaling pathways that augment drug resistance⁵¹. For example, hypoxia activates sphingosine kinase 2, which in turn promotes the synthesis and release of sphingosine-1-phosphate (S1P)⁵⁵. S1P then binds to S1P receptors, thereby activating p42/44 mitogen-activated protein kinase and protecting lung cancer cells from etoposide cytotoxicity⁵⁵.

Hypoxia also leads to increased expression of hypoxia inducible factor-1 α (HIF-1 α), which in turn augments resistance of NSCLC cell lines to cisplatin⁵⁶, doxorubicin⁵⁶, and paclitaxel⁵², alters conformation and dynamics of microtubules⁵², and fosters tumor regrowth through stimulating angiogenesis by up-regulation of expression of vascular endothelial growth factor (VEGF) and platelet derived growth factor (PDGF)⁵⁷.

Clinically, in patients with advanced NSCLC treated with platinum-based regimens, there was no evidence that therapy efficacy was increased by the addition of any of pentoxifylline (which may improve blood flow by decreasing red blood cell membrane rigidity)⁵⁸, tiripazamine (which sensitizes hypoxic tissues to therapy)⁵⁹, or motexafin gadolinium (which increases the formation of superoxide and other reactive oxygen species in the presence of oxygen)⁶⁰. Limited clinical data suggested possible benefit for NSCLC patients from addition of the hypoxic cell sensitizer metronidazole to doxorubicin, carmustine or mitomycin-C single agent chemotherapy, with responses in 4 of 8 patients⁶¹, although little efficacy was seen in NSCLC or other tumor types when metronidazole was added to epirubicin⁶², carboplatin⁶³ or etoposide⁶⁴ concurrently with multiple other potential resistance modulators including ketoconazole⁶²⁻⁶⁴, dipyridamole⁶²⁻⁶⁴, tamoxifen⁶²⁻⁶⁴, pentoxifylline⁶³, novobiocin⁶³ and cyclosporin⁶²⁻⁶⁴. While administration of the vasodilator nitroglycerin prior to surgical removal of NSCLCs did reduce the rate of immunohistochemistry (IHC) positivity for HIF-1 α and VEGF⁶⁵, imaging studies using (18)F-Fluoromisonidazole (which concentrates in hypoxic tissues) suggested that the hypoxic cell fraction of primary NSCLC is low in humans, and there was no significant correlation between hypoxia and glucose metabolism as assessed by (18)F-Fluorodeoxyglucose (FDG) scanning⁶⁶. Furthermore, in operable NSCLC patients treated with neoadjuvant cisplatin-gemcitabine, HIF-1 α mRNA in post chemotherapy resected specimens did not correlate with patient survival⁶⁷, serum or plasma levels of VEGF did not correlate with survival in patients with advanced NSCLC receiving platinum-based chemotherapy alone⁶⁸ or combined with tirapazamine⁶⁹ or bevacizumab⁷⁰, and tumor IHC expression of VEGF did not correlate with response or survival in advanced NSCLC patients treated with a variety of cisplatin-based combinations⁷¹. Overall, it remains unclear whether tumor blood flow and hypoxia-related factors play a major role in resistance of human lung cancers to chemotherapy.

In addition to trying to improve delivery of drugs and/or oxygen to tissues by manipulations that could alter blood flow, other strategies have also been tested. Liposome encapsulation of drug overcame resistance to cisplatin⁷², daunorubicin⁷³ and vinorelbine⁷⁴ in lung cancer

cell lines. However, administration of liposome-encapsulated cisplatin^{75–77}, docetaxel plus gemcitabine combined with liposomal doxorubicin⁷⁸ or docetaxel plus liposomal doxorubicin plus radiotherapy⁷⁹ to patients with NSCLC did not appear to augment efficacy in phase II trials, and pegylated liposomal doxorubicin was inactive in recurrent SCLC⁸⁰. Inhaled aerosolized liposomal cisplatin has also undergone phase I trials, but none of 16 patients experienced tumor regression⁸¹.

Drug administration via nanoparticles has also been tested. Preclinical studies suggested that cisplatin delivery via inhaled biotinylated-EGF-modified gelatin nanoparticles would improve delivery to EGFR-expressing tumor cells⁸², and a micellar nanoparticle formulation increased the ability of paclitaxel to radiosensitize lung cancer cell lines⁸³. Nanoparticle albumin-bound paclitaxel (NAB-paclitaxel) reached higher tumor concentrations and was more active than cremophor-based paclitaxel in preclinical models that included lung cancer⁸⁴. In a phase I/II trial of NAB-paclitaxel, the observed response rate of 30% was somewhat higher than what would be expected with standard taxane therapy⁸⁵, and a phase II trial of NAB-paclitaxel with carboplatin and bevacizumab yielded a median survival time (16.8 months) which was longer than generally seen with advanced NSCLC⁸⁶. Hence, while liposomal administration of drug did not appear to improve outcome, preliminary clinical data with nanoparticle administration are suggestive of possible enhancement of efficacy. Randomized phase III trials are ongoing to assess this further.

3.0 Extracellular pH

Extracellular pH (which may be manipulated by systemic administration of a glucose load, by medications such as amiloride or by bicarbonate⁸⁷) may also be important (Fig. 2). Tumor extracellular pH is often acidic, while tumor intracellular pH is often neutral or alkaline. A low extracellular pH favors uptake into tumor cells and enhances cytotoxicity of weak acids such as cisplatin⁸⁸ and alkylating agents⁸⁹, while a higher extracellular pH favors uptake into tumor cells of weak bases such as doxorubicin⁸⁹ and vinca alkaloids⁸⁷, and has little net effect on zwitterions like paclitaxel⁸⁹. Hence, both dietary factors and concurrent medications could potentially contribute to tumor resistance by altering tumor pH.

4.0 Drug uptake

Tumor uptake of some drugs is by passive diffusion, whereas other drugs enter cells by active transport or by a combination of active transport and passive diffusion (Fig.2).

4.1 Cisplatin

Reduced uptake of cisplatin has been noted in many resistant NSCLC^{32, 90–94} and SCLC^{32, 93, 95} cell lines, although it is not a universal finding. Cisplatin-resistant lung cancer cell lines with reduced platinum uptake have also been noted to have increased intracellular chloride⁹⁶ or zinc content⁹¹, increased cell membrane rigidity⁹⁷, increased sphingomyelin content⁹⁷, and increased density of membrane lipid packing, with reduced long chain and unsaturated fatty acids⁹⁸. These changes could potentially alter passive diffusion of cisplatin into cells or might reduce the activity of membrane transporters. Culturing cisplatin-resistant SCLC cells in long chain fatty acids (that were then incorporated into cell phospholipids) increased cisplatin cellular uptake and DNA binding, and reduced resistance⁹⁹. Dipyrindamole increased the concentration of both cisplatin and its active aquated species in cells through mechanisms that were uncertain¹⁰⁰. However, when dipyrindamole was added to high dose cisplatin in the treatment of chemo-naïve NSCLC, the response rate was only

14%, with a median survival time of 8 months, suggesting that clinical activity was not increased substantially¹⁰¹.

4.1.1 CTR1 and CTR2—Platinum-resistance may be associated with broad cross-resistance and with down-regulation of expression of a wide range of membrane transporters¹⁶. Platinums may be transported into cells by the copper transporter CTR1, and platinum resistance in lung cancer may be associated with decreased CTR1 expression¹⁰². A role for the related copper transporter CTR2 in platinum uptake has also been suggested in other tumor types¹⁰³ but has not yet been assessed in lung cancer. Decreased CTR1 expression was noted in patients with a variety of malignancies who had been exposed to a wide range of chemotherapy and targeted agents within the previous 3 months¹⁰⁴ suggesting that exposure to many types of drugs could lead to platinum resistance by down-regulating expression of CTR1^{104, 105}.

4.1.2 Na⁺, K⁺ ATPase—Na⁺, K⁺ ATPase and agents that activate it are also associated with increased intracellular accumulation and/or efficacy of cisplatin, with the association being stronger in NSCLC than in SCLC cell lines^{106–108}. Inhibition of factors (such as thromboxane A₂^{108, 109}) that antagonize Na⁺, K⁺ ATPase also increased cisplatin uptake and cytotoxicity. Inhibition of thromboxane A₂ also resulted in upregulation of expression of interleukin-1 β -converting enzyme, the expression of which is reduced in some platinum-resistant NSCLC cell lines¹⁰⁹. Whether Na⁺, K⁺ ATPase plays a direct role in cisplatin cellular uptake or whether it instead exerts an effect indirectly through alteration of intracellular pH¹¹⁰ or other mechanisms is unclear. In NSCLC cell lines, the glucose metabolite sorbitol decreased cisplatin cytotoxicity, Na⁺, K⁺-ATPase activity, and cisplatin uptake, suggesting a possible mechanism for cisplatin resistance in poorly controlled diabetes¹¹¹.

Tumor retention of Thallium-201 (T201) on SPECT scanning may reflect Na⁺, K⁺ ATPase activity, and in one study in which SCLC patients were treated with cisplatin-based chemotherapy, response correlated with pretreatment T201 retention¹¹², but this was not noted in another study in which SCLC patients were treated with a broader spectrum of chemotherapy agents¹¹³, and no correlation between T201 retention and outcome was seen in NSCLC patients treated with mitomycin-vindesine-cisplatin¹¹⁴. Hence, it is unclear if T201 scanning is of any value in this setting.

4.2 Taxanes, vinca alkaloids and etoposide

There is relatively little information available on mechanisms by which taxanes, vinca alkaloids or etoposide enter cancer cells, nor on the potential role of decreased drug influx in resistance, although etoposide uptake is significantly higher in sensitive SCLC cell lines than in more resistant NSCLC lines¹¹⁵. The nonionic detergent Tween-80 augmented uptake and cytotoxicity of etoposide in NSCLC cell lines¹¹⁶, and some etoposide-resistant cell lines have greatly increased content of cholesterol¹¹⁷, which would increase cell membrane rigidity. Transfection into NSCLC cells of the human HERG K⁺ channel gene significantly increased the cytotoxicity of vincristine, paclitaxel and hydroxycamptothecin (but not cisplatin)¹¹⁸, but it is unknown if it plays any role in drug transport.

4.3 Gemcitabine

For gemcitabine, human equilibrative nucleoside transporter 1 (hENT1) plays a role in cellular uptake, and hENT 1-deficient cells are resistant to gemcitabine¹¹⁹. A deficiency in hENT 1 may be more important in intrinsic than in acquired gemcitabine resistance¹²⁰. Liposome encapsulation may augment gemcitabine uptake and efficacy¹²¹.

While pretreatment hENT-1 expression did not correlate with response or survival in one NSCLC study of gemcitabine-based chemotherapy in which only 16% expressed hENT 1 by IHC¹¹⁹, in another trial, no NSCLC patient in whom hENT 1 was absent by IHC responded to gemcitabine-based therapy¹²².

4.4 Pemetrexed

For the multitargeted antifolate pemetrexed, the proton-coupled folate receptor¹²³, the reduced folate carrier^{123, 124} and the folate receptor- α ¹²⁵ all appear to play a role in cellular uptake, although they have not been extensively assessed in lung cancer. Pemetrexed is more active in lung adenocarcinomas than in squamous cell carcinomas clinically⁴², possibly related to the fact that adenocarcinomas have significantly higher expression of folate receptor- α and a strong trend towards increased expression of the reduced folate carrier compared to squamous cell carcinomas¹²⁶.

Overall, low drug uptake may be an important cause of resistance, and uptake may vary with extracellular pH. With cisplatin, CTR1, Na⁺, K⁺ ATPase, and membrane fluidity may play a role in uptake and resistance, while hENT1 may play a role with gemcitabine and folate carriers may play a role with pemetrexed. Little is known regarding how other drugs enter cells.

5.0 Drug efflux

More is known about the role of efflux (Fig. 3) than about the role of uptake in drug resistance. Various membrane efflux pumps have been assessed that may play a role in reducing drug intracellular concentrations.

5.1 Multidrug resistance protein (MRP)

A high proportion of SCLC^{127, 128} and NSCLC^{127, 129} cell lines express MRP mRNA, with greater MRP protein and/or mRNA expression in NSCLC than in SCLC cell lines^{130, 131}. MRP expression is associated with decreased cellular drug accumulation of cisplatin⁹⁵, paclitaxel¹³² and other agents¹³³. In NSCLC heterotransplants in nude mice, there was a statistically non-significant trend toward a higher response rate to paclitaxel in MRP-negative tumors¹³⁴. In both SCLC^{95, 127, 128, 130–132} and NSCLC^{29, 127, 129–133, 135} cell lines, MRP mRNA or protein expression correlated significantly with resistance to anthracyclines^{95, 127, 128, 130, 131, 133, 136} vinca alkaloids^{95, 129, 130, 133, 136}, etoposide^{127, 130, 131, 136}, docetaxel²⁹, paclitaxel¹³², gemcitabine¹³⁵ and cisplatin^{95, 130}. However, an association between MRP expression and resistance was not seen in some cell lines^{137–139}, and in some, MRP was not associated with resistance to cisplatin despite contributing to resistance to other agents^{127, 133}. Paradoxically, in still other lines, resistance to gemcitabine was decreased instead of being increased by MRP1 expression¹³⁹. Similarly, in another study, the anti-inflammatory agent indomethacin induced apoptosis preferentially in SCLC cells that were doxorubicin resistant and that overexpressed MRP1, and this apoptosis was decreased if MRP1 was inhibited by siRNA¹⁴⁰.

MRP1¹³⁰, MRP3¹³⁰, and MRP7^{129, 132} may be particularly important in resistance, with less of a role for MRP2^{129, 130} and MRP3129. The MRP7 inhibitor sulfin-pyrazone increased NSCLC cell line sensitivity to vinorelbine¹²⁹. 5-Fluorouracil may sensitize cells to cisplatin by inhibiting MRP expression¹⁴¹, and verapamil may also modulate MRP effect¹³¹. Depleting cells of glutathione reversed resistance associated with MRP in one study, suggesting that MRP functions as a glutathione S-conjugate (“GS-X”) pump that exports molecules that are bound to glutathione¹³⁶. Increased GS-X pump activity is associated with cisplatin resistance and decreased cellular cisplatin accumulation¹⁴².

MRP mRNA and/or protein by IHC was found in 32–100% of clinical NSCLC tumor specimens^{127, 143–145}, but gene amplification has not been noted¹²⁷. MRP expression was significantly higher in more differentiated tumors than in less differentiated tumors¹⁴³, in squamous cell carcinomas than in other NSCLCs¹⁴⁵, and in tumors with mutant p53¹⁴⁴. MRP expression is also common in SCLC clinical tumor samples¹²⁸.

There is not a consensus on whether tumor MRP expression correlates with clinical outcome, although tumor type and chemotherapy type may have a bearing on the relationship. In SCLC patients treated with platinum-based combinations, response rates were significantly lower for tumors that were IHC-positive vs –negative for MRP1^{146, 147} or MRP2¹⁴⁸. After being taken up by cells, Tc-99m methoxyisobutyl isonitrile (MIBI) and technetium-99m tetrofosmin (Tc-TF) may be exported from cells by either MRP or by MDR1/p-glycoprotein, and retention of these compounds on SPECT scanning has been used clinically as an indicator of efflux pump activity. Higher tumor uptake of MIBI¹⁴⁹ and Tc-TF¹⁵⁰ on SPECT scanning was associated with better response to chemotherapy, and, in keeping with preclinical predictions, uptake of MIBI and Tc-TF correlated negatively with tumor MRP and MDR1 expression^{149–151}). Furthermore, MRP1 expression in SCLC tumors was markedly increased at relapse after cisplatin-etoposide compared to chemo-naïve values¹⁴⁶ or there was a strong trend towards an increase in MRP IHC positivity after treatment with etoposide or teniposide¹⁵², suggesting that chemotherapy exposure either upregulates expression of MRP or else selects for resistant, MRP-positive cells. Similarly, in autopsy NSCLC tumor tissues, mRNA expression levels of MRP3¹⁵³ and MRP5¹⁵⁴ (but not MRP4¹⁵³) were significantly higher in patients who had been exposed to platinum drugs *ante mortem* than in patients who had not received platinum agents.

In advanced NSCLC, response rates to cisplatin plus irinotecan were higher and survival was longer in patients with some MRP2 host genotypes than with other genotypes¹⁵⁵, tumor MRP2 (but not MRP1 or MRP3) IHC expression correlated with survival (but not with response) in patients treated with platinum-based combination chemotherapy¹⁵⁶, and tumor MRP expression was associated with poor survival in patients treated with vindesine plus etoposide^{144, 145}. However, in another NSCLC study, MRP mRNA expression only correlated inversely with response in lung adenocarcinoma, and not in squamous cell carcinoma¹⁵⁷, there was no correlation between MRP IHC expression and response to platinum-based combinations in other studies^{156, 158}, MIBI scanning did not correlate with response to chemotherapy in a study involving 14 NSCLC and 9 SCLC patients¹⁵¹, and tumor MRP1 or MRP2 IHC expression did not correlate with survival in patients with resected NSCLC receiving adjuvant cisplatin plus a vinca alkaloid or etoposide¹⁵⁹.

Overall, preclinical data strongly support a role for MRP in resistance to several types of chemotherapy. Clinical observations suggest that MRP is probably associated with resistance to chemotherapy in SCLC. The clinical data remain less convincing in NSCLC, but are suggestive of a possible role for MRP in chemoresistance, and the increased MRP expression seen in treated NSCLC and SCLC tumors suggest the possibility that it may play a greater role in acquired than in intrinsic resistance.

5.2 MDR1/p-glycoprotein (P-gp)

Like MRP, P-gp may also render tumors resistant to chemotherapy by transporting drugs out of cells. In NSCLC cell lines, increased MDR1 mRNA and/or protein expression levels were associated with resistance to anthracyclines^{25, 160, 161}, vinca alkaloids^{25, 129, 160–162}, etoposide^{160, 161}, and taxanes^{25, 160, 161, 163, 164}. With occasional exceptions¹⁶⁵, MDR1/P-gp expression did not correlate significantly with sensitivity to cisplatin^{25, 92, 137} or carboplatin¹⁶⁰, nor with intracellular^{92, 137} or intranuclear¹³⁷ cisplatin accumulation, and cisplatin and carboplatin may actually increase cellular concentrations of some other agents

by inhibiting P-gp¹⁶⁶. Increased expression of P-gp is also frequently seen in cell lines that are sensitive to cisplatin despite resistance to paclitaxel¹⁶⁷. In addition, some NSCLC and SCLC cell lines transfected with the MDR1 gene had augmented sensitivity to gemcitabine, and this augmented sensitivity was reversed by the P-gp inhibitor verapamil¹³⁹. MDR1 gene amplification was detected in some resistant lines²⁵.

MDR1 gene overexpression was also seen in SCLC cell lines selected for resistance by exposure to paclitaxel¹³², anthracyclines³³ or etoposide^{168, 169}, although MDR1 mRNA expression was relatively uncommon in SCLC cell lines not selected for resistance¹²⁸.

Some resistant lines that overexpress P-gp also overexpressed caveolin 1, and P-gp localized to caveolin-rich membrane domains in these cells¹¹⁷. P-gp expression correlated with HIF-1 α expression in NSCLC cell lines¹⁷⁰ and in resected NSCLC tumors⁶⁵. P-gp expression was increased when lung adenocarcinoma cells were cultured in hypoxia¹⁷⁰ and was reduced in tumors of patients who had nitroglycerin patches applied to improve tumor blood flow and oxygenation prior to surgical resection⁶⁵.

Expression of Connexin 32 in a NSCLC cell line significantly potentiated vinorelbine-induced cytotoxicity and induction of apoptosis by down-regulating expression of MDR1¹⁶². Connexins are important in gap junctions and normally play a role in electrical signaling between cells.

Clinically, in chemo-naïve NSCLC patients, MDR1 mRNA and/or P-gp were expressed in 11–32% of surgical specimens^{152, 165, 171–173}, but were expressed in 61% of tumors that had been treated with chemotherapy¹⁷². MDR1 expression in NSCLC did not correlate with histopathology or with clinical details¹⁷¹. In SCLC, MDR1 expression was seen in 13–60% of tumor biopsy samples^{128, 152, 173}, and in 50% of SCLCs xenografted from treated and untreated patients into nude mice¹⁷⁴.

With MDR1/P-gp, as with MRP, there is stronger evidence of an association of expression with outcome in SCLC than in NSCLC, so we will discuss SCLC first. In SCLC, there was a negative correlation between expression of P-gp and response^{146, 147, 149, 173, 175, 176} and/or survival^{176, 177} in patients treated with cisplatin-etoposide^{146, 147, 149, 173, 175, 176}, cyclophosphamide-doxorubicin-vincristine (CAV)^{173, 176}, or other doxorubicin or etoposide regimens¹⁷⁷, and P-gp expression was significantly increased in tumors previously exposed to therapy compared to pretreatment expression^{146, 152}. Response of SCLC patients to cisplatin plus etoposide also correlated with MDR1 host polymorphisms, with a significantly better chemotherapy response in patients with the 3435 CC genotype (exon 26) compared with the combined 3435 CT and TT genotypes, and patients harboring the 2677G-3435C haplotype also responded significantly better than did other patients¹⁷⁸. Similarly there was a strong correlation between uptake of MIBI^{113, 149, 179} or Tc-TF^{150, 175, 180} on SPECT scanning and response of SCLC to cisplatin plus etoposide^{149, 150, 175, 179, 180} (EP), to EP plus doxorubicin¹⁸¹, and to CAV plus mitomycin-C and vindesine¹¹³, or there was a trend towards a correlation of response with MIBI^{182, 183}. Patients with SCLC demonstrated significantly greater MIBI uptake than those with NSCLC¹⁸⁴.

In NSCLC, there was a significant correlation of tumor P-gp IHC expression with response in 2 studies using paclitaxel and a platinum^{185, 186} and in one study using cisplatin, ifosfamide, vinblastine and radiation¹⁸⁷, and P-gp expression also correlated inversely with survival in this latter study¹⁸⁷. The MDR1 3435 CC host genotype was associated with a significantly better response to cisplatin-vinorelbine compared with the combined 3435 CT and TT genotypes, and patients with the 2677G-3435C haplotype also had significantly better responses to chemotherapy compared to patients with other haplotypes combined¹⁸⁸. In another NSCLC study, tumor MDR1 mRNA expression did correlate with shortened

survival even though it did not correlate with response to chemotherapy¹⁵⁷. In NSCLC, while tumor P-gp status did not correlate with MIBI uptake on SPECT scanning in some studies^{189, 190}, it did correlate in other studies^{185, 191}. Furthermore, MIBI^{114, 185, 190, 191} and Tc-TF^{192, 193} uptake on SPECT scanning correlated significantly with response to chemotherapy with cisplatin, mitomycin-C plus vindesine^{114, 190}, with paclitaxel¹⁸⁵, with paclitaxel-based regimens¹⁹¹, or with radiation combined with cisplatin plus etoposide¹⁹³, and also correlated with survival¹⁹⁰.

However, in a number of other studies involving patients with advanced^{156, 173, 194} or locally advanced¹⁹⁵ NSCLC, P-gp expression by IHC did not correlate with response to cisplatin-based regimens^{156, 173, 194} or carboplatin-based regimens¹⁹⁵ that also included vinca alkaloids^{156, 173, 195}, irinotecan¹⁵⁶, taxanes¹⁵⁶, gemcitabine¹⁵⁶ or radiation¹⁹⁵, and host MDR1 C3435T polymorphisms did not correlate with outcome in patients with advanced NSCLC treated with docetaxel-cisplatin¹⁹⁶ despite their association with efficacy of cisplatin-vinorelbine in another study, as noted above¹⁸⁸. Similarly, preoperative MIBI uptake did not correlate with in vitro sensitivity (for etoposide, doxorubicin, vindesine, cyclophosphamide and cisplatin) of resected specimens¹⁸⁹, nor with clinical response to chemotherapy¹⁵¹ in some studies.

P-gp antagonists have been assessed in both NSCLC and in SCLC. The P-gp antagonist verapamil augmented paclitaxel accumulation in human lung cancer cells overexpressing P-gp¹³⁸, increased vinorelbine efficacy in NSCLC cell lines overexpressing MDR1¹²⁹, and improved the efficacy of a cyclophosphamide, cisplatin, doxorubicin, and etoposide regimen against SCLC xenografts¹⁹⁷. The epidermal growth factor receptor (EGFR) inhibitor gefitinib also reversed P-gp mediated taxane resistance in NSCLC cell lines^{164, 198}, but did not improve efficacy of chemotherapy in NSCLC in randomized clinical trials^{199, 200}.

In a phase II clinical trial in which the P-gp antagonist cyclosporine A was added to paclitaxel in NSCLC patients (18 of whom had received one prior chemotherapy regimen and 8 of whom were chemonaïve), the response rate was 26%, median time to progression was 3.5 months and median overall survival was 6 months, suggesting a possible modest beneficial impact of the cyclosporine on paclitaxel efficacy²⁰¹. However, when cyclosporine was added to etoposide plus cisplatin as front line therapy in advanced NSCLC, there was no evidence of benefit, with a response rate of 22.7% and a 2-year survival rate of 8%, and better outcome in patients receiving a lower vs higher dose of cyclosporine²⁰².

In a small randomized phase II trial with 54 NSCLC patients, addition of the P-gp antagonist dexverapamil to vindesine plus etoposide was associated with a response rate of 31.3 % compared to only 11.1 % for the chemotherapy alone²⁰³, and addition of verapamil to vindesine plus ifosfamide/mesna in a randomized study involving 72 NSCLC patients was associated with an increase in response rate from 18% to 41% (p=0.057) and the overall survival time was increased significantly (p=0.02). However the calcium channel blocker nifedipine (which reverses resistance to chemotherapy both through P-gp antagonism plus through other mechanisms, as previously reviewed²⁰⁴) plus the methylxanthine pentoxifylline did not reverse resistance to combination chemotherapy in previously-treated patients with NSCLC and other malignancies⁵⁸. Similarly, while hydroxyurea is thought to reverse *MDR1*-associated resistance by breaking down double minute chromosomes carrying amplified *MDR1*²⁰⁵, the addition to paclitaxel of hydroxyurea in previously-treated patients with advanced NSCLC did not appear to improve outcome, with a response rate of only 3% and a median survival time of 4.6 months²⁰⁶.

In randomized clinical trials in SCLC, the P-gp modulator megestrol acetate did not improve outcome with chemotherapy²⁰⁷ and verapamil did not improve response or survival rates in patients receiving cyclophosphamide, doxorubicin, vincristine plus etoposide²⁰⁸.

Based on the above, we can conclude that there is strong preclinical evidence of an association between MDR1/P-gp expression and resistance to several agents in lung cancer cell lines, but probably no increased resistance to platinum agents, and possible collateral sensitivity to gemcitabine. Clinically, there is relatively strong evidence of an association between MDR1/P-gp expression in SCLC and resistance to combination chemotherapy. The clinical evidence is inconclusive for an association with outcome in NSCLC. With respect to P-gp modulation, verapamil or dexverapamil may possibly increase chemotherapy efficacy in NSCLC, but other strategies have been ineffective, and neither verapamil nor megestrol helped in SCLC.

5.3 Lung Resistance Protein/Major Vault Protein (LRP)

Vaults are ribonucleoparticles involved in nuclear-cytoplasmic transport, cell signaling and immune responses, and LRP is a major component of vaults²⁰⁹. In some NSCLC cell lines, LRP expression correlated significantly with resistance to cisplatin^{210, 211} or etoposide²¹². However, in other NSCLC cell lines, resistance to cisplatin^{92, 137} and cisplatin intracellular concentration^{92, 137} did not correlate with LRP mRNA^{92, 137} and protein¹³⁷ expression. LRP expression also did not correlate with resistance of NSCLC cell lines to anthracyclines^{210, 211}, etoposide^{210, 211, 213}, vinca alkaloids^{210, 211}, bleomycin^{210, 211} or the irinotecan metabolite SN-38²¹¹.

In some clinical studies, NSCLC tumor LRP expression was associated with reduced response to platinum-based chemotherapy^{157, 172, 214}. However, this association was only noted in squamous cell carcinomas in one study²¹⁴, and in still other studies, there was no correlation with response to platinum-based chemotherapy (including platinum combinations with taxanes and epipodophyllotoxins^{158, 215}) or to taxane-based chemotherapy²¹⁶. Autopsy tumor LRP gene expression was not increased by prior platinum exposure²¹⁷. Tumor LRP expression correlated with MRP expression in one NSCLC study¹⁵⁷, but it did not correlate with MIBI¹⁹¹ or Tc-TF^{150, 192} uptake on SPECT scanning.

In SCLC, there was a statistically insignificant trend towards decreased response to CAV in patients whose tumors overexpressed LRP¹⁴⁶, while in other studies, there was no apparent association between tumor LRP expression and response to CAV²¹⁵ or with response to cisplatin plus etoposide¹⁴⁷. Overall, there is no conclusive evidence that LRP plays a major role in resistance of lung cancer to chemotherapy.

5.4 P-type adenosine triphosphatase (ATP 7B)

As recently reviewed⁴⁸, the copper transporter ATP 7B is thought to play a role in cisplatin efflux. While ATP 7B mRNA and IHC expression significantly correlated with cisplatin resistance in NSCLC xenografts²¹⁸, it did not correlate with resistance in some lung cancer cell lines¹⁰². There is evidence of a link between ATP 7B expression and resistance to cisplatin-based chemotherapy in some other tumor types²¹⁹, but data from clinical tumor specimens are not yet available for lung cancer. It remains uncertain whether ATP 7B is important in lung cancer resistance to chemotherapy.

5.5 Breast Cancer Resistance Protein (BCRP)

In advanced NSCLC patients undergoing platinum-based chemotherapy, tumor IHC expression of BCRP (a transporter and member of the ATP binding cassette family) was significantly associated with shortened survival in two studies^{156, 220}, and blood BCRP

concentrations were significantly higher in chemoresistant patients than in chemosensitive patients²²¹. Tumor BCRP IHC expression correlated with lack of response in one study²²², but not in another study²²⁰. Overall, the data suggest a possible role for BCRP in lung cancer chemotherapy resistance.

5.6 Ral-interacting protein (RLIP76) (RALBP1)

In NSCLC and SCLC cell lines, overexpression of the non-ABC glutathione-conjugate transporter RLIP76 (which is involved in receptor-ligand endocytosis and in multispecific drug transport) was associated with augmented efflux, decreased intracellular concentrations and resistance to vinorelbine²²³ and doxorubicin^{224–226}, and its inhibition enhanced cisplatin-vinorelbine efficacy in lung cancer xenografts²²⁷. RLIP76 transport activity appeared to be regulated by protein-kinase-C- α (PKC- α)-mediated phosphorylation^{226, 228}. The greater resistance to doxorubicin in NSCLC cells compared to SCLC cells may be mediated through differential phosphorylation of RLIP76 in NSCLC vs SCLC²²⁶. Lung cancer clinical data are unavailable.

6.0 Drug detoxification

Drugs may also be neutralized or detoxified by various cellular mechanisms (Fig. 3).

6.1 Glutathione (GSH)

GSH may bind cisplatin, thereby decreasing formation of platinum-DNA adducts. It may also play a role in increased repair of platinum-DNA adducts²²⁹ and it plays a role in drug efflux via pumps that expel GSH-conjugates from cells (“GS-X pumps”). In lung cancer cell lines that had been rendered resistant to anthracyclines, vinca alkaloids and etoposide by transfection with the MRP gene, GSH depletion by DL-buthionine-S,R-sulfoximine (BSO) reversed resistance, suggesting that MRP functions as a GS-X pump¹³⁶. While efficacy of cisplatin against NSCLC cell lines⁹⁰, NSCLC xenografts²³⁰, or SCLC cell lines²³¹,²³² did not correlate with GSH content in some studies, in several other NSCLC cell lines^{92, 93, 233, 234} and SCLC cell lines^{32, 93, 142, 233, 235–237}, increase in GSH content was accompanied by increase in cisplatin resistance^{32, 92, 93, 142, 233–237}, with decreased platinum-DNA binding^{93, 236, 237}, and with decreased intracellular platinum accumulation³². Factors that reduced cellular GSH augmented sensitivity to cisplatin^{93, 141} or other agents²³⁸.

Etoposide-resistant and doxorubicin-resistant NSCLC cell lines also may have increased GSH content²³⁸, and in some cases, cisplatin resistance and increased GSH content was accompanied by cross-resistance to vinca alkaloids²³⁶, anthracyclines³², etoposide³², camptothecins³², mitomycin-C³², alkylating agents³², methotrexate³², and radiation²³⁴, while in other instances, resistance to cisplatin with augmented GSH was accompanied by lack of cross-resistance to anthracyclines or epipodophyllotoxins²³⁶, or by collateral sensitivity to vinca alkaloids and 5-fluorouracil³².

Expression of genes involved in GSH synthesis (glutamate-cysteine ligase [GCL], also known as gammaglutamylcysteine synthetase [GGCS]) in NSCLC xenografts also enhanced resistance to cisplatin^{239, 240}, and inhibition of GCL decreased cisplatin resistance²⁴¹. The expression level of the GCL light chain subunit gene was significantly higher in NSCLC than in SCLC²³⁹. Survival of NSCLC patients treated with cisplatin-based regimens was significantly associated with host genotype for some GSH metabolic genes²⁴².

In vitro sensitivity testing of lung cancers that had been resected demonstrated increased resistance to cisplatin for tumors that were IHC positive for GSH peroxidase and GSH reductase, and IHC expression also varied significantly with lung cancer histologic types²⁴³.

Overall, the preponderance of preclinical evidence supports a role for GSH in resistance to cisplatin and perhaps other agents in both NSCLC and SCLC, although the relationship is not consistent across all studies, and clinical data are limited.

6.2 Glutathione-S-transferase-pi (GST-pi)

GST catalyzes the binding of GSH to various compounds. There has been particular interest in the GST-pi variant in chemotherapy resistance, and SCLC cell lines had lower GST-pi expression than did NSCLC cell lines^{244, 245}. Cisplatin resistance correlated with GST-pi expression^{244–246}, GST activity²³¹, or absence of GST inhibitor^{232, 247} in cells harvested from resected NSCLC tumors²⁴⁶, in some NSCLC cell lines²⁴⁴, and in some SCLC cell lines^{231, 232, 244, 245, 247}, but in other NSCLC cell lines^{90, 92, 93, 160, 248} and SCLC cell lines^{93, 95, 236}, resistance to cisplatin^{90, 92, 93, 95, 236, 248}, anthracyclines^{95, 160}, vinca alkaloids^{95, 160, 236}, etoposide^{160, 248}, irinotecan²⁴⁸, taxanes²⁴⁸, 5-fluorouracil¹⁶⁰ and radiation⁹⁵ did not correlate with GST-pi expression.

Clinically, NSCLC tumors were positive for GST-pi or had high GST-pi IHC expression in 56–69% of patients^{246, 249–251}. Some clinical studies showed a correlation between lack of response^{187, 249–251} or increased risk of relapse or shortened survival¹⁸⁷ and high tumor^{187, 249–251} or serum²⁵² GST-pi expression in NSCLC patients treated with platinum-vindesine²⁴⁹, platinum-etoposide²⁵⁰, cisplatin, ifosfamide, vinblastine and radiation¹⁸⁷ or various platinum-based regimens^{251, 252} for advanced disease^{249–252}, for locally advanced disease¹⁸⁷, or with adjuvant cisplatin-based chemotherapy for resected disease²⁴⁶. NSCLC patients treated with platinum-based regimens who had the GST-pi exon 6 wild type host genotype (Ala/Ala) had significantly worse survival than did patients with the variant genotype (Ala/Val or Val/Val), while GSTpi exon 5 genotype was not associated with survival²⁵³.

On the other hand, some other NSCLC studies showed no correlation between tumor GST-pi IHC expression and response^{194, 195} or survival¹⁹⁵ in patients with locally advanced disease undergoing radiotherapy combined with vinorelbine +/- carboplatin¹⁹⁵ or in patients with advanced disease receiving platinum-based chemotherapy^{194, 254}. Furthermore, a second pharmacogenetic study failed to demonstrate an association between GST-pi exon 5 and exon 6 polymorphisms and response to chemotherapy or survival, although patients possessing the variant alleles GSTP1 105Val or GSTP1*B demonstrated trends toward inferior response and survival²⁵⁵.

Overall, as with GSH, the association of GST-pi with resistance of lung cancer to platinum and other agents is suggestive but is not consistent across studies, and there is little experience with attempts to clinically modulate GST-pi in lung cancer patients receiving chemotherapy.

6.3 Metallothioneins

Metallothioneins are a family of cysteine-rich low molecular weight proteins that are involved in zinc homeostasis and may also be involved in chemotherapy binding and detoxification. Increased metallothionein expression was noted in some cisplatin-resistant NSCLC⁹² and SCLC²³² cell lines, and metallothionein expression also correlated with resistance to etoposide²⁵⁶. Exposure of lung cancer cell lines to cadmium or zinc increased metallothionein synthesis and increased resistance to etoposide²⁵⁶.

In patients with SCLC treated with alternating cisplatin-etoposide/CAV, IHC for metallothionein (45% positive) correlated significantly with short survival²⁵⁷. Overall, IHC expression of metallothionein in human tumors was less common in SCLC than in NSCLC, and it increased post exposure to chemotherapy²⁵⁸.

6.4 Dihydrodiol dehydrogenase (DDH)

NSCLC cells overexpressing or transfected with DDH (a cytoplasmic aldo-keto oxidoreductase enzyme that plays an important role in the detoxification process²⁵⁹ and in the metabolism of steroid hormones, prostaglandins and xenobiotics²⁶⁰) had increased resistance to cisplatin^{259–261}, doxorubicin^{259, 261}, vincristine²⁶¹, melphalan²⁶¹ and radiation²⁵⁹. While some assessments suggested an association between DDH expression and paclitaxel resistance²⁶¹, a systematic review of cell lines with an inverse resistance relationship between cisplatin and paclitaxel (resistant to cisplatin but sensitive to paclitaxel) suggested that increased expression of DDH was a common characteristic of such cells¹⁶⁷. Proinflammatory mediators such as interleukin-6 induced DDH expression in NSCLC cells, but this induction could be prevented by protein kinase C inhibitors²⁶⁰. DDH expression inversely correlated with the DNA repair factor Nijmegen breakage syndrome 1 (NBS1) and with apoptosis-inducing factor (AIF)²⁶⁰. DDH is often overexpressed in NSCLC cells, and patients with DDH overexpression are at increased risk of recurrence²⁵⁹, and have a poor prognosis and chemoresistance²⁶⁰. Hence, preclinical data and limited clinical data suggest a role for DDH in lung cancer resistance.

6.5 Deoxycytidine deaminase

Gemcitabine is activated by conversion to the metabolite gemcitabine triphosphate (see below), and gemcitabine triphosphate may then be inactivated by the enzyme deoxycytidine deaminase. Deoxycytidine deaminase activity was lower in SCLC^{33, 139} and NSCLC¹³⁹ variants that were sensitive to gemcitabine compared to resistant variants. In pharmacogenetic analyses in patients with advanced NSCLC treated with cisplatin-gemcitabine, a cytidine deaminase host polymorphism associated with significantly reduced enzymatic activity correlated with better clinical benefit, toxicity and longer time to progression and overall survival²⁶².

6.6 Other potential detoxifying mechanisms

Down-regulation of expression of the redox factor peroxiredoxin V in lung cancer cell lines augmented efficacy of etoposide and doxorubicin, suggesting a role for redox reactions in resistance to these agents²⁶³. In NSCLC and other cell lines, thymidine administration inhibited cytotoxicity of pemetrexed, and dipyrindamole prevented this rescue by blocking thymidine transport²⁶⁴. High extracellular folate pools also markedly decreased cell killing by pemetrexed²⁶⁵. The clinical importance of these observations is uncertain.

7.0 Drug activation and binding

Resistance may also occur if there is defective drug activation (Fig. 2 and 4).

7.1 Deoxycytidine kinase

Some NSCLC^{21, 120, 135, 139, 266, 267} and SCLC^{33, 139} cell lines resistant to gemcitabine had decreased activity of the enzyme deoxycytidine kinase which is responsible for conversion of gemcitabine to its active metabolite, gemcitabine triphosphate. Observations in some lines suggested that deoxycytidine kinase deficiency may play more of a role in acquired gemcitabine resistance than in intrinsic resistance¹²⁰.

MRP1 expression was associated with increased deoxycytidine kinase expression and with augmented sensitivity to gemcitabine, and antagonism of MRP1 by verapamil reduced sensitivity to gemcitabine, possibly by decreasing expression of deoxycytidine kinase^{139, 268}. Corticosteroids alone decreased deoxycytidine kinase activity and also decreased gemcitabine efficacy in NSCLC cell lines²⁶⁸. Synergism between gemcitabine and pemetrexed in NSCLC cell lines appears to be mediated in part through the up-regulation of

expression of deoxycytidine kinase expression by pemetrexed^{135, 267}, although upregulation of expression of the gemcitabine transporter (hENT1) and decreased phosphorylation of Akt may also play a role²⁶⁷. Clinical data on the importance of deoxycytidine kinase remain limited.

7.2 DNA binding of platinum agents and intracellular pH

DNA binding of cisplatin (ie, platinum-DNA adduct formation) was increased in some cisplatin sensitive NSCLC^{93, 94, 269} and SCLC^{93, 99, 232, 237, 270} cell lines compared to resistant variants, although this was not seen in all cases^{93, 233}. Through mechanisms that are unclear, the antifungal agent amphotericin B increased sensitivity to cisplatin while increasing cisplatin uptake and DNA binding in both NSCLC and SCLC cell lines²⁷¹. As outlined in an earlier review⁴⁸, when cisplatin or carboplatin enter tumor cells, chloride adducts are lost in the low-chloride intracellular milieu and are replaced by water molecules to form highly reactive, positively charged aquated species or relatively non-reactive, neutral hydroxy moieties. A more acidic intracellular pH favors formation of the positively charged species, and it is the positively charged species that is the active form of cisplatin that binds to DNA. An acidic intracellular pH was also associated with a reduction in cisplatin efflux in NSCLC cell lines, possibly due to both more rapid intracellular binding as well as decreased ability to passively diffuse back out across cell membranes²⁶⁹. Resistant lung cancer variants with decreased DNA binding had a more alkaline intracellular pH compared to sensitive variants^{269, 272}. However, the proton pump inhibitor AG-2000 did not alter sensitivity of a cisplatin-resistant NSCLC cell line²⁷³. In more resistant lines with higher intracellular pH, platinum tended to concentrate more in acidic organelles due to their tropism for an acidic environment²⁷².

In NSCLC patients treated with daily low dose cisplatin and XRT, cisplatin-DNA-adduct staining in buccal cells was an independent prognostic factor, with shorter survival times for patients with low adduct staining²⁷⁴. Overall, preclinical data support a role for reduced DNA-adduct formation and for increased intracellular pH in lung cancer resistance to platinum, but clinical data remain limited.

8.0 Increased, decreased or altered target

Depending on the drug and its target, resistance may also occur if drug target is increased, decreased or mutated (Fig. 4).

8.1 Tubulin

NSCLC cell lines rendered resistant to taxanes had significantly increased expression of class III β -tubulin^{275–279} encoded by the H β 4 gene²⁷⁹, and may also have had increased α -tubulin²⁷⁸. Findings were less consistent in NSCLC lines rendered resistant to vinca alkaloids, in that some had significantly increased β -tubulin²⁸⁰ while others instead had significantly decreased expression of class III β -tubulin²⁷⁵. Increased expression of β -tubulin after taxane exposure was inhibited in the presence of wild type p53²⁷⁶.

In two NSCLC cell lines, siRNA vs β III-tubulin inhibited its expression, and increased sensitivity not only to the tubulin binding agents paclitaxel, vincristine, and vinorelbine, but also to cisplatin, doxorubicin, and etoposide²⁸¹. In some NSCLC cell lines that were more resistant to paclitaxel under hypoxic conditions than under normoxic conditions, the expression level of β -tubulin was comparable under normoxic and hypoxic conditions, but the distribution of β -tubulin and cell morphology were changed according to HIF-1 α expression levels, suggesting that HIF-1 influences the conformation and dynamics of microtubules⁵².

Polo-like kinases (PLKs) play a role in mitotic entry, spindle pole function and cytokinesis²⁸². Expression of PLK 1 was elevated in NSCLC, and inhibiting it abolished microtubule polymerization, led to abnormalities in spindle formation and to abnormalities in staining for α -tubulin, arrested cells in G2/M, induced apoptosis, and substantially potentiated the efficacy of vinorelbine²⁸³.

In NSCLC²⁸⁴ and SCLC²⁸⁵ cell lines resistant to taxanes, there was increased microtubule dynamic instability²⁸⁴ or increased acetylation of α -tubulin²⁸⁵ and partial dependence on taxane for growth^{284, 285}. In addition, histone deacetylase 6 (HDAC6) decreased microtubule stability and antagonized the effect of paclitaxel on NSCLC cells, while the farnesyl transferase inhibitor lonafarnib blocked the effect of HDAC6 on tubulin and was synergistic with paclitaxel²⁸⁶.

In IHC assessments of advanced NSCLC, all tumors stained with pan- β tubulin antibody and class I tubulin isotype²⁸⁷. A majority of the tumor samples expressed class II and class III tubulins, although the percentage of positive cells varied significantly between tumors²⁸⁷. Expression of delta2 α tubulin was uncommon²⁸⁷. In another study involving European patients, β -tubulin mutations in exons 1 or 4 were noted in 33% of NSCLC patients, and β -tubulin mutations were also detected in serum DNA of 42% of 131 NSCLC patients (vs none of a control group)²⁸⁸, although they were uncommon in tumors of Japanese patients with NSCLC or SCLC²⁸⁹. In NSCLC patients treated with surgery alone, high class III β -tubulin expression was associated with poorer relapse-free and overall survival²⁹⁰.

In locally advanced or metastatic NSCLC treated with taxane-containing regimens, patients with tumor β -tubulin mutations had substantially lower response rates²⁸⁸ or a trend towards lower response rates²⁸⁷ than did patients with wild type β -tubulin. Response rates were also lower in patients with high tumor expression of β -tubulin III compared to those with low expression^{291, 292}. There was also a longer time to progression^{287, 291, 292} and longer survival²⁹² with low β -tubulin III mRNA expression in patients treated with taxane-containing regimens, but not in patients receiving non-tubulin-binding regimens²⁹².

Advanced NSCLC patients treated with vinorelbine/cisplatin who had low β -tubulin III mRNA expression also had a significantly longer time to progression^{291, 293}, lower likelihood of tumor progression while on therapy²⁹³ and longer overall survival²⁹³ than patients with high expression. Low Delta2 α -tubulin expression was also associated with a significantly longer overall survival in patients treated with cisplatin/vinorelbine²⁹³, while tubulin II levels did not correlate with patient outcome²⁹³. Conversely, when cisplatin/vinorelbine was administered as adjuvant therapy to patients who had undergone surgical resection of their NSCLC tumors, greater benefit of the therapy was seen in patients with high vs low class III β -tubulin, and the adjuvant therapy appeared to overcome the negative prognostic effect of high β -tubulin²⁹⁰. It is unclear why the effects of tubulin on vinorelbine efficacy appear to be different in the adjuvant setting than in advanced disease.

Overall, the available data suggest that elevated expression or mutation of class III β -tubulin (and possibly α -tubulin) increases resistance to taxanes, with somewhat more equivocal data for its effect on resistance to vinca alkaloids.

8.2 Topoisomerase II- α (topo II- α)

Ordinarily, topo II temporarily breaks DNA, permitting it to unwind, then puts it back together again. Epipodophyllotoxins such as etoposide and teniposide, and anthracyclines such as doxorubicin and epirubicin bind to topo II and permit it to break DNA, but block its ability to rejoin the cleaved strands, leading to potentially lethal DNA breaks. A variety of topo II- α abnormalities have been found in resistant lines.

On average, SCLC cell lines are more sensitive to topo II inhibitors than are NSCLC cell lines^{115, 294}. SCLC cell lines had only a modest increase in topo II- α levels and topo II catalytic activity compared to NSCLC cell lines in one study, and no clear association across all unrelated cell lines between sensitivity and topo II- α levels or topo II activity²⁹⁴. However, in another cell line comparison, nuclear topo II activity was twofold higher in SCLC cell lines than in NSCLC cell lines¹¹⁵, and when resistant variants were compared to their sensitive parent line in matched comparisons, expression of topo II- α mRNA and protein levels were lower in the resistant variants²⁹⁵.

One etoposide-resistant SCLC cell line had no alteration of topo II activity, but did have increased topoisomerase I (topo I) activity (in addition to increased GSH and GST activity)³², with cross-resistance to platinum, mitomycin-C, camptothecins, alkylating agents, methotrexate and anthracyclines, but collateral sensitivity to vinca alkaloids and 5-fluorouracil³². Another etoposide-resistant SCLC cell line had decreased nuclear localization of topo II- α , with a shift of the enzyme from nucleus to cytoplasm, but no change in overall cellular catalytic activity²⁹⁶. Other etoposide-resistant SCLC cell lines derived from a patient who developed drug-resistant relapse after initial response to an etoposide regimen had cross-resistance to doxorubicin, reduced topo II unknotting activity, and reduced topo II α expression²⁹⁷.

Still other etoposide-resistant SCLC^{169, 236, 298–301} or NSCLC^{299, 302} cell lines with cross-resistance to doxorubicin^{299, 301} or teniposide^{236, 299} (but not to cisplatin²⁹⁸ or vinca alkaloids^{236, 298}) had mutated topo II- α ^{236, 298, 299, 302} (in some cases with relocalization of topo II- α to cytoplasm from the nucleus³⁰²) and reduced levels of topo II- α protein and/or mRNA^{236, 298, 299, 301}, or reduced topo II activity^{169, 300}, compared to their sensitive parent lines. Some also had decreased cleaved complex formation in the presence of etoposide^{298, 300, 301} or decreased expression of topo II- β ³⁰¹.

Drug-resistant variants selected by exposure of a NSCLC cell line to doxorubicin frequently had reduced topo II- α mRNA and protein levels, whereas clones selected with vincristine showed normal levels of topo II- α ³⁰³. No alterations of topo II β levels were detected³⁰³. On the other hand, some SCLC cell lines resistant to cisplatin^{95, 236} with cross-resistance to radiation⁹⁵ and vinca alkaloids^{95, 236} had increased expression of topo II- α ^{95, 236} (but continued sensitivity to etoposide²³⁶) in keeping with a possible role for topo II- α in repair of DNA damage caused by cisplatin or radiation.

In clinical tumor specimens, there was a significantly greater topo II- α expression by IHC in SCLC than in NSCLC^{152, 304}, and topo II- α IHC expression decreased significantly in SCLC tumors after therapy with etoposide or teniposide¹⁵². A topo II- α mutation (transversions of G to A at codon 486) associated with resistance in cell lines was detected in tumor samples obtained from a patient with SCLC previously treated with etoposide^{152, 305}.

In patients with advanced NSCLC¹⁵⁸, or with limited^{306, 307} or extensive disease SCLC³⁰⁷ treated with a platinum^{158, 306, 307} combined with either paclitaxel¹⁵⁸, teniposide¹⁵⁸, etoposide^{158, 306, 307}, ifosfamide¹⁵⁸ or other agents³⁰⁷, patients with high topo II- α expressing tumors had a significantly worse survival^{158, 307} or a significantly lower response rate³⁰⁶ than did patients with tumors with low topo II- α expression, while high topo II- β expression was associated with lower response rates in one study³⁰⁷.

Overall, preclinical data suggest that reduced expression or mutation of topo II- α may increase resistance to epipodophyllotoxins and anthracyclines (putatively since these drugs rely on an intact topo II system to break DNA strands) while high topo II expression may increase resistance to platinum, radiation and some other agents (potentially through the

role topo II may play in DNA repair). Clinical data are inconclusive. On the one hand, higher topo II- α expression in SCLC than in NSCLC might help explain the greater sensitivity of SCLC to topo II inhibitors. Conversely, the majority of clinical studies assessing impact of topo II expression within a tumor type support, if anything, a role of topo II in increasing resistance to platinum-based combination therapy, whether or not the platinum is combined with a topo II inhibitor.

8.3 Ribonucleotide reductase M1 (RRM1)

RRM1 (a major target for gemcitabine) encodes the regulatory subunit for ribonucleotide reductase³⁰⁸. Because of the role of ribonucleotide reductase in synthesis of deoxyribonucleotides, this enzyme is important in cell growth and DNA repair. RRM1 encodes the regulatory subunit of ribonucleotide reductase and is a molecular target of gemcitabine³⁰⁸. While an association has not been found in all lung cancer cell line studies³⁰⁹, RRM1 gene polymorphisms³¹⁰, gene amplification³¹¹, and mRNA over-expression^{308, 312} did correlate with NSCLC cell line resistance to gemcitabine in other studies. RRM1 over-expression was also associated with a modest degree of platinum resistance in NSCLC cell lines³⁰⁸. Bexarotene (which prevents or reverses RRM1 gene amplification³¹¹) and RRM1 siRNA¹³⁵ both reduced resistance to gemcitabine. In a panel of 15 NSCLC and 5 SCLC cell lines, significantly higher RRM1 mRNA expression was found in SCLC vs NSCLC³⁰⁹.

In tumors from patients with advanced NSCLC^{313–316} and SCLC³⁰⁶, there was a strong correlation between expression levels of RRM1 and of the nucleotide excision repair pathway factor ERCC1^{306, 313–316}, and in some studies a correlation was also noted with the DNA repair factor XPD³¹⁵. While no significant correlation between RRM1 mRNA and survival was seen in patients treated with gemcitabine plus vinorelbine and ifosfamide in one study³¹³, a number of other studies have suggested a link between tumor RRM1 expression and gemcitabine efficacy. For example, in NSCLC patients treated with gemcitabine alone³¹⁴ or combined with a platinum^{291, 308, 313–315}, with docetaxel³¹⁷, or with pemetrexed³¹⁸, RRM1 mRNA expression levels correlated inversely with survival^{291, 313–315, 317}, time to progression^{291, 317}, or response^{308, 315, 318}, and patients with low levels of both RRM1 and ERCC1 mRNA expression had significantly longer survival than did patients in whom both were high^{313, 314}. The related factor RRM2 also correlated inversely with response in one study³¹⁷, and patients whose tumors had high expression of both RRM1 and RRM2 had significantly lower response rates and shorter time to progression and overall survival than did patients in whom both were low³¹⁷.

In NSCLC pharmacogenetic studies, while RRM1 host gene polymorphisms did not correlate with outcome in patients treated with cisplatin plus docetaxel¹⁹⁶, the RRM1 promoter host allelotype in patients treated with gemcitabine +/- a platinum did correlate significantly with response (but not with overall survival or progression-free survival)³¹⁹.

In limited disease and extensive disease SCLC patients treated with a platinum plus etoposide, tumor RRM1 mRNA expression did not correlate with response or survival³⁰⁶.

Overall, available preclinical and clinical data indicate that tumor RRM1 expression probably is associated with resistance to gemcitabine-based therapy in NSCLC, and further studies are underway to explore RRM1 tumor expression as a tool to help in selecting therapy for patients with advanced NSCLC. Data are insufficient in SCLC to draw any conclusions.

8.4 Folate pathway

Pemetrexed cytotoxicity is mediated through the inhibition of the enzymes thymidylate synthase (TS), dihydrofolate reductase (DHFR), and glycinamide ribonucleotide formyltransferase (GARFT), and increased expression of these enzymes was associated with decreased efficacy of pemetrexed in NSCLC cell lines²⁶⁷.

8.5 Stathmin

Stathmin (oncoprotein 18) is a protein that plays an important regulatory role in tubulin dynamics. Transfection of the gene into lung cancer cells increased sensitivity to vinca alkaloids³²⁰, but in patients with advanced NSCLC treated with vinorelbine plus cisplatin, time to progression was shorter in patients with high stathmin than with low stathmin mRNA expression²⁹¹. Hence, its role in resistance remains unclear.

9.0 DNA repair

While some resistant cell lines appear to survive exposure to cisplatin and other agents by developing tolerance to drug-induced DNA damage, other resistant lines show evidence of significant repair of DNA damage⁹³ (Fig. 4). Clinically, several genes associated with DNA repair are overexpressed in NSCLCs³²¹. DNA repair pathways that could potentially be relevant for selected chemotherapy agents include the nucleotide excision repair pathway, homologous recombination repair, non-homologous end-joining repair, base excision repair, and mismatch repair⁴⁸.

9.1 Nucleotide Excision Repair Pathway

Components of the nucleotide excision repair pathway include XPA-XPG, ERCC1, Replication Protein A (RPA), Rad23A, DNA polymerases δ and ϵ , and DNA ligase.

9.1.1 Excision repair cross-complementation group 1 protein (ERCC1)—As outlined in a recent review⁴⁸, ERCC1 probably plays a major role in repair of cisplatin-induced DNA damage. In chemosensitivity testing of resected NSCLC tumors³²² and in a panel of NSCLC cell lines³²³, high NER activity³²³ and ERCC1 expression³²² correlated with cisplatin resistance, but ERCC1 expression did not correlate with NSCLC in vitro sensitivity to paclitaxel, vinorelbine, etoposide, irinotecan, 5-fluorouracil or gemcitabine³²², and in another panel of 15 NSCLC and 5 SCLC cell lines, there were no correlations between mRNA expression for ERCC1 or ERCC2 and chemosensitivity to cisplatin, carboplatin or gemcitabine³⁰⁹.

The ERCC1 protein was detected by IHC in approximately 40–55% of patients with early stage³²⁴ or advanced^{325–327} NSCLC. ERCC1 mRNA^{308, 314, 328–330} or IHC^{220, 325, 331, 332} expression was assessed in tumors from patients with advanced^{220, 308, 314, 325, 328–331} or resected^{324, 326, 332} NSCLC treated with therapeutic^{220, 308, 314, 325, 328–331}, neoadjuvant^{326, 332} or postoperative adjuvant³²⁴ regimens that combined a platinum with a taxane^{326, 329, 330, 332}, etoposide^{324, 326, 329}, a vinca alkaloid^{324, 325, 329, 330}, gemcitabine^{308, 314, 325, 327–329, 331}, irinotecan³³², mitomycin-C plus ifosfamide, radiation^{326, 332}, or with a variety of agents²²⁰. Patients treated with a combination of epirubicin plus gemcitabine have also been assessed³³¹. ERCC1 expression did not correlate with response^{220, 328–332}, progression-free survival²²⁰ or survival^{331, 332} in some studies. However, there was a significantly higher response rate³³² or a trend towards a higher response rate in ERCC1-negative tumors in other studies^{308, 325}. Furthermore, in some studies, median progression-free survival³²⁹ and/or overall survival^{220, 314, 324, 326–329} was significantly longer in patients whose tumors had low (or negative) vs high (or positive) ERCC1 expression in tumors, or there was a trend towards longer progression-free

survival³²⁶ or overall survival^{325, 330}. Patients with resected ERCC1-negative tumors derived substantially more survival benefit from platinum-based adjuvant chemotherapy than did patients with ERCC1-positive tumors³²⁴. Assigning patients with advanced NSCLC who had high ERCC1 mRNA expression to chemotherapy with docetaxel plus gemcitabine instead of to docetaxel plus cisplatin resulted in a significant increase in response rate³³³.

In patients with advanced NSCLC treated with platinum-based regimens, ERCC1 C8092A^{334, 335} and ERCC1 C118T^{262, 336} host genetic polymorphisms did not correlate independently with treatment outcome in some analyses, but ERCC1 C118T genotype was significantly associated with response³³⁷ or survival^{196, 335, 338} in other studies, and in still other NSCLC studies, the ERCC1 C8092A genotype was significantly associated with survival^{336, 339}.

In patients with limited disease SCLC treated with platinum combinations (mainly combined with etoposide³⁰⁶, etoposide plus ifosfamide³⁴⁰ or irinotecan³⁴⁰) high expression of ERCC1 was associated with poor overall survival. Little³⁴⁰ or no³⁰⁶ significant association with therapy efficacy was seen in extensive SCLC.

Overall, while not all studies agree, the data suggest a probable role for ERCC1 in resistance of NSCLC to platinum-based chemotherapy, while there are insufficient clinical data to draw conclusions in SCLC. Additional studies are underway assessing ERCC1 as a marker for allocation of patients to therapy with a platinum vs other agent.

9.1.2 Other components of the NER pathway—Antisense to xeroderma pigmentosum group A (XPA) gene reduced cellular NER capacity in NSCLC cells and sensitized cells to cisplatin³⁴¹, and the cell cycle checkpoint abrogator UCN-01 inhibited DNA repair by attenuating the interaction of XPA and ERCC1³⁴². Sensitivity to cisplatin was also increased by silencing of the NER component RAD23A³⁴³.

In clinical pharmacogenetic analyses of components of the NER pathway in advanced NSCLC patients receiving platinum-based chemotherapy, response rates varied significantly with XPC SS vs LL host genotype³³⁴, XPD Asp312Asn genotype³⁴⁴, XPD *A haplotype³⁴⁵, and with XPG 46His/His genotype³⁴⁶. However, in other studies, response did not vary with XPDAsp312Asn genotype²⁶², with XPD Lys751Gln genotype³³⁴, with XPG 1104His/Asp genotype³⁴⁶, and survival did not correlate with XPDAsp312Asn genotype^{262, 338} or with XPD Lys751Gln genotype^{196, 338, 347}. Even if individual polymorphisms did not correlate with outcome, there were significant interactions between XPC, XPD and ERCC1 genetic polymorphisms³³⁴ or between XPD and the base excision repair pathway component XRCC1³⁴⁸ in some studies, and survival worsened significantly as a patient's number of different unfavorable genotypes within the NER pathway increased, with ERCC1 8092, XPC intron 9, XPD 751 and ERCC6 1097 each contributing to outcome³³⁹. This latter model was further strengthened when polymorphisms for XPG 1104, NQO1, p53 and GSTpi exon 5 were added³³⁹.

Overall, the pharmacogenetic data available suggest a role for XPC and XPD in resistance of NSCLC to platinum-based chemotherapy.

9.2 Base excision repair

Components of the base excision repair pathway include DNA glycosylases, AP endonucleases, DNA polymerase β , XRCC1, poly(ADP-ribose) polymerase (PARP) and DNA ligase III. As outlined below, data are limited, but suggest a possible role for base excision repair in NSCLC resistance to platinum agents.

9.2.1 XRCC1—In clinical pharmacogenetic analyses in advanced NSCLC patients receiving platinum-based chemotherapy, response rates varied significantly with XRCC1 Arg194Trp genotype^{346, 348}. While response did not vary with XRCC1 399Arg/Gln genotype³⁴⁶, survival did vary significantly with this genotype in both NSCLC and SCLC patients treated with platinum-based regimens³⁴⁷. The pharmacogenetic data suggest a possible role for XRCC1 in resistance of NSCLC to platinum-based chemotherapy.

9.2.2 Apurinic/apyrimidinic endonuclease (APE1)—APE1 is involved in base excision repair and in redox signaling. Exposure of NSCLC cells to cisplatin in vitro resulted in increased expression of APE1, and APE1 inhibition by siRNA sensitized cells to cisplatin³⁴⁹. In NSCLC patients treated with cisplatin-based regimens, 84% of resistant tumors had high APE1 expression, compared to only 8% of responding tumors, and survival was better in patients with low APE1 expression ($p < 0.01$)³⁴⁹.

9.2.3 PARP—PARP inhibitors potentiated the effect of temozolomide³⁵⁰, toptecan³⁵⁰ and cisplatin³⁵¹ in lung cancer cell lines, suggesting that PARP played a role in resistance. Clinical trials of PARP inhibitors are in progress.

9.3 Homologous recombination repair

Components of the homologous recombination repair pathway include Rad51, DMC1, XRCC2, XRCC3, BRCA1, BRCA2, Eme 1 endonuclease, Fanconi Anemia proteins and DNA polymerase eta.

9.3.1 Rad51—In SCLC cell lines, resistance to etoposide correlated with protein levels of RAD51, and downregulation or overexpression of the RAD51 gene altered both etoposide efficacy and repair of etoposide-induced DNA double strand breaks³⁵².

Rad51 was expressed in 41% of clinical NSCLC tumor samples (particularly in squamous cell and poorly differentiated tumors)³²². In NSCLC cell lines, Rad51 expression was associated with reduced platinum efficacy, particularly if co-expressed with ERCC1, while its expression did not correlate with resistance to paclitaxel, etoposide, vinorelbine, gemcitabine, 5-FU, or irinotecan³²². In NSCLC cell lines, cisplatin exposure increased ERK1/2 activation³⁵³ and Rad51 protein induction³⁵³. Increased ERK1/2 signaling augmented expression of Rad51 protein, and blockage of ERK1/2 signaling activation by administering the EGFR tyrosine kinase inhibitor gefitinib concurrently with cisplatin potentiated the efficacy of cisplatin by decreasing cisplatin-induced augmentation of Rad51 protein expression through reduction of Rad51 mRNA and destabilization of the Rad51 protein³⁵³. Si-RNA reduction of Rad51 also significantly increased cell kill by cisplatin³⁵³. However, in one clinical study involving patients with NSCLC treated with cisplatin plus gemcitabine, hRad51 expression by IHC did not correlate with response or survival³³¹.

Overall, available preclinical data suggest that Rad51 might play a role in lung cancer resistance to platinum and etoposide, although this has not been confirmed clinically, and its role in resistance to other agents has not been explored in any detail.

9.3.2 Breast Cancer 1 (BRCA1)—BRCA1 interacts with Rad51 during repair of DNA double strand breaks. Its overexpression in resected early stage NSCLC was associated with poor survival³⁵⁴, and in cell lines, high expression correlated with resistance to platinum but sensitivity to antimicrotubule agents^{167, 355}. In patients with advanced NSCLC treated with cisplatin-gemcitabine or epirubicin-gemcitabine, IHC for tumor BRCA1 expression did not correlate with response or survival³³¹, and in patients treated with gemcitabine plus docetaxel, response rate increased and probability of tumor progression decreased with

higher tumor BRCA1 mRNA expression³⁵⁶. On the other hand, in NSCLC patients who received neoadjuvant cisplatin plus gemcitabine, survival was worse with high than with low tumor BRCA1 mRNA levels³⁵⁷, and high tumor BRCA1 mRNA expression was associated with worse progression-free survival in advanced NSCLC patients receiving second line platinum-based therapy³⁵⁶. In an assessment of impact of BRCA1 host genotype, patients with stage III-IV NSCLC treated with platinum regimens also had significantly worse survival if they had 2 copies of the wild-type BRCA1 AACC haplotype than if they had none or one copy, particularly if they had squamous cell lung cancer³⁵⁸. Overall, available data suggest that BRCA1 may increase resistance to platinum regimens but may increase sensitivity to taxanes.

9.3.3 Fragile histidine triad gene (FHIT)—FHIT blocks DNA homologous recombination repair and promotes apoptosis in cells with damaged DNA³⁵⁹. FHIT increased sensitivity to cisplatin in NSCLC cells that possessed wild type p53 and partially restored sensitivity to cisplatin in resistant cells that overexpressed Bcl-2- and Bcl-x(L)³⁶⁰. FHIT also increased sensitivity of lung cancer cells to paclitaxel in some assessments³⁶¹, but not in others³⁶⁰. However, transfection of FHIT-negative NSCLC cells with FHIT reduced sensitivity to etoposide, doxorubicin, and topotecan due to Fhit-induced downregulation of DNA topoisomerases I and II³⁶⁰.

9.3.4 Other homologous recombination repair pathway components—The Eme1 endonuclease was associated with resistance to cisplatin in lung cancer and other cell lines³⁶². In vitro sensitivity of resected lung cancer samples to cisplatin and carboplatin varied significantly with XRCC2 C41657T host genotype, but not with the XRCC2 G4234C genotype, although sensitivity was significantly greater with the 41567T/4234G haplotype than with the 41567T/4234C haplotype³⁶³. Expression of FANCD2 protein (a marker for Fanconi Anemia pathway functioning) was detected by IHC in 32% of tumor specimens from NSCLC patients, but did not correlate with response to platinum-based chemotherapy or with patient survival³⁶⁴.

9.4 Non-homologous end-joining repair

Components of the non-homologous end-joining repair pathway include Ku70, Ku80, DNA ligase IV, XRCC4, XRCC5, XLF, DNA-dependent protein kinase (DNA-PK(cs)) protein, and DNA polymerases λ and μ . Etoposide efficacy and etoposide-induced double strand breaks in SCLC cell lines varied with expression of DNA-PK(cs) proteins, suggesting a role for non-homologous end-joining repair in etoposide resistance³⁵². However, in vitro sensitivity of resected lung cancers to cisplatin and carboplatin did not vary with XRCC5 G74582A and C74468A host genotypes³⁶³.

9.5 Mismatch repair

Components of the mismatch repair pathway include MSH2, MSH3, MSH6, MLH1 and PMS1. As recently reviewed⁴⁸, cells deficient in mismatch repair may be paradoxically resistant to platinum agents, possibly since attempts at mismatch repair trigger apoptosis. The tumor suppressor gene NPRL2 is thought to be involved in DNA mismatch repair³⁶⁵. The expression of NPRL2 in NSCLC cell lines correlated with cisplatin sensitivity, transfection with NPRL2 gene augmented cisplatin sensitivity in NSCLC cell lines, and systemic administration of NPRL2 nanoparticles to mice bearing an orthotopic lung cancer model significantly enhanced the therapeutic efficacy of cisplatin and overcame cisplatin-induced resistance³⁶⁵.

In patients with stage III NSCLC receiving vinorelbine +/- cisplatin with radiotherapy, response to induction chemotherapy and survival were worse in patients with low vs high

IHC expression of hMSH2, while outcome did not correlate with hMLH1 expression¹⁹⁵. In NSCLC patients receiving gemcitabine with cisplatin, hMLH1 and hMSH2 expression by IHC did not significantly impact response or survival³⁶⁶. Hence, preclinical data and some clinical data support a role for mismatch repair deficiency in lung cancer resistance, but further clinical data are needed.

9.6 Deoxyribonucleotides needed for DNA repair and cell division: Thymidylate synthase (TS) and dihydropyrimidine dehydrogenase (DPD)

In resected NSCLC tumor samples, in vitro sensitivity to cisplatin and carboplatin correlated significantly with mRNA expression of TS and DPD, 2 enzymes that are critical in nucleic acid metabolism³⁶⁷, and that could thereby play a role in DNA repair. The correlation between TS expression and platinum sensitivity was particularly important for tumors from patients with some specific TS polymorphisms³⁶⁷.

9.7 High mobility group box 2 (HMG2)

HMG2 are chromatin-associated proteins that bend DNA and may be involved in DNA repair. However, NSCLC cells transfected with HMG2 gene actually have increased sensitivity to cisplatin compared to non transfected cells, with increased cellular platinum levels and decreased repair of DNA interstrand cross-links³⁶⁸.

10.0 Damage tolerance

In addition to resistance being due to a decrease in ability of chemotherapy to cause damage or an increase in ability to repair damage, resistance may also arise from increased damage tolerance, such that cellular damage occurs and is not repaired, but fails to cause cell death³⁶⁹. This could occur due to defective proapoptotic factors or to increased antiapoptotic factors (Fig. 5).

11.0 Reduced apoptotic response (Fig. 5)

11.1 P53

Mutations in p53 are very common in lung cancer cell lines³⁷⁰ and mutations were also found in 40–90% of resected NSCLC tumors^{371–373}. A significant increase in radiosensitivity was found for mutations in p53 exon 7 compared with mutations in other p53 exons³⁷⁰. While no correlation was found between type of p53 mutation and sensitivity to chemotherapy agents in some studies^{370, 372}, ability of p53 mutations to induce chemotherapy resistance did vary with the mutation type in another study³⁷⁴. NSCLC cell lines^{371, 374–376} or heterotransplants¹³⁴ with mutant (MT) p53 were resistant to cisplatin^{371, 374–376} (particularly low dose cisplatin³⁷⁴), cyclophosphamide³⁷¹, 5-fluorouracil³⁷⁵, etoposide^{374, 375}, methotrexate³⁷⁵, anthracyclines³⁷⁵, and bleomycin³⁷⁵, but were not resistant to paclitaxel^{134, 375}. Introduction of MTP53 into NSCLC cell lines bearing wild type (WT) p53 rendered the cells more resistant to cisplatin^{377, 378}, etoposide³⁷⁸, and camptothecin³⁷⁸ in some instances, while transfection of WTP53 into cells bearing MTP53 augmented sensitivity^{375, 378, 379}. Presence of MTP53 in NSCLC cell lines correlated significantly with overexpression of MRP¹⁴⁴.

Transfection of WTP53 into p53 null NSCLC cell lines only modestly increased sensitivity to carboplatin in one trial³⁸⁰, while transfection with WTP53 into p53 null^{379, 381–386} or WTP53^{381, 384} cells did augment sensitivity to cisplatin^{379, 381–386}, 5-fluorouracil^{379, 384}, doxorubicin³⁸⁴, taxanes^{379, 384}, irinotecan^{379, 384}, and etoposide³⁸⁴ in other NSCLC cell lines. Induced expression of p53 in p53 null NSCLC cells led to cellular senescence^{385, 387}, enhanced the cytotoxic effect of cisplatin³⁸⁵ but protected against etoposide³⁸⁵ and

paclitaxel³⁸⁷ cytotoxicity, and decreasing p53 expression by siRNA increased resistance to paclitaxel in NSCLC cells in one study¹⁶³. Induction of p21waf1 overexpression conferred increased resistance to both etoposide and cisplatin³⁸⁵. Similarly, transfection of p73 (a homologue of p53) into p53 null and Wtp53 NSCLC cells enhanced cell sensitivity to cisplatin and increased apoptosis, independently of p53³⁸⁸. The presence of hypoxia may elicit a downstream transcriptional response to p53 activation that favors cell cycle arrest over apoptosis³⁸⁹.

Exposure of Wtp53 NSCLC cell lines to docetaxel strongly induced expression of p53 mRNA, and docetaxel induced increased β -tubulin gene transcription in p53 null cells, but not in Wtp53 cells unless p53 was silenced by siRNA²⁷⁶. This suggests that p53 plays a role in preventing a compensatory increase in β -tubulin production in NSCLC cells exposed to taxanes.

Overall, the NSCLC cell line data suggest that Mtp53 may confer resistance to several agents. Loss of p53 function may possibly be associated with increased sensitivity to taxanes (instead of resistance) but the data are inconclusive.

In lung cancer cell lines, higher mRNA expression of the proapoptotic p53-binding protein 2 (53BP2) was also associated with increased sensitivity to cisplatin and radiation³⁹⁰.

Approximately 31%–63% of NSCLC tumors expressed p53 by IHC^{71, 173, 194, 327, 391, 392}. Positive IHC staining for p53 tended to be more frequent in tumors bearing p53 mutations, with a 70% concordance between IHC overexpression of p53 protein and mutation in p53³⁷¹, although this concordance was not seen in all studies³⁹³. Because of the frequent concordance between IHC positivity and p53 mutation status, IHC positivity is often used as a surrogate indicator of p53 mutation. However, correlations of drug resistance with tumor p53 IHC positivity do not reflect well the correlations seen between p53 mutation and resistance in cell lines. With in vitro sensitivity testing of resected NSCLC tumors, positive IHC expression of p53 correlated with a high degree of resistance to etoposide and doxorubicin but with reduced resistance to platinum, taxanes and gemcitabine in one study⁶. In another study, tumors that were p53 IHC positive had significantly greater in vitro resistance to 5FU and slightly higher resistance to doxorubicin than p53 negative tumors, while p53 IHC expression did not correlate with in vitro sensitivity to cisplatin, etoposide, vindesine or mitomycin-C³⁹⁴. In other in vitro sensitivity testing, cells from resected NSCLC tumors that had p53 mutations determined by gene sequencing were significantly more resistant to combined cyclophosphamide, etoposide plus epirubicin than were tumors with Wtp53, while sensitivity to carboplatin plus paclitaxel or etoposide did not correlate with p53 mutation status³⁹⁵.

Several NSCLC clinical studies have also assessed the relationship between tumor p53 mutations or IHC expression and outcome, and findings have been inconsistent across studies. In many of these studies, platinum agents were combined with one or more other agents, and in studies using more than one combination, there was generally no breakdown with respect to interaction (if any) of p53 abnormality and individual agent added to the platinum. Hence, it remains possible that some of the inconsistency is due to differences between different agents with respect to impact of the p53 abnormality. In patients receiving preoperative neoadjuvant cisplatin plus etoposide and radiation³⁹⁶ or in patients receiving cisplatin-based combinations for advanced disease³⁹³, presence of Mtp53 in tumor (defined by DNA sequencing) was associated with significantly lower response rates and shorter overall survival compared to patients with Wtp53, while there was no significant association between response and tumor p53 mutation status in patients with locally advanced NSCLC treated with radiotherapy and a taxane (without a platinum)³⁷³.

NSCLC studies assessing p53 IHC positivity have included patients receiving neoadjuvant therapy^{397, 398} and therapy for locally advanced^{195, 274, 399, 400} or metastatic^{71, 173, 194, 214, 327, 329, 391, 401–406} disease. P53 IHC positivity was associated with a lower probability of response^{71, 173, 194, 195, 400, 401, 406}, with a trend towards a lower response rate³⁹⁷, or with significantly shorter survival^{71, 194, 195, 329, 401, 402} for some studies combining a platinum with a vinca alkaloid^{173, 329, 401}, a taxane³²⁹, etoposide^{71, 401}, gemcitabine^{71, 329}, ifosfamide⁴⁰¹, mitomycin-C/ifosfamide⁷¹, mitomycin-C/vindesine⁴⁰⁰, 5FU/folinic acid⁴⁰¹, a vinca alkaloid plus radiotherapy (with or without a platinum)¹⁹⁵, or unspecified platinum combinations^{194, 406}. In one study in which an association was noted between p53 IHC expression and shorter overall survival, no significant association was seen with progression-free survival³²⁹.

On the other hand, there was no significant correlation between tumor p53 IHC expression and response^{391, 393, 398, 399, 403} or survival^{327, 391, 393, 398, 399, 404} in other studies in which a vinca alkaloid alone⁴⁰³ or combined with gemcitabine³⁹¹ was used, or in which a platinum was combined with etoposide⁴⁰⁴, gemcitabine^{327, 404}, irinotecan³⁹¹, radiotherapy²⁷⁴, a vinca alkaloid^{398, 399}, a vinca alkaloid plus radiotherapy³⁹⁹, mitomycin-C/ifosfamide⁴⁰⁴ or unspecified concurrent agents³⁹³. In 2 studies, response^{214, 405} or survival⁴⁰⁵ were paradoxically increased in patients whose tumors expressed p53 by IHC and who received a platinum combined with a vinca alkaloid²¹⁴, a taxane²¹⁴, etoposide²¹⁴, irinotecan²¹⁴, mitomycin-C²¹⁴, or unspecified platinum combinations⁴⁰⁵. In a metaanalysis of 16 published studies involving 1070 NSCLC patients treated with cisplatin-based regimens the association of p53 expression with response did not achieve significance, with a combined odds hazard ratio of 1.37 (0.84–2.24)³⁹².

SCLC tumors were positive for p53 by IHC in 48%–63% of patients^{173, 257}. Positivity for p53 correlated with positivity for metallothioneins²⁵⁷. Tumor p53 IHC positivity has been assessed in SCLC patients treated with a platinum combined with other agents including etoposide^{173, 257, 340, 402, 407}, irinotecan³⁴⁰, ifosfamide³⁴⁰, cyclophosphamide²⁵⁷, doxorubicin²⁵⁷, and vincristine²⁵⁷. It has also been assessed in SCLC patients treated with CAV without a platinum^{173, 402}, and with unspecified regimens¹⁴⁸. In SCLC, high p53 IHC expression did not correlate with patient outcome in some trials^{148, 173, 340, 402}, although there was a trend towards shorter survival in patients whose tumors were p53 IHC positive in another trial²⁵⁷, and significantly shorter survival in another⁴⁰⁷.

The possible importance of host p53 genotype in patient outcome was supported by the observation that in patients with advanced NSCLC treated with cisplatin combinations, p53 mRNA expression was significantly higher in the lymphocytes of nonresponders than in the lymphocytes of responders, and p21waf1 mRNA expression in lymphocytes was also significantly higher in non-responders⁴⁰⁸. In patients with advanced NSCLC treated with platinum-based chemotherapy, a higher response rate was seen in patients with the p53 codon 72 Pro allele and in those with p73 exon 2 G4C14/A4T14 or A4T14/A4T14 genotypes compared to the G4C14/G4C14 genotype⁴⁰⁹. The response rate in those carrying the WT genotypes for both p53 and p73 was only 7.7%, whereas the response rates in patients carrying 1, 2, or more than 2 variant alleles of p53 and p73 were 34.8%, 42.2% and 40.7%, respectively⁴⁰⁹. However, in another study, patients with p53 codon 72 Pro/Pro genotype had a significantly lower response rate to cisplatin plus irinotecan and shorter survival than did patients with the Arg/Pro or Arg/Arg genotypes³⁹¹. The p53 genotype did not correlate with response to gemcitabine plus vinorelbine³⁹¹. For MDM2 (a protein that binds to p53), SNP309 genotype did not correlate significantly with response to cisplatin-irinotecan or gemcitabine-vinorelbine, but the TT genotype was associated with significantly better survival than were the TG or GG genotypes³⁹¹.

Injection of adenovirus/WTp53 into tumors of NSCLC patients receiving carboplatin plus paclitaxel or cisplatin plus vinorelbine did not increase response rate, although the injected lesions did have a greater decrease in tumor size than did non injected lesions, particularly for patients receiving cisplatin plus vinorelbine⁴¹⁰.

Overall, there is fairly strong preclinical evidence for a role of MTP53 in lung cancer resistance to chemotherapy. While there have been few studies of actual p53 mutational status, the data from the available studies do support an association between p53 mutation and resistance to platinum-based therapy in NSCLC. Limited host genotyping studies have given conflicting results. IHC positivity for p53 (which generally correlates with p53 mutational status, but which is not necessarily synonymous with p53 mutation) correlated with benefit of platinum based therapy in some NSCLC and SCLC trials but not in others, and a metaanalysis in NSCLC was associated with a statistically insignificant trend towards an unfavorable outcome with p53 IHC positivity. Together the data indicate that clinical decisions re therapy choices should not be made based on p53 IHC positivity but that more studies should be done assessing actual p53 mutational status.

11.2 Caspases

In NSCLC cell lines, chemotherapy resistance was associated with defects in expression of caspase-3, -8, and -9⁴¹¹. NSCLC xenograft transfection with caspase-8 and -9 strongly induced apoptosis and also sensitized the tumors to cisplatin-induced cell death⁴¹¹. In other studies, NSCLC and SCLC cell lines exhibited normal caspase-9 activation in response to cisplatin, and while some NSCLC lines expressed the caspase-9 inhibitor TUCAN/CARDINAL/CARD8, siRNA down-regulation of TUCAN did not affect cisplatin-induced caspase-9 activation or cisplatin sensitivity⁴¹². In NSCLC cells, inhibition of caspase 9 by XIAP did not block apoptosis induction by cisplatin, topotecan, and gemcitabine, but stable expression of caspase-8 inhibitors (such as cytokine response modifier A) almost completely abrogated apoptosis and enhanced clonogenic survival⁴¹³.

Dexamethasone increased resistance of tumor cells from resected lung cancer specimens to both cisplatin and gemcitabine⁴¹⁴. Glucocorticoids induced chemotherapy resistance in lung carcinoma cells by down-regulating proapoptotic elements of the death receptor and mitochondrial apoptosis pathways, resulting in a decreased activity of caspase-8, caspase-9, and caspase-3⁴¹⁵. Transfection of glucocorticoid-treated cells with caspase-8 and -9 resensitized tumor cells and xenografts to cisplatin induced cell death⁴¹⁵. Clinical data on role of caspase expression in lung cancer resistance are very limited, but clinical assessments are warranted based on the preclinical data.

11.3 SAPK/c-Jun N-terminal kinase (JNK) and c-Jun

Activation of JNK plays a role in TRAIL-induced apoptosis⁴¹⁶. JNK phosphorylates 14-3-3, a cytoplasmic anchor of the proapoptotic protein Bax, thereby promoting apoptosis by releasing Bax for translocation to mitochondria⁴¹⁷. In NSCLC cells, gemcitabine induced activation of JNK (for which the transcription factor target is c-Jun) in sensitive parental cells, but not in gemcitabine-resistant variants⁴¹⁸. JNK inhibitors abrogated gemcitabine-induced apoptosis in sensitive cells, while transfection with JNK partially restored gemcitabine sensitivity in the resistant variant⁴¹⁸. Cisplatin also induced activation of JNK, and this in turn mediated induction of apoptosis⁴¹⁹.

In SCLC cells, cisplatin^{420, 421}, tumor suppressive anti-GD2-monoclonal antibodies⁴²¹, and other anticancer agents⁴²¹ stimulated activity of JNK^{420, 421}, suppressed tumor cell growth⁴²¹ and induced apoptosis⁴²¹. The anti-GD2-antibodies were synergistic with cisplatin in phosphorylating JNK and inducing apoptosis⁴²¹. The JNK inhibitor curcumin

decreased the effect of cisplatin and anti-GD2 antibodies⁴²¹. On the other hand, transfection with mutant JNK or overexpression of the JNK target, c-Jun, significantly protected SCLC cells from platinum compounds, but did not alter expression of genes involved in DNA repair, glutathione synthesis, or drug accumulation⁴²⁰.

11.4 β ig-h3

Expression of β ig-h3 (a secreted protein induced by transforming growth factor- β that may modulate cell adhesion and tumor formation, and that upregulates IGFBP3) was absent or reduced by more than two-fold in 35% of primary lung carcinomas relative to normal lung tissues⁴²². Lung cancer cells with low β ig-h3 expression were resistant to etoposide, and restoration of β ig-h3 expression resulted in a significant increase in sensitivity to etoposide, and also significantly suppressed tumor cell growth and tumorigenicity⁴²².

11.5 FUS1

In NSCLC cells deficient in FUS1, a tumor suppressor gene located in the human chromosome 3p21.3 region, restoration of function inhibited tumor cell growth, sensitized cells to cisplatin in vitro and in an orthotopic xenograft mouse model, down-regulated MDM2, and led to accumulation of p53 and activation of the Apaf-1-dependent apoptosis pathway⁴²³. Clinical trials are underway in NSCLC and SCLC to restore FUS1 by intravenous infusion of the *FUS1* gene entrapped in nanoparticles.

11.6 Glycosylphosphatidylinositol-anchored molecule-like (GML) protein gene

The GML gene, which is induced by WT p53, may participate in apoptosis. GML gene mRNA was detected in 30% of resected NSCLC tumor samples, and tumors that were p53 IHC-negative or WT were significantly more likely to express GML than were p53 positive or mutated tumors⁴⁰⁶. Cisplatin sensitivity in vitro and response to cisplatin-based chemotherapy clinically correlated with tumor expression of GML and with lack of IHC staining for p53⁴⁰⁶.

12.0 Apoptosis inhibitors

In some NSCLC cell lines, cisplatin⁴²⁴, etoposide⁴²⁵, radiation⁴²⁵, or Fas ligand⁴²⁵ induced normal activation of caspase-8⁴²⁴, -9^{424, 425} and -3^{424, 425}, and normal release of cytochrome c^{424, 425}, but did not demonstrate cleavage of nuclear substrates of caspase-3, such as PARP⁴²⁵, and did not demonstrate relocation of caspase-3 from cytosol to nucleus⁴²⁵ or other aspects of apoptosis inducibility⁴²⁴. This suggests that the inhibition of apoptosis in NSCLC occurs downstream of mitochondrial changes and caspase activation, and upstream of nuclear events⁴²⁵. Several factors in the cell could augment resistance to anticancer therapies by specifically or non-specifically opposing apoptosis:

12.1 Antiapoptotic factors (Fig. 5)

12.1.1 Cyclooxygenase-2 (COX-2)—NSCLC cells have constitutively high expression of COX-2, cytosolic phospholipase A2 (cPLA2), and prostaglandin (PGE2), and many also express 12-lipoxygenase and 12(S)-hydroxyeicosatetraenoic acid⁴²⁶. Constitutive expression of COX-2 resulted in increased expression of the apoptosis inhibitor survivin⁴²⁷. Exposure to cisplatin induced COX-2 expression in p53 wild type NSCLC cells, but not in p53 mutant cell, while paclitaxel exposure induced COX-2 expression in both p53 wild type and mutant cells⁴²⁸. Many non-steroidal anti-inflammatory drugs (NSAIDs) inhibit COX-2, and NSAIDs may also have effects on lung cancer cell lines that are mediated by targets other than COX-2⁴²⁹.

In one study using human NSCLC cell lines, indomethacin, Sulindac, tolmetin and other NSAIDs significantly increased the cytotoxicity of anthracyclines, epipodophyllotoxins, and vincristine (independently of their COX inhibitory ability, and possibly through impact on MRP), but did not enhance efficacy of the other vinca alkaloids, various antimetabolites, alkylating agents, platinum, paclitaxel and camptothecin⁴²⁹. However, in other studies, the COX-2 inhibitor celecoxib significantly potentiated the effect of docetaxel against NSCLC cell lines⁴³⁰, ⁴³¹ and xenografts⁴³¹. Sulindac sulfide (a COX-1 and COX-2 inhibitor), sulindac sulfone (exisulind, a proapoptotic agent that does not inhibit COX), and nordihydroguaiaretic acid (a lipoxygenase inhibitor) each inhibited growth of NSCLC and SCLC cell lines, and were synergistic with paclitaxel, cisplatin, and 13-cis-retinoic acid⁴²⁶. The COX-2 inhibitor nimesulide inhibited proliferation and induced apoptosis in NSCLC cell lines (particularly in cell lines with high COX-2 expression), and potentiated the effect of docetaxel, cisplatin or etoposide⁴³². The NSAID indomethacin substantially augmented the efficacy of vincristine in resistant NSCLC cells, and its ability to modulate efficacy varied with the chemotherapy agent used⁴³³. The COX-2 inhibitor NS-398 enhanced the efficacy of gemcitabine against a NSCLC cell line, and this potentiation was associated with up-regulation of the cyclin dependent kinase inhibitors p21WAF1 and p27KIP1 protein⁴³⁴. Growth hormone releasing hormone (GHRH) antagonists that inhibited growth of NSCLC xenografts also significantly decreased protein expression of COX-2, as well as decreasing expression of various other factors⁴³⁵.

From a prognostic perspective, COX-2 expression in NSCLC tumors has only a minor negative impact on survival, with the greatest negative impact seen in stage I patients in a meta-analysis⁴³⁶. However, it may increase resistance to chemotherapy in patients with advanced disease. NSCLC IHC expression of COX-2 correlated strongly with expression of the efflux pumps P-gp, MRP1 and BCRP⁴³⁷. Patients with advanced NSCLC receiving platinum-based chemotherapy had significantly worse survival if they had high baseline serum levels of the inflammatory marker C-reactive protein, and patients who had a reduction in serum C-reactive protein over the course of therapy had substantially better survival than did patients who did not have a decrease in serum C-reactive protein⁴³⁸. Similarly, in a phase II trial adding celecoxib to carboplatin plus paclitaxel in advanced NSCLC, responding patients had a greater drop in urinary excretion of PGE-M (a major metabolite of prostaglandin E₂) within one week of starting celecoxib compared to nonresponders, and very high initial urinary levels of PGE-M were associated with poor response to the chemotherapy⁴³⁹. In another trial in which celecoxib was combined with docetaxel in previously treated NSCLC patients, patients with the greatest reduction in urinary PGE-M had the longest survival⁴⁴⁰. In a randomized study combining the COX-2 inhibitor celecoxib with chemotherapy, COX-2 expression was not a negative prognostic factor for patients receiving celecoxib with chemotherapy, but it was a negative prognostic factor for those receiving chemotherapy alone⁴⁴¹. In pharmacogenetic analyses, there was a trend towards better outcome in patients with NSCLC treated with vinorelbine if they possessed the COX-2 1195G genotype¹⁸⁸. Overall, the data suggest that biomarkers related to COX-2 are associated with decreased chemotherapy efficacy in advanced NSCLC.

In clinical trials in which COX-2 inhibitors were added to chemotherapy, a few have suggested a beneficial impact of the COX-2 inhibitor, but most have not. In nonrandomized trials, patients with stage IB-IIIa NSCLC treated with preoperative carboplatin, paclitaxel plus celecoxib, had a response rate of 65%, which was somewhat better than expected with chemotherapy alone⁴⁴², and in patients with platinum-refractory NSCLC treated with docetaxel plus celecoxib, the response rate of 24%, median time to progression of 5 months and median survival of 11 months were superior to outcomes typically seen with docetaxel alone⁴⁴³. However, in other nonrandomized trials, there was no indication of benefit from addition of celecoxib to carboplatin/paclitaxel⁴³⁹ or to docetaxel in chemo-naïve patients

with advanced NSCLC⁴⁴⁴, from addition of celecoxib to docetaxel in previously treated NSCLC patients^{440, 445, 446}, nor from addition of celecoxib to cisplatin-etoposide in patients with SCLC⁴⁴⁷.

In randomized trials, addition of celecoxib to carboplatin plus gemcitabine in advanced NSCLC improved overall survival and progression-free survival for patients whose tumors expressed COX-2, while having no significant impact on those whose tumors did not express COX-2⁴⁴¹. Addition of the COX-2 inhibitor rofecoxib to gemcitabine did not significantly prolong survival but it did significantly improve response rate and quality-of-life⁴⁴⁸. Addition of celecoxib to irinotecan plus docetaxel vs gemcitabine in previously treated NSCLC patients led to a median survival of only 6.3 months in patients receiving celecoxib vs 9 months in patients receiving chemotherapy alone⁴⁴⁹.

Overall, both preclinical and clinical data suggest that COX-2 and related factors impact efficacy of some chemotherapy agents in NSCLC. However, COX-2 inhibitors have had little impact on chemotherapy efficacy. The data available suggest that any further trials of COX-2 inhibitors should be limited to patients whose tumors overexpress COX-2.

12.1.2 Bcl-2 and related proteins

12.1.2.1 Bcl-2: In sensitive NSCLC cells, cisplatin exposure resulted in activation of the mitochondrial death pathway, with activation of caspase-9 and downregulation of the antiapoptotic protein Bcl-2 through dephosphorylation, ubiquitination and enhanced proteosomal degradation⁴⁵⁰. The downregulation of Bcl-2 in sensitive cells appeared to be mediated by cisplatin-induced generation of peroxide and other reactive oxygen species, and this Bcl-2 downregulation was prevented by the antioxidant enzymes catalase and glutathione peroxidase⁴⁵⁰. Bcl-2 downregulation was also prevented by nitric oxide, which induced Bcl-2 S-nitrosylation, thereby inhibiting its ubiquitination and degradation⁴⁵¹.

In some NSCLC^{451–454} and SCLC^{455, 456} cell lines, high expression of the antiapoptotic protein Bcl-2 was associated with increased resistance to cisplatin^{451–456}, camptothecin⁴⁵³, doxorubicin^{453, 455, 456} and etoposide^{455, 456}, and Bcl-2 was constitutively phosphorylated in a cisplatin-sensitive parental SCLC cell line, but not in the cisplatin-resistant variant⁴⁵⁷. In NSCLC cell lines, nicotine exposure resulted in increased phosphorylation of Bcl-2 and resistance to cisplatin plus etoposide⁴⁵⁸. However, some other NSCLC¹⁶⁰ and SCLC⁴⁵⁷ cell lines demonstrated no relationship between Bcl-2 expression and resistance to doxorubicin¹⁶⁰, vincristine¹⁶⁰, etoposide^{160, 457}, paclitaxel^{160, 457}, 5-fluorouracil¹⁶⁰, or carboplatin¹⁶⁰.

In NSCLC cell lines^{453, 459–462} and orthotopic xenografts⁴⁶³ and in SCLC cell lines⁴⁵⁶, downregulation of Bcl-2 expression by siRNA^{459, 462}, by an antisense oligonucleotide^{453, 456, 463}, or by an aurora kinase inhibitor⁴⁶¹ increased spontaneous apoptosis⁴⁵⁹ and reduced resistance to cisplatin^{453, 456, 459, 460}, etoposide^{456, 460, 461}, anthracyclines^{453, 456, 460, 461}, camptothecin⁴⁵³, and vinorelbine⁴⁶³, but was paradoxically antagonistic with cisplatin, doxorubicin and etoposide in one SCLC cell line with low baseline bcl-2 expression⁴⁵⁶.

Taxanes may interact with Bcl-2 in a different manner than do other agents. Some NSCLC cell lines demonstrated no correlation between Bcl-2 expression and sensitivity to paclitaxel^{160, 457}, and cells derived from NSCLC tumor samples demonstrated increased sensitivity to docetaxel in the presence of high Bcl-2 expression, rather than increased resistance¹⁶⁵. In other NSCLC studies involving tumors heterotransplanted from patients into nude mice, all tumors that responded to paclitaxel were Bcl-2 positive at baseline, while only 43% of non-responding tumors were Bcl-2 positive ($p = 0.02$)¹³⁴. Furthermore, unlike the situation with most other chemotherapy agents, Bcl-2 downregulation did not increase

efficacy of docetaxel⁴⁶². Docetaxel inactivated NSCLC cellular Bcl-2 by phosphorylation, while cisplatin exposure led to Bcl-2 dephosphorylation⁴⁶².

Expression of Bcl-2 was seen in 5–37% of NSCLC tumors^{71, 165, 327, 464}. Despite the frequent association between tumor cell Bcl-2 expression and resistance in lung cancer cell lines, Bcl-2 expression was associated with a significant improvement (rather than worsening) in NSCLC prognosis across different stages and histologies in a meta-analysis⁴⁶⁵ and in individual large studies⁴⁶⁶. In locally advanced^{195, 274} or advanced^{71, 214, 327, 403, 464} NSCLC and in limited and extensive SCLC³⁴⁰, tumor Bcl-2 expression by IHC did not correlate with response^{195, 214, 403, 464} or survival^{195, 274, 327, 340, 464}, or was unexpectedly associated with significantly improved survival⁷¹ in patients treated with radiation plus vinorelbine +/- carboplatin¹⁹⁵, radiation plus daily low dose cisplatin²⁷⁴, docetaxel plus vinorelbine⁴⁶⁴, vinorelbine alone⁴⁰³, etoposide or irinotecan +/- ifosfamide³⁴⁰, or a platinum combined with a vinca alkaloid²¹⁴, taxane²¹⁴, etoposide^{71, 214}, irinotecan²¹⁴, gemcitabine^{71, 327}, mitomycin-C²¹⁴ or mitomycin-C/ifosfamide⁷¹. However, in SCLC patients receiving cyclophosphamide, epirubicin, and etoposide or cyclophosphamide, epirubicin and vincristine alternating with carboplatin and etoposide, shorter survival was seen with high tumor Bcl-2 IHC expression in multivariate analysis³⁰⁷.

In patients receiving neoadjuvant etoposide-cisplatin, tumor Bcl-2 IHC expression decreased following treatment⁴⁶⁷, while serum Bcl-2 levels did not change with therapy in patients with advanced NSCLC receiving platinum-based chemotherapy, and bcl-2 serum levels did not correlate with survival⁶⁸.

In a randomized trial in extensive SCLC of carboplatin plus etoposide +/- the bcl-2 antisense oligonucleotide oblimersen, the oblimersen did not alter response rates and was associated with worsening of failure-free survival and overall survival⁴⁶⁸, due in part to increased Myelosuppression and toxic deaths in the oblimersen arm.

Overall, despite substantial preclinical data suggesting that Bcl-2 is associated with resistance to chemotherapy, there is little indication that Bcl-2 expression is associated with worsening of outcome clinically. Across all stages high tumor Bcl-2 expression was associated with favorable survival, and most clinical studies have not found it to be a predictive factor negatively associated with response to chemotherapy.

12.1.2.2 Bcl-xL: Overexpression of the antiapoptotic protein Bcl-xL was found in NSCLC cell lines^{160, 469} and xenografts⁴⁶⁹ resistant to doxorubicin¹⁶⁰, vincristine¹⁶⁰, etoposide¹⁶⁰, paclitaxel¹⁶⁰, 5-fluorouracil¹⁶⁰ and gemcitabine⁴⁶⁹, and Bcl-xL expression was up-regulated following exposure to gemcitabine in a gemcitabine-resistant cell line⁴⁷⁰. However, a cisplatin-resistant SCLC cell line with reduced apoptosis in response to cisplatin also had reduced Bcl-xL levels compared to the sensitive parent, rather than having increased levels⁴⁵⁷.

In resistant NSCLC cell lines, downregulation of Bcl-xL expression by siRNA^{470, 471}, by an antisense oligonucleotide⁴⁷², by an aurora kinase inhibitor⁴⁶¹, or by a histone deacetylase inhibitor⁴⁷³, or Bcl-xL inhibition by a small molecule inhibitor⁴⁷⁴, augmented sensitivity to gemcitabine⁴⁷⁰, cisplatin^{471, 474}, paclitaxel^{472, 474}, etoposide^{461, 473, 474}, doxorubicin^{461, 474}, and vincristine⁴⁷². In keeping with this, augmentation of expression of Bcl-xL in response to FGF-2 augmented chemoresistance in SCLC cells⁴⁷⁵.

Clinically, response of NSCLC to vinorelbine did not correlate with tumor Bcl-xL expression⁴⁰³. Overall, preclinical data suggest a possible role for Bcl-XL in lung cancer chemoresistance, but clinical data are limited.

12.1.2.3 Myeloid cell leukemia-1 (Mcl-1) protein: NSCLC cells express abundant amounts of the antiapoptotic Bcl-2 family member Mcl-1, and resected NSCLC tumors expressed elevated levels of Mcl-1 protein compared with normal adjacent lung tissue⁴⁷⁶. Lung cancer cells overexpressing Mcl-1 were less sensitive to apoptosis induced by cisplatin, etoposide, paclitaxel and gefitinib, and depletion of Mcl-1 levels by antisense Mcl-1 oligonucleotides induced apoptosis in NSCLC cells and sensitized cells to apoptosis induced by chemotherapy agents and radiation⁴⁷⁶. Epidermal growth factor (EGF) enhanced Mcl-1 protein levels in an ERK-dependent manner⁴⁷⁶.

The pan-Bcl-2 inhibitor GX15-070 is a small molecule agent that binds to anti-apoptotic Bcl-2 proteins and interferes with their ability to interact with pro-apoptotic proteins⁴⁷⁷. GX15-070 disrupted Mcl-1:Bak interactions, induced apoptosis, and was synergistic with cisplatin in NSCLC cells⁴⁷⁷.

12.1.2.4 Other Bcl-2 family members: In still other NSCLC cell lines, apoptosis was increased on exposure to etoposide, paclitaxel, doxorubicin or cisplatin if a transfected gene for the pro-apoptotic Bcl-2 family member BAK was turned on vs off⁴⁷⁸, and in heterotransplants of resected NSCLC tumors from patients into nude mice, there was a trend toward a higher response rate in tumors positive for Bax (another pro-apoptotic Bcl-2 family member)¹³⁴. A cisplatin-resistant SCLC cell line also had a slight reduction in Bax levels⁴⁵⁷. However, overexpression (rather than under expression) of Bax has also been noted in resistant lines¹⁶⁰. Bax expression was reported in 63–68% of NSCLC tumor samples⁴⁶⁴. In patients with advanced NSCLC treated with vinorelbine, response rate did not vary with tumor expression of Bad⁴⁰³, Bak⁴⁰³, Bid⁴⁰³ or Bax⁴⁶⁴. Furthermore, in patients with advanced NSCLC treated with cisplatin-gemcitabine, survival did not correlate with Bax expression³²⁷, and outcome in SCLC patients treated with a platinum combined with etoposide or irinotecan +/- ifosfamide did not correlate with tumor bax expression by IHC³⁴⁰.

12.1.3 Survivin—Survivin expression in SCLC cells was increased by exposure to cisplatin or by constitutive activation of Akt⁴⁷⁹. In resistant NSCLC cells^{480–482}, SCLC cells⁴⁷⁹ and NSCLC xenografts⁴⁸³, transfection with an siRNA^{479, 481} or antisense oligonucleotide^{480, 482} to survivin caused apoptosis⁴⁸⁰, enhanced caspase-3 activity⁴⁸⁰, and enhanced efficacy of cisplatin^{479–481, 483}, etoposide⁴⁸² and paclitaxel⁴⁸¹. Nicotine protected NSCLC cells from apoptosis induced by gemcitabine, cisplatin, and paclitaxel by mediating the recruitment of E2F1, which led to dissociation of the retinoblastoma tumor suppressor protein (Rb) from the survivin promoter, thereby leading to induction of XIAP and survivin⁴⁸⁴. In patients with NSCLC treated with cisplatin plus etoposide, survivin expression by in situ hybridization correlated significantly with lack of response and with shorter survival⁴⁸⁵.

12.1.4 Livin—The anti-apoptotic protein Livin (also called ML-IAP or KIAP) is expressed in a significant proportion of NSCLC tissue samples and cell lines, and Livin siRNA sensitized Livin-expressing NSCLC cells to various pro-apoptotic stimuli, such as UV-irradiation and etoposide⁴⁸⁶.

12.1.5 X-linked Inhibitor of Apoptosis Protein (XIAP) and Smac/DIABLO—XIAP suppresses apoptosis by binding to caspases-3, -7, and -9. Exposure of NSCLC cells to nicotine led to Akt-mediated upregulation of expression of XIAP and increased resistance to cisplatin plus etoposide, and this antiapoptotic effect of nicotine was blocked by inhibitors of mitochondrial anion channels, Akt or MAPK⁴⁵⁸. The apoptogenic protein Smac/DIABLO^{487, 488} (or small molecule Smac mimics⁴⁸⁷ or an IAP-binding peptide⁴⁸⁸) overcame the effect of XIAP, enhancing cisplatin-induced⁴⁸⁷ or etoposide-induced⁴⁸⁸

apoptosis, with an increase in caspase-3 activity⁴⁸⁷. However, Smac deficiency does not appear to play a major role in resistance⁴⁸⁸. FGF-2 increased expression of XIAP and Bcl-xL and triggered resistance to etoposide in SCLC cells in a process mediated through the formation of a complex consisting of B-Raf, PKCepsilon and S6K2⁴⁷⁵.

12.1.6 Inhibitor of Apoptosis Proteins (IAPs)—In NSCLC cells (unlike SCLC cells), the apoptotic process was blocked downstream of caspase activation⁴⁸⁹. NSCLC cell lines had a stronger cIAP-2 expression than SCLC cell lines, while the SCLC cell lines had a higher level of XIAP protein⁴⁸⁹. However, following exposure to etoposide or radiation, expression of cIAP-1, cIAP-2, and XIAP did not appear to be sufficient to explain differences in sensitivity between NSCLC and SCLC cell lines⁴⁸⁹.

In NSCLC cell lines, exposure to gemcitabine decreased expression of IκB-α protein, thereby leading to increased activity of NF-κB, which in turn led to increased expression of the caspase inhibitor IAP-1⁴⁹⁰. Suppression of NF-κB activation blocked the increase of IAP-1 protein and potentiated the action of gemcitabine, while overexpression of IAP-1 restored resistance to gemcitabine⁴⁹⁰.

12.1.7 Telomeres and Telomerase—Many cancers have increased telomerase expression. This permits them to maintain telomeres, and this in turn permits ongoing propagation without apoptosis being triggered in response to telomere shortening. In NSCLC cells, inhibition of telomerase led to increased baseline apoptosis and enhanced induction of apoptosis by cisplatin, docetaxel and etoposide⁴⁹¹, and a telomerase-specific oncolytic adenovirus OBP-301 enhanced the effect of gemcitabine⁴⁹² and docetaxel⁴⁹³ in NSCLC xenografts. A histone deacetylase inhibitor that inhibited expression of mRNA for human telomerase reverse transcriptase (hTERT) also inhibited growth of SCLC cells that were resistant to etoposide, irinotecan and cisplatin⁴⁹⁴. Four of four NSCLC clinical samples expressed flap endonuclease I (FEN1), a protein involved in DNA repair and in preservation of telomere stability⁴⁹⁵. In multivariate analyses, serum hTERT levels were an independent predictor of short time to progression and overall survival in NSCLC patients treated with cisplatin plus docetaxel⁴⁹⁶, while high tumor expression of RAP1 (which negatively regulates telomere length) was associated with improved survival in patients undergoing NSCLC resection⁴⁹⁷, and tumor hTERT gene amplification⁴⁹⁸ and high tumor telomerase activity⁴⁹⁹ were associated with worse recurrence-free survival. However, results have not been consistent across all studies: in other studies of early stage NSCLC patients treated with surgery alone, high tumor hTERT mRNA expression⁵⁰⁰ was associated with improved 5-year survival, and telomere shortening was associated with worse outcome⁵⁰¹. The telomerase antisense compound GRN163L is currently undergoing early clinical trials in combination with chemotherapy in advanced NSCLC⁵⁰².

12.1.8 Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) Decoy receptor 1 (DcR1) and 2 (DcR2)—The TRAIL receptors DcR1⁵⁰³ and DcR2⁵⁰⁴ suppress TRAIL-induced apoptosis and their overexpression conferred resistance to doxorubicin and etoposide in lung cancer cell lines^{503, 504}, while their silencing enhanced chemotherapy-induced apoptosis⁵⁰⁴.

12.2.0 Growth Factor Receptors

12.2.1 Epidermal growth factor receptor (EGFR)—In some NSCLC cell lines^{505, 506} or resected tumors tested in vitro for chemosensitivity²⁴³, higher EGFR expression correlated with increased resistance to cisplatin^{243, 505}, doxorubicin⁵⁰⁵, etoposide⁵⁰⁵, vinorelbine⁵⁰⁶, paclitaxel⁵⁰⁶, camptothecin⁵⁰⁶, and 5-fluorouracil⁵⁰⁶. However, in other NSCLC cell lines³²³ or resected NSCLC tumor samples⁶, EGFR expression did not

correlate with cisplatin resistance³²³, or EGFR positive tumors were actually less likely to have extreme resistance to platinum than were EGFR negative tumors⁶. In NSCLC heterotransplants into nude mice, there was a statistically insignificant trend toward a lower response rate to paclitaxel in EGFR-positive tumors¹³⁴.

EGFR down-regulation by siRNA⁵⁰⁷ or inhibition by tyrosine kinase inhibitors (TKIs)^{198, 505, 508–513} or by the anti-EGFR monoclonal antibody cetuximab^{514, 515} increased sensitivity of NSCLC cell lines^{198, 505, 507–511, 513} or xenografts^{512, 514, 515} to platinum^{505, 507, 511, 512, 514, 515}, doxorubicin^{505, 507}, etoposide⁵⁰⁵, taxanes^{198, 507, 508, 511–515}, or pemetrexed^{509, 510} and did not alter efficacy of gemcitabine in one study⁵¹², while it did increase gemcitabine efficacy in another⁵¹⁴. The addition of the EGFR TKI erlotinib to pemetrexed inhibited the increase in EGFR phosphorylation⁵⁰⁹ and the induction of an EGFR-mediated activation of the PI3-kinase/AKT pathway⁵¹⁰ seen when cells were treated with pemetrexed alone⁵⁰⁹. Erlotinib also significantly reduced the expression and activity of the pemetrexed target thymidylate synthase⁵⁰⁹. The presence of P-gp overexpression did not prevent the EGFR TKI gefitinib from reversing resistance to paclitaxel¹⁹⁸.

EGFR TKI augmentation of chemotherapy efficacy was schedule-dependent. For docetaxel, the optimum schedule was docetaxel followed by the EGFR TKI gefitinib⁵⁰⁸ or erlotinib⁵¹³. Similarly, with pemetrexed, synergism was seen in NSCLC cell lines when erlotinib was administered concurrently with and/or following pemetrexed^{509, 510}, while antagonism (associated with erlotinib-induced G1 blockade) was seen if erlotinib preceded pemetrexed⁵¹⁰ or docetaxel⁵¹³. In one series of NSCLC cell lines, gefitinib antagonized the effect of cisplatin in 13 of 15 lines tested, and was also antagonistic to cisplatin combined with paclitaxel⁵¹⁶. The antagonism appeared to possibly be related to a gefitinib-induced reduction in cisplatin uptake at higher cisplatin doses, and was not seen if the gefitinib was delayed until 24 hours after the cisplatin⁵¹⁶.

The synergism (or antagonism⁵¹⁶) between an EGFR TKI and pemetrexed^{509, 510}, cisplatin^{511, 516} or paclitaxel⁵¹¹ did not vary with EGFR mutation status^{509–511} or with sensitivity to erlotinib alone⁵¹⁰, whereas cetuximab improved efficacy of cisplatin or paclitaxel against NSCLC xenografts only in models that were sensitive to cetuximab alone⁵¹⁵. In some studies, the sensitization to chemotherapy by EGFR inhibitors was seen only in cell lines with high EGFR expression, and not in lines with low EGFR expression⁵⁰⁵. In other studies, the cell lines that were most likely to display synergism between the EGFR TKI gefitinib and cisplatin or paclitaxel were those that exhibited an increase in phospho-EGFR (pEGFR) following exposure to chemotherapy alone, while cell lines that showed no change or a decrease in pEGFR following chemotherapy exposure were more likely to exhibit an antagonistic or additive interaction between gefitinib and the chemotherapy⁵¹¹.

Approximately 49–54% of NSCLC tumor samples express EGFR⁵¹⁷. In a NSCLC meta-analysis, EGFR expression was significantly associated with poor survival when EGFR expression was assessed by IHC⁵¹⁷. However, when EGFR expression was assessed by Northern blot, PCR or ligand competition methods, there was no correlation with survival⁵¹⁷.

In patients with locally advanced¹⁹⁵ or advanced^{518–520} NSCLC undergoing treatment with chemoradiotherapy¹⁹⁵ or with front-line chemotherapy^{518–520}, response rate^{195, 518–520}, progression-free survival⁵¹⁹ and overall survival^{195, 519} did not correlate with EGFR IHC expression^{195, 518, 519} or with EGFR gene copy number by FISH^{519, 520}. While presence of EGFR mutations did not correlate with response to chemotherapy or time to progression in

one NSCLC study⁵²¹, patients with advanced NSCLC treated with chemotherapy in another study did survive significantly longer if their tumor had an EGFR mutation rather than being wild type⁵²², and in still another study, among patients with EGFR gene mutations, response to chemotherapy was observed only in those with exon 19 deletion⁵¹⁸.

In advanced NSCLC, addition of gefitinib to gemcitabine plus cisplatin¹⁹⁹ or to paclitaxel plus carboplatin²⁰⁰ did not improve survival, time to progression, or response rate, and erlotinib added to cisplatin and gemcitabine also failed to improve outcome⁵²³, while erlotinib added to carboplatin plus paclitaxel improved outcome only in patients who had never smoked⁵²⁴.

Overall, preclinical studies suggest that EGFR expression may increase resistance to chemotherapy and that antagonizing EGFR may reduce resistance to chemotherapy, with the interaction between chemotherapy and EGFR inhibitors being schedule-dependent. However, clinical studies suggest that EGFR inhibitors generally do not potentiate the effect of chemotherapy, and that EGFR expression has little impact on resistance to chemotherapy, but tumors with EGFR mutations (particularly exon 19 deletions) have increased sensitivity to chemotherapy.

12.2.2 HER-2/neu (erbB-2)—HER-2/neu expression is present more frequently in NSCLC cell lines than in SCLC (where it is present infrequently)⁵²⁵. In NSCLC cell lines, high expression of glycoprotein p185neu (the product of *HER-2/neu* gene expression, a membrane-bound receptor with tyrosine kinase activity)^{372, 526, 527} or high HER-2/neu mRNA expression⁵²⁸ correlated with resistance to etoposide^{372, 526–528}, doxorubicin^{372, 526, 528}, cisplatin^{372, 526–528}, and other agents⁵²⁸. High p185neu expression also correlated with low S-phase fraction³⁷², and long doubling time³⁷². In NSCLC heterotransplants in nude mice, resistance to paclitaxel correlated strongly with HER-2/neu expression¹³⁴. High HER-2/neu expression was not associated with resistance to gemcitabine in NSCLC cell lines, and the combination of gemcitabine plus cisplatin had greater cytotoxicity against the high- than the low-p185neu expressors⁵²⁷. High HER-2/neu expression (but not EGFR expression) correlated with nucleotide excision repair pathway activity and with cisplatin resistance³²³, and HER-2/neu depletion down-regulated DNA repair mechanism⁵²⁹.

Her-2/neu depletion⁵²⁹ or inhibition by a TKI^{526, 530} or by the monoclonal antibody trastuzumab⁵²⁵ increased sensitivity of NSCLC cells to cisplatin^{525, 526, 529, 530}, gemcitabine⁵²⁵, paclitaxel⁵²⁵, vinorelbine⁵²⁵, doxorubicin^{526, 530} and etoposide^{526, 530} in Her-2/neu positive NSCLC cell lines.

In a meta-analysis, overexpression of Her-2/neu appeared to be a potential marker of poor prognosis for survival in NSCLC⁵³¹. In patients with advanced NSCLC, response rate to front-line chemotherapy did not correlate with HER2 gene copy number by FISH⁵¹⁸. Similarly, Her-2/neu IHC expression did not correlate significantly with response to induction or neoadjuvant chemotherapy^{195, 397, 399, 532, 533} or with survival^{195, 399, 532, 533} in patients with stage II³⁹⁷ or III^{195, 397, 399, 532, 533} NSCLC treated with a vinca alkaloid +/- cisplatin plus radiation^{195, 399}, neoadjuvant cisplatin-vinorelbine³⁹⁷ or neoadjuvant cisplatin plus etoposide (followed by postoperative radiotherapy plus either cisplatin, etoposide⁵³² or carboplatin, vindesine⁵³³). However, there was a nonsignificant trend towards chemoresistance in tumors that were Her-2/neu positive in two studies^{397, 533}, and in one of these two studies, chemoresistance was observed statistically significantly more often when one or both of Her-2/neu and p53 were positive³⁹⁷. There was also a trend to a higher proportion of tumors being positive after therapy vs pre therapy in one study, suggesting induction of Her-2/neu expression or else selection of positive tumor cells⁵³³.

Overall, preclinical data suggest an association between Her-2/neu expression and chemoresistance in NSCLC, while clinical data are at best equivocal.

12.2.3 Insulin-like growth factor-1 receptor (IGF-1R)—In a NSCLC cell line and in a NSCLC xenograft model, inhibition of IGF-1R significantly enhanced sensitivity to cisplatin and increased apoptosis⁵³⁴. Similarly, in SCLC cell lines, IGF-1R was a potent growth factor and inhibited etoposide- and carboplatin-induced apoptosis through activation of the PI3K/Akt pathway⁵³⁵. Clinically in a randomized phase II trial in advanced NSCLC, addition of the anti-IGF-1R antibody CP-751,871 to paclitaxel plus carboplatin was associated with an increase in response rate from 42% with chemotherapy alone to 54% with the addition of the IGF-1R antagonist, with the greatest apparent benefit being seen in squamous cell lung cancer⁵³⁶. Response of squamous cell carcinoma to single agent CP-751,871 was also seen in 2 patients⁵³⁶.

12.2.4 $\beta(1)$ -integrins, extracellular matrix and cell adhesion—Interaction of cells with extracellular matrix (ECM) proteins such as fibronectin (FN) initiates clustering of integrin receptors in the cell membrane, leading to activation of signaling pathways regulating survival, proliferation, differentiation, adhesion, and migration⁵³⁷. Cisplatin, paclitaxel, mitomycin-C or radiation stimulated clustering of $\beta(1)$ integrins in NSCLC cells only if the cells were attached to FN, and attachment to FN led to a significant reduction in sensitivity to these agents⁵³⁷.

Attachment of SCLC cells to ECM^{485, 538, 539}, or stromal cells⁵³⁹ or the ECM components FN⁵⁴⁰, laminin⁵⁴⁰ or collagen IV⁵⁴⁰ conferred resistance to apoptosis induction by etoposide^{485, 538–541}, cisplatin⁵⁴⁰, doxorubicin⁵⁴⁰, cyclophosphamide⁵⁴¹ and radiation^{485, 541}. SCLC cellular attachment was also associated with morphological changes, with down-regulation of neuroendocrine markers, and with up-regulation of several factors, including epithelial differentiation markers (cyclin D1 and endothelin), and cell adhesion molecules (CD 44 and integrin subunits $\alpha 2$, $\beta 3$ and $\beta 4$)⁵⁴¹. Transfection of SCLC cells with the gene for osteopontin (a hypoxia-associated cytokine that plays a role in cell adhesion and binds to integrin- α -V / integrin- β -3) reduced sensitivity to cisplatin-induced apoptosis⁵⁴². Similarly, transfection with hyaluronan/CD44 (a receptor for hyaluronate, osteopontin, collagen and matrix metalloproteinases that plays a role in cell adhesion) increased cisplatin resistance of NSCLC cells cultured on hyaluronate-coated plates by inducing increased MRP2 expression⁵⁴³.

SCLC cells also express high levels of CXCR4 receptors for the chemokine stromal-cell-derived factor-1 (SDF-1/CXCL12)⁵³⁹. CXCL12-induced integrin activation resulted in an increased adhesion of SCLC cells to fibronectin and collagen, mediated by $\alpha 2$, $\alpha 4$, $\alpha 5$, and $\beta 1$ integrins along with CXCR4 activation⁵³⁹. CXCR4 inhibitors decreased the protection from etoposide-induced apoptosis afforded by stromal cells⁵³⁹.

Cell attachment was also associated with augmented activation of phosphoinositide-3-OH kinase (PI3K)⁵³⁸, Akt⁵⁴¹, MAP kinase⁵⁴¹, other protein tyrosine kinases⁵⁴⁰, Bad protein⁵⁴¹ and nuclear factor- κ B (NF- κ B)⁵⁴¹. These adhesion-related events led to suppression of caspase-3 activation and apoptosis⁵³⁸, and prevention of etoposide- and radiation-induced G2/M cell cycle arrest (by blocking up-regulation of p21Cip1/WAF1 and p27Kip1 and by down-regulation of cyclins E, A and B)⁵³⁸. The ability of adhesion to ECM to block therapy effects was abrogated by PI3K inhibition⁵³⁸. Cultivation of adherent SCLC sublines on uncoated surfaces reversed their adherent phenotype immediately, and Akt activity reverted to low levels⁵⁴¹. Similarly, conditioned medium from primary tumor fibroblasts decreased efficacy of paclitaxel (but not of cisplatin) against NSCLC cells, and this resistance appeared to be mediated by activation of ERK 1/2 and Akt⁵⁴⁴.

In patients with advanced NSCLC treated with cisplatin/paclitaxel +/- bevacizumab, high vs low baseline plasma levels of the integrin ligand intercellular adhesion molecule (ICAM) were associated with significantly lower response rates and shorter overall survival time, and beneficial impact of bevacizumab on survival was greatest for patients with low ICAM levels⁷⁰. Overexpression of matrix metalloproteinase-7 (MMP-7, matrilysin) (which helps maintain ECM homeostasis and stabilizes cell-matrix interactions) in patients with advanced NSCLC treated with platinum regimens was associated with a significant reduction in response rates and survival, particularly in patients with lung adenocarcinomas⁵⁴⁵. Matrilysin exposure also increased resistance of a NSCLC cell line to cisplatin⁵⁴⁶. For patients with advanced NSCLC on both arms of a randomized trial of carboplatin/paclitaxel +/- tiripazamine, progression-free and overall survival was significantly longer and response probability was higher in patients with low vs high plasma osteopontin levels⁶⁹. Patient outcome and plasma osteopontin levels did not correlate with tumor osteopontin levels⁶⁹.

Overall, available preclinical data suggest that adherence and integrins are important in resistance, and limited clinical data support this.

12.2.5 Other receptors—In NSCLC orthotopic xenografts, cells were positive for growth hormone releasing hormone (GHRH) receptors and peptide, and inhibition of GHRH decreased tumor growth, significantly decreased protein expression of K-Ras, COX-2 and pAkt, and was synergistic with docetaxel⁴³⁵. In SCLC patients treated with cisplatin plus etoposide, time to progression and survival was longer for patients positive for c-kit by IHC than for those negative⁵⁴⁷. Imatinib inhibited the growth of a cisplatin-resistant NSCLC cell line, and synergistically augmented tumor cell killing by cisplatin, probably through its ability to inhibit phosphorylation of platelet derived growth factor receptor (PDGFR) α ⁵⁴⁸. By IHC of clinical samples, 89% of squamous lung carcinomas, 100% of lung adenocarcinomas, and 100% of small cell lung cancers expressed PDGFR α ⁵⁴⁸.

12.3 Growth factors

Hepatocyte growth factor (HGF) (the ligand for the c-Met receptor) is frequently overexpressed in NSCLC, and addition of HGF to NSCLC cell lines increased cisplatin resistance⁵⁴⁹. This augmentation of cisplatin resistance appeared to be mediated by suppression of apoptosis-inducing factor (AIF) via the HGF receptor's downstream effector, focal adhesion kinase (FAK)⁵⁴⁹. Fibroblast growth factors (FGF)-2 increased the expression of the antiapoptotic proteins, XIAP and Bcl-X(L), and rendered SCLC cells resistant to etoposide⁴⁷⁵. These effects were mediated through the formation of a specific multiprotein complex comprising B-Raf, PKCepsilon and S6K2⁴⁷⁵. In patients with advanced NSCLC treated with platinum-based regimens, expression in blood of PC cell-derived growth factor (PCDGF) (a growth factor originally derived from the PC teratoma cell line) was somewhat higher in chemoresistant patients than in chemosensitive patients, and tumors expressing PCDGF by IHC were also more resistant to platinum-based chemotherapy²²².

12.4.0 Signaling pathways

12.4.1 Phosphatase and tensin homolog (PTEN)/Phosphoinositide 3-kinase (PI3K)/ Protein kinase B (Akt)/mammalian target of rapamycin (mTOR) pathway—The PI3K/Akt pathway is important in many cellular functions, and activating mutations of PI3K are present in many malignancies. The tumor suppressor gene PTEN inhibits PI3K, but PTEN may be mutated, deleted or hypermethylated in cancers, or may be unable to silence PI3K if the PI3K gene has an activating mutation.

12.4.1.1 PTEN/PI3K: In NSCLC cell lines, SiRNA inactivation of PTEN increased resistance to paclitaxel¹⁶³. Similarly, hypoxia activated the PI3K/Akt pathway and induced resistance to UV light and etoposide⁵¹. This hypoxia-induced resistance was reversed by blocking activation of PI3K/Akt⁵¹. In SCLC cell lines, β 1 integrin (expressed following adhesion to ECM⁵³⁸) and components of important SCLC autocrine loops (stem cell factor and insulin-like growth factor⁵⁵⁰) potentially activated PI3K⁵³⁸. 550, and activated PI3K prevented etoposide-induced caspase-3 activation and subsequent apoptosis⁵³⁸.

Inhibition of PI3K sensitized SCLC cell lines to etoposide⁵⁵⁰, and sensitized NSCLC cell lines^{51, 454, 551–553} or xenografts⁵⁵⁴ to apoptosis induced by paclitaxel^{551, 552, 554}, docetaxel⁴⁵⁴, cisplatin⁵⁵², etoposide^{51, 552, 553}, gemcitabine⁵⁵², trastuzumab⁵⁵² and radiation⁵⁵².

12.4.1.2 Akt: Akt is a key factor in the suppression of anoikis and in the modulation of chemotherapy-induced apoptosis⁵⁵⁵, and a high proportion of NSCLC cell lines have PI3K-dependent constitutive activation of Akt1⁵⁵². Akt1 overexpression⁵⁵⁶, gene amplification⁵⁵⁶ or constitutive activation^{552, 557} rendered NSCLC cell lines resistant to cisplatin^{556, 557}, doxorubicin⁵⁵⁷, mitoxantrone⁵⁵⁷, paclitaxel⁵⁵⁷, etoposide⁵⁵², 5-fluorouracil⁵⁵⁷ and radiation⁵⁵². Akt modulated the apoptotic threshold of several apoptotic pathways towards increasing the threshold of onset⁵⁵⁷. Nicotine exposure led to increased Akt-mediated resistance to cisplatin/etoposide-induced apoptosis in NSCLC cells⁴⁵⁸. Following exposure to cisplatin, increased expression of Bcl-x(l) and a delayed onset of the p53 pathway was noted in resistant Akt1-expressing cells⁵⁵⁷.

Akt activity^{551, 552} and phosphorylation⁵⁵² were decreased by PI3K inhibition, and inhibition of Akt by PI3K inhibitors sensitized NSCLC cell lines to a variety of chemotherapy agents and radiation, as outlined above in the PI3K section. The augmentation by PI3K inhibition of chemotherapy-induced apoptosis was seen predominantly in cells with high Akt levels⁵⁵². The effect of PI3K inhibitors on etoposide efficacy in SCLC cell lines was reversed by expression of constitutively activated Akt⁵⁵⁰. Inhibition of Akt by transfection with dominant negative Akt^{552, 556}, by siRNA⁵⁵⁸ or by growth hormone releasing hormone antagonists⁴³⁵ also decreased NSCLC cell resistance to etoposide⁵⁵², cisplatin^{552, 556, 558}, docetaxel⁴³⁵ and paclitaxel⁵⁵², and significantly decreased ERK activity⁵⁵⁸. The c-Src antagonist dasatinib decreased expression of Akt in SCLC cells and sensitized them to the anthracycline amrubicin⁵⁵⁹.

Adhesion of SCLC cells to ECM components increased Akt activation^{541, 560} and decreased sensitivity to a variety of chemotherapy agents⁵⁴¹. Inhibitors of the PI3K/Akt/mTOR pathway abrogated ECM-mediated survival⁵⁶⁰, and cultivation of cells on uncoated surfaces resulted in reversion of Akt activity to low levels⁵⁴¹.

In patients with advanced NSCLC, response rate to front-line chemotherapy did not correlate with p-Akt status by IHC^{518, 521}.

12.4.1.3 mTOR: In NSCLC cell lines, Akt1-induced resistance to cisplatin appeared to be mediated through mTOR⁵⁵⁶, and the mTOR inhibitor CCI-779 enhanced the effect of cisplatin in cisplatin resistant cell lines⁵⁶¹.

12.4.1.4 P70 S6 kinase: Activation of p70 S6 kinase by mTOR results in phosphorylation of the S6 ribosomal protein, and this in turn induces protein synthesis by ribosomes. In SCLC cells, levels of phosphorylated p70S6K and S6 (but not total p70S6K or S6) were elevated in a cisplatin-resistant variant compared to the sensitive parent⁵⁶². Cell exposure to cisplatin

activated p70S6K, and downregulation of p70S6K or its inhibition by rapamycin augmented cisplatin-induced apoptosis⁵⁶².

Overall, there is substantial preclinical evidence that the PTEN/PI3K/Akt/mTOR pathway can render lung cancer cells resistant to several chemotherapy agents, but clinical data remain very limited.

12.4.2 K-ras—In a panel of 20 NSCLC cell lines, presence of K-ras mutation did not correlate significantly with resistance to doxorubicin, cisplatin, melphalan, or carmustine, and mutant lines were slightly more sensitive to etoposide and mitomycin-C than were K-ras wild type lines⁵²⁸. In lung cancer cell lines with both mutant and wild-type K-ras, the farnesyl transferase inhibitor lonafarnib was synergistic with paclitaxel when administered after the paclitaxel, but was antagonistic when administered before paclitaxel⁵⁶³. Lonafarnib prevents post-translational modifications of K-ras protein that are required for localization of K-ras in membrane, where it can participate in signaling. RasGTPase-activating protein (RasGAP) (the main regulator of Ras GTPase family members) was cleaved at low caspase activity into an N-terminal fragment that triggered potent anti-apoptotic signals via activation of the Ras/PI3K/Akt pathway⁵⁶⁴. When caspase activity was increased, RasGAP fragment N was further processed into two fragments that effectively potentiated apoptosis⁵⁶⁴. Hence, whether RasGAP was anti-apoptotic or pro-apoptotic depended on the degree of caspase activation. SCLC cell lines (which were sensitive to therapy) had lower RasGAP expression levels and higher spontaneous cleavage than did more resistant NSCLC cell lines⁵⁶⁴. Constitutive formation of RasGAP fragment N could potentially contribute to primary resistance of NSCLC and to acquired resistance in SCLC⁵⁶⁴.

K-ras mutations were noted in approximately 21% of patients with NSCLC⁵²² and in 26% of lung adenocarcinomas⁵⁶⁵. In a meta-analysis, there was a significantly worse survival for patients with NSCLC whose tumors had K-ras mutations than with K-ras wild type⁵⁶⁶, and in a phase II trial of paclitaxel in NSCLC, K-ras mutations appeared to correlate with resistance⁵⁶⁷. However, in patients with advanced NSCLC treated with a mesna, ifosfamide, carboplatin, etoposide combination⁵⁶⁵ or with carboplatin plus paclitaxel⁵²², K-ras mutations did not correlate with response or survival^{522, 565}, although there was a trend towards a lower response rate in patients with K-ras mutations in one trial of advanced NSCLC patients treated with platinum-based chemotherapy⁵⁶⁸. In patients with resected NSCLC, adjuvant chemotherapy with cisplatin plus vinorelbine was associated with significant improvement in survival in patients whose tumors were K-ras wild type, with a hazard ratio of 0.69, while in patients with K-ras mutant tumors, chemotherapy did not significantly improve survival (hazard ratio = 0.95)⁵⁶⁹. However, in multivariate analysis in this study, there was no significant interaction between the impact of chemotherapy and the presence of a K-ras mutation ($p=0.27$), and K-ras mutation was not found to be a prognostic factor⁵⁶⁹. In patients treated with adjuvant cisplatin plus etoposide plus radiotherapy after NSCLC resection, there was also a trend towards worse outcome in patients with K-ras mutant tumors, while K-ras mutational status was not associated with outcome in patients treated with postoperative radiotherapy alone⁵⁷⁰. Survival was significantly shorter in advanced NSCLC patients with K-ras mutations (but not in K-ras wild type patients) if the EGFR TKI erlotinib was added to chemotherapy compared to chemotherapy alone⁵²².

In NSCLC patients with taxane-refractory NSCLC who received the farnesyltransferase inhibitor lonafarnib (which inhibits mutant K-ras function) combined with paclitaxel, 10% of patients experienced a response and 38% had stable disease⁵⁷¹, suggesting that paclitaxel efficacy may have been modestly augmented by the lonafarnib.

Overall, available data suggest a possible role for K-ras mutations in lung cancer resistance to chemotherapy, but the data are not conclusive. Available data do suggest a harmful effect of adding an EGFR TKI to chemotherapy in patients with mutant K-ras.

12.4.3 Extracellular signal-regulated kinases 1 and 2 (ERK1/2)—ERK1 and ERK2 are examples of mitogen-activated protein kinases (MAPKs), and are downstream from Ras, Raf and MEK in the Ras pathway. Some etoposide-resistant SCLC cell lines had markedly increased MAPK activity⁵⁴¹. Hypoxia activated the ERK pathway and increased resistance of NSCLC lines to UV light or etoposide, and blocking the ERK pathway reversed this hypoxia-induced resistance⁵¹. Inhibition of ERK in NSCLC cell lines with constitutive ERK1/2 activity also potentiated paclitaxel-induced apoptosis (suggesting that constitutive ERK1/2 activity in NSCLC cells promotes cell survival and chemotherapy resistance), while MEK inhibitors had no effect on apoptosis⁵⁷². In taxane-sensitive cells, taxanes inhibited MAPK activity and p34cdc2 kinase activity, and this kinase inhibition in turn played a role in altering microtubule dynamics by enhancing the interaction of microtubule-associated proteins with α and β tubulins⁵⁷³. This inhibition of MAPK was not seen in a taxaner-resistant SCLC cell line, suggesting an alteration in the MAPK cascade in resistant cells⁵⁷³.

However, in other studies, inhibition of ERK reduced sensitivity of NSCLC cell lines to paclitaxel⁵⁷⁴, and exposure of NSCLC cells to cisplatin led to a marked increase in ERK activity, while pretreatment of these cells with a selective MEK inhibitor reduced the level of cisplatin-induced apoptosis, implying that cisplatin-induced MEK/ERK activation mediates apoptotic cell death⁵⁵⁸.

In clinical studies, response rates and time to progression for platinum-based and paclitaxel-based regimens in advanced NSCLC were not influenced by p-Erk IHC expression⁵²¹. While all responders to gemcitabine exhibited p-Erk IHC expression, time to progression for gemcitabine was not affected by p-Erk expression⁵²¹. Overall, the role (if any) of ERK/ MAPK in chemotherapy resistance is unclear.

12.4.4 Mitogen-activated protein kinase (MAPK) phosphatase-1 (MKP1)—MKP1 negatively regulates MAPK signaling⁵⁷⁵, ERK⁵⁷⁶, N-terminal-c-Jun kinase (JNK)⁴¹⁹, ⁵⁷⁶ and p38⁵⁷⁶, and may play a role in cell survival in response to stressful stimuli⁵⁷⁵. MKP1 was expressed in a high proportion of NSCLC tumor samples⁴¹⁹, ⁵⁷⁶, and was expressed more commonly in NSCLC than in SCLC cell lines⁵⁷⁶. Cisplatin induced MKP1 in human lung cancer cell lines, and overexpression of MKP1 rendered human lung cancer cells resistant to cisplatin⁵⁷⁵. Cisplatin-induced cell death was inhibited by blocking the apoptosis induction mediator JNK (which is negatively regulated by MPK1), while MKP1 inhibition sensitized lung cancer cell lines to cisplatin⁴¹⁹, ⁵⁷⁵. This sensitization was associated with a marked increase in cisplatin-induced activation of JNK and p38⁴¹⁹. Similarly, inhibition of MKP1 in NSCLC xenografts decreased the rate of tumor growth and increased sensitivity to cisplatin⁴¹⁹.

12.4.5 Protein kinase C (PKC)—Impact of PKC on chemotherapy resistance may depend on the PKC isoform present and on the agents used. In SCLC cell lines, paclitaxel resistance was not associated with alteration of expression of any of the PKC isozymes, while cisplatin-resistant and etoposide-resistant SCLC cells had significant reduction (rather than increase) in PKC activity⁵⁷⁷. A cisplatin-resistant SCLC variant had a decrease in PKC- α and - β expression and no change in PKC-zeta or -iota⁵⁷⁷, while an etoposide-resistant SCLC variant had decreased PKC β expression⁵⁷⁷. On the other hand, PKC-delta expression was increased in cisplatin- and etoposide-resistant SCLC variants⁵⁷⁷, and PKC-epsilon expression was associated with resistance to etoposide and doxorubicin, with

blockage of apoptosis⁵⁷⁸. PKC-epsilon formed a multiprotein complex with B-raf and S6K2 that increased expression of the antiapoptotic proteins XIAP and Bcl-xL and increased resistance to etoposide in SCLC cells⁴⁷⁵.

Expression of PKC-epsilon was also associated with chemotherapy resistance in NSCLC cell lines⁵⁷⁸, and down-regulation of PKC-eta reduced resistance to vincristine and paclitaxel and augmented vincristine- and paclitaxel-induced caspase-3 activity⁴⁷², while expression of other PKC isoforms did not correlate with resistance in NSCLC cell lines⁵⁷⁸. Although PKC- α antisense augmented sensitivity to carboplatin, vincristine, doxorubicin and other agents in NSCLC cell lines⁵⁷⁹, addition of PKC- α inhibitors to cisplatin plus gemcitabine in phase II trials in NSCLC yielded response rates and survival times similar to what one would expect with the chemotherapy alone^{580, 581}.

Overall, preclinical data suggest that PKC-epsilon expression confers resistance in both SCLC and NSCLC cell lines, resistance may be associated with PKC-delta expression in SCLC and with PKC-eta in NSCLC, while PKC- α and - β expression may be associated with chemotherapy sensitivity in SCLC cell lines.

12.5.6 Caveolin-1—Caveolin is a principal component of caveolae membranes, and interaction of the caveolin scaffolding domain with signaling molecules can functionally inhibit the activity of these molecules⁵⁸². Caveolin 1 expression is downregulated in various cancer cell lines, but its expression is substantially increased in several drug-resistant cell lines¹¹⁷. Significantly increased expression of caveolin-117, 582, caveolae organelles⁵⁸², and cholesterol¹¹⁷ (the major lipid component of caveolae) (but not of caveolin-2582) was reported in NSCLC cell lines that were resistant to paclitaxel⁵⁸² and etoposide¹¹⁷, and paclitaxel exposure led to upregulation of caveolin-1 expression in paclitaxel-sensitive NSCLC cells⁵⁸².

In patients with advanced NSCLC treated with gemcitabine plus cisplatin or epirubicin or with gemcitabine alone, caveolin-1 IHC expression was found in 16.4% of patients⁵⁸³. Patients with caveolin-1 expression had a significantly lower response rate and significantly shorter progression-free survival and overall survival compared to those without caveolin-1 expression⁵⁸³.

12.4.7 Other signaling pathway components—Gangliosides (cell membrane components composed of glycosphingolipid with one or more sialic acids linked on the sugar chain) modulate cell signal transduction. A cisplatin-resistant SCLC cell line showed a simplified ganglioside pattern, expressing only gangliosides GM3 and GM2, and had a marked increase in ganglioside GM3 compared to the parent line⁵⁸⁴. Annexins are proteins that undergo calcium-dependent binding to negatively charged phospholipids, and annexin IV plays a role in ion channel regulation, exocytosis and calcium-dependent signal transduction⁵⁸⁵. In a paclitaxel-resistant NSCLC cell line, annexin IV (but not annexin II) was overexpressed⁵⁸⁶. Short-term treatment of NSCLC cells with paclitaxel resulted in induction of annexin IV expression, and transfection of annexin IV cDNA into sensitive NSCLC cells augmented their resistance to paclitaxel⁵⁸⁶. Clinically, amplification and overexpression of c-myc was associated with poor prognosis in SCLC⁵⁸⁷ and amplification of N-myc in SCLC cell lines was associated with increased resistance to cisplatin⁵⁸⁸. Inhibition of c-myc increased sensitivity of a cisplatin-resistant SCLC cell line to cisplatin, but not to doxorubicin or vincristine⁵⁸⁹.

12.5.0 Stress proteins and chaperones

12.5.1 Heat shock proteins (HSPs)—HSPs are chaperones that protect cellular proteins from degradation under hostile intracellular conditions. Hsp90 inhibitors, such as 17-

allylamino-17-demethoxygeldanamycin (17-AAG), induced the degradation of Hsp90-interacting proteins (including mutant EGFR), and significantly potentiated the efficacy of paclitaxel against NSCLC xenografts⁵⁹⁰. In a panel of lung carcinoma cell lines, siRNA to HSP72 did not sensitize cells to cisplatin, etoposide or ionizing radiation⁵⁹¹, but in NSCLC patients receiving front-line single agent vinorelbine, there was a trend towards Hsp27-positive tumors being more likely to show progression ($p=0.068$)⁴⁰³.

12.5.2 Clusterin—Clusterin is a stress-associated cytoprotective chaperone protein that is up-regulated by various apoptotic triggers in many cancers and confers treatment resistance when overexpressed⁵⁹². Clusterin was expressed by IHC in more than 80% of human NSCLCs⁵⁹². Clusterin antisense or siRNA significantly enhanced sensitivity of NSCLC cell lines to paclitaxel, and clusterin antisense enhanced the efficacy of paclitaxel and gemcitabine against NSCLC xenografts⁵⁹².

12.5.3 Nrf2/heme oxygenase-1 (HO-1)—HO-1 is a molecular chaperone protein that plays an important role in tumor cell growth through anti-oxidative and anti-apoptotic effects, and HO-1 expression was mediated by transcriptional activation by the transcription factor Nrf2⁵⁹³. Inhibition of HO-1 by siRNA or by MAPK inhibitors increased apoptosis, degradation of procaspase-3, cisplatin cytotoxicity and cisplatin-induced production of reactive oxygen species in a resistant NSCLC cell line that expressed high levels of HO-1, suggesting that HO-1 may modulate the chemosensitivity of NSCLC cells to cisplatin through the MAPK-Nrf2 pathway⁵⁹³. In another study, treatment of lung cancer cells with cisplatin augmented HO-1 expression, inhibitors of EGFR or of Akt blocked this augmentation of HO-1 expression, and HO-1 inhibition augmented cisplatin cytotoxicity⁵⁹⁴.

Decrease in Keap1 activity (eg, due to mutation) resulted in constitutive activation of Nrf2 in lung cancer cell lines^{595, 596}. Increased expression of Nrf2 led to resistance to cisplatin^{595, 597}, carboplatin⁵⁹⁶, doxorubicin⁵⁹⁷ and etoposide^{596, 597} by causing up-regulation of expression of cytoprotective genes encoding multidrug resistance pumps, phase II detoxifying enzymes, and antioxidative stress enzymes/proteins^{595, 596}.

12.5.4 Glucose-regulated protein78 (GRP78)—In NSCLC cell lines, induction of increased expression of GRP78 (a member of the endoplasmic reticulum chaperone family) increased resistance to etoposide⁵⁹⁸.

13.0 Cell cycling

Factors related to cell cycling may also contribute to resistance (Fig. 5).

13.1 Cell cycle phase

In NSCLC cells, maximal sensitivity to cisplatin occurred with exposure during the G1 phase of the cell cycle, and least effect was seen with exposure during G2⁵⁹⁹. Exposure to cisplatin led to a prolonged S-phase and accumulation of cells at the S/G2 transition⁵⁹⁹. While quiescent cells are resistant to some agents, platinum drugs retain the ability to evoke apoptosis in quiescent cells⁵⁴.

Unlike cisplatin, etoposide and doxorubicin were least cytotoxic to a murine lung cancer cell line during G1, possibly due to the disappearance of topoisomerase II during G1 phase⁶⁰⁰. Similarly, pretreatment of NSCLC cells with tumor necrosis factor⁶⁰¹ or erlotinib⁵¹⁰ blocked cells in G1^{510, 601} or G0⁶⁰¹ and proved antagonistic to pemetrexed⁵¹⁰ and doxorubicin⁶⁰¹, while not altering efficacy of cisplatin⁶⁰¹.

One NSCLC variant with resistance to vinorelbine, cisplatin, fluorouracil, paclitaxel, etoposide, decarbonizes, ifosfamide, and epirubicin (but not to gemcitabine or irinotecan) had a significantly higher proportion of cells in G0/G1 and significantly fewer in S phase than did the sensitive parent²⁶. Treatment of NSCLC cells with a monoclonal antibody that significantly increased the percentage of cells in the G2 phase of the cell cycle led to increased sensitivity to taxanes⁶⁰². On the other hand, transfection of WT p53 gene into p53-null NSCLC cells induced senescence, cell cycle arrest in G1 and decreased sensitivity to paclitaxel³⁸⁷.

13.2 Mitotic spindle checkpoint and mitotic slippage/aneuploidy

Ordinarily, cells with abnormal mitoses undergo G2-M arrest. The mitotic spindle checkpoint, which blocks segregation of abnormal chromosomes, is often defective in human lung cancer cell lines, and this is thought to contribute to chromosomal instability⁶⁰³. Anti-microtubule agents such as vinca alkaloids and taxanes activate the mitotic spindle checkpoint⁶⁰³. In NSCLC cell lines, impairment of the mitotic spindle checkpoint was associated with marked reduction in the ability of vinorelbine and docetaxel to induce apoptosis, compared to cell lines with an intact mitotic spindle checkpoint, while the status of the mitotic spindle checkpoint had no impact on the ability of cisplatin to induce apoptosis⁶⁰³.

Cells may also escape from the G2-M arrest without undergoing cell division ("mitotic slippage"), resulting in the formation of aneuploidy G1 cells, and this process of mitotic slippage may also trigger apoptosis⁶⁰⁴. Microtubule-stabilizing agents such as taxanes and related agents produced aneuploidy populations in NSCLC cell lines that had defective mitotic blocks, and aneuploidy cells were diminished in resistant variants⁶⁰⁴. In contrast, microtubule-destabilizing agents such as the vinca alkaloids were unable to initiate aneuploidy⁶⁰⁵. Hence, factors permitting aberrant mitoses and aneuploidy may reduce resistance to taxanes, while factors that promote a mitotic block (thereby reducing aberrant mitoses and aneuploidy) may augment resistance⁶⁰⁵.

13.3 CHK2

CHK2 kinase is a tumor suppressor that plays an important role in DNA damage signaling, cell cycle regulation and DNA-damage-induced apoptosis. DNA damage leads to its phosphorylation, and the activated protein then blocks entry of cells into mitosis. Cisplatin-resistant NSCLC cell lines had decreased expression of CHK2, related to hypermethylation of the CHK2 promoter⁶⁰⁶. Hypermethylation of the CHK2 promoter was also found in all of 10 NSCLC tumor samples assessed, and decreased IHC staining for CHK2 was found in 83% of 87 NSCLC samples assessed⁶⁰⁶.

13.4 Hus1

Hus1, a component of the radiation sensitive (Red) machinery that plays a role in DNA repair and cell cycle G2/M checkpoint control pathways, forms a discrete complex with the Red family members, Rad1 and Rad9. Antisense-mediated downregulation of expression of Hus1 sensitized p53 deficient NSCLC cells to cisplatin⁶⁰⁷, suggesting that Hus1 may play a role in cisplatin resistance.

13.5.0 Cell cycle regulators

13.5.1 Retinoblastoma protein (RB)—The retinoblastoma tumor suppressor (RB) is a key regulator of cell cycle progression and is functionally inactivated in the majority of NSCLC tumors⁶⁰⁸. Hypophosphorylated RB blocks cell cycle progression, and phosphorylation of RB (to form pRB) inactivates RB, permitting progression through the

cell cycle. Transfection of RB into RB-deficient NSCLC cells enhanced the G1 checkpoint response to chemotherapy⁶⁰⁸. Transfection of RB into RB-deficient NSCLC⁶⁰⁸ or SCLC⁶⁰⁹ cells also significantly increased resistance⁶⁰⁸ or resulted in a trend towards increased resistance⁶⁰⁹ to cisplatin^{608, 609}, etoposide^{608, 609}, doxorubicin⁶⁰⁹ and 5-fluorouracil⁶⁰⁸, while dexamethasone-induced dephosphorylation of pRB in NSCLC cells was associated with antagonism of paclitaxel-induced cytotoxicity⁵⁷⁴. On the other hand, one study found that expression of RB protein in cell lines derived from biopsies of patients with NSCLC or SCLC did not correlate with in vitro chemotherapy resistance, nor with response to chemotherapy or survival of the patients from whom the cells were derived⁶⁰⁹.

By IHC, 38% of pre-chemotherapy advanced NSCLC tumors⁷¹ and 10% of limited and extensive SCLC tumors²⁵⁷ expressed RB. Response rates to chemotherapy (cisplatin combined with gemcitabine, etoposide or with mitomycin-c plus ifosfamide) were significantly lower in RB positive vs negative NSCLC patients⁷¹, while survival did not correlate with RB expression in either these NSCLC patients⁷¹ nor in SCLC patients treated with alternating cisplatin-etoposide/CAV²⁵⁷. Hence, RB expression is associated with chemotherapy resistance preclinically and with reduced lung cancer clinical response to chemotherapy, but did not correlate with patient survival.

13.5.2 14-3-3—14-3-3 is a family of highly conserved regulatory proteins that bind to numerous signaling proteins and appear to play a role in cell signaling, cell cycle control, and apoptotic death⁶¹⁰. Down-regulation of 14-3-3zeta in lung cancer cells significantly increased sensitivity to cisplatin⁶¹⁰.

In NSCLC patients, increased 14-3-3zeta expression was positively correlated with a more advanced pathologic stage and grade⁶¹⁰. Methylation of the mitotic checkpoint gene 14-3-3sigma in serum-derived circulating tumor DNA was associated with significant prolongation of overall survival and time to progression, and with a trend to increased probability of response ($p=0.06$) in NSCLC patients treated with cisplatin plus gemcitabine⁶¹¹.

Overall, the limited data available suggest that 14-3-3 might contribute to resistance.

13.5.3 The cyclin-dependent kinase inhibitors P27kip1 and P21WAF1/CIP1—In patients with advanced NSCLC treated with platinum-based chemotherapy regimens, higher tumor IHC expression of the cyclin-dependent kinase inhibitor p27Kip1 correlated with improved response⁶¹² or survival^{612, 613}. In keeping with this, overexpression in NSCLC cells of S-phase kinase-associated protein 2 (SKP2) (a member of the F-box family of ubiquitin-protein ligase complexes that controls the stability of several cell cycle-related proteins⁶¹⁴) was associated with reduced expression of each of p27kip1, p21WAF/cip1 and cyclin E1, with increased S-phase cells and with chemoresistance against camptothecin, cisplatin, and other agents⁶¹⁴.

On the other hand, in patients with resected NSCLC, adjuvant chemotherapy (cisplatin plus vinca alkaloid or etoposide) resulted in significantly longer overall survival compared with controls among patients whose tumors were IHC negative for p27Kip1, while overall survival was not different between patients treated with cisplatin-based chemotherapy and controls in patients with p27Kip1-positive tumors⁶¹⁵. By IHC, other cell cycle regulators including p16INK4A, cyclin D1, cyclin D3, cyclin E, and Ki-67 did not predict benefit of adjuvant cisplatin-based chemotherapy, and none of these biomarkers was significantly associated with overall survival of the patients in the total study population⁶¹⁵.

Furthermore, a NSCLC cell line resistant to camptothecin, doxorubicin and cisplatin had markedly attenuated caspase-3-like protease activity and elevated expression of p21Waf1/Cip1⁴⁵³. Transfection of p21Waf1/Cip1 cDNA into the parental sensitive cell line resulted in resistance to apoptosis, while p21Waf1/Cip1 antisense oligonucleotides restored sensitivity, particularly if combined with bcl-2 antisense oligonucleotides⁴⁵³. Induction of p21Waf1/Cip1 overexpression in a p53 null NSCLC cell line also conferred increased resistance to killing by etoposide or cisplatin³⁸⁵ and high pre-chemotherapy expression of p21WAF1/CIP1 in tumors of stage IIIA-B NSCLC patients undergoing neoadjuvant platinum-based chemotherapy was associated with shorter time to failure⁶¹⁶.

Hence, some data suggest that cyclin-dependent kinase inhibitors decrease chemotherapy resistance while other data suggest that they increase resistance.

13.5.4 Other cell cycle regulators—Cells in human lung cancers that survived induction chemotherapy (and hence were presumably at least partially resistant) overexpressed Cdc2/Cdk1617. Host polymorphisms for the cyclin D1 gene correlated significantly with tumor response or stabilization⁶¹⁸, and patients with advanced NSCLC treated with an antimetabolic agent plus a platinum had significantly higher response rates if their tumors were IHC positive vs negative for cyclin B1 or for the microtubule motor protein Eg5 (which functions in bipolar spindle assembly)⁶¹⁹.

14.0 Transcription factors

Several transcription factors have also been linked to chemotherapy resistance (Fig. 5).

14.1 Nuclear factor- κ B (NF- κ B)

NF- κ B augments the expression of a variety of anti-apoptotic proteins, including IAPs^{490, 551}, Bcl-XL^{461, 551} and Bcl-2⁴⁶¹, and NF- κ B activity in NSCLC cells was significantly increased by exposure to cisplatin⁶²⁰, taxanes⁶²⁰, doxorubicin⁵⁰³, etoposide⁵⁰³, and gemcitabine^{490, 621, 622}. This suggests that inhibition of NF- κ B activity could augment chemotherapy efficacy by blocking an NF- κ B-mediated upregulation of expression of antiapoptotic proteins. In keeping with this, introduction of the NF- κ B antagonist I κ B α into resistant NSCLC cells^{623, 624}, upregulation of I κ B via inhibition of PI3K⁵⁵¹, presence of I κ B in a sensitive variant⁶²², inhibition of the I κ B antagonist Aurora-A kinase⁴⁶¹, and exposure to BAY11-0782⁵⁵¹, to genistein⁶²⁰, or to a proteasome inhibitor^{621, 625} blocked NF- κ B activation^{461, 551, 620, 623, 624}, reduced expression of NF- κ B-regulated antiapoptotic proteins (including IAPs^{490, 551}, Bcl-XL^{461, 551} and Bcl-2⁴⁶¹), and augmented apoptosis induced by cisplatin^{620, 626}, doxorubicin^{461, 620, 626}, etoposide⁴⁶¹, gemcitabine^{490, 621, 622} and taxanes^{551, 620, 623–625}. Overexpression of IAP-1 protein in NSCLC cells expressing I κ B α restored resistance to gemcitabine, suggesting that IAP-1 may be responsible for the chemoresistance induced by NF- κ B⁴⁹⁰. Transfection with I κ B α ⁶²⁴ or exposure to a proteasome inhibitor⁶²¹ also blocked paclitaxel⁶²⁴ or gemcitabine⁶²¹-induced upregulation of expression of p65 (which heterodimerizes with NF- κ B to form the NF- κ B complex)⁶²⁴, NF- κ B binding to DNA⁶²¹ and transcription of several NF- κ B-regulated genes⁶²¹. Proteasomal inhibition also potentiated the effect of gemcitabine against NSCLC xenografts⁶²¹. However, exposure of NSCLC cells to doxorubicin and etoposide induced the NF- κ B-dependent expression of the pro-apoptotic proteins TRAIL and DR5 (in addition to increasing the NF- κ B-dependent expression of anti-apoptotic proteins such as IAPs), and inhibition of NF- κ B led to loss of cell surface expression of TRAIL and DR5, and to chemoresistance of tumor xenografts in nude mice⁵⁰³. Hence, because of the range of factors regulated by NF- κ B, inhibition of it could result in either increased or decreased chemotherapy efficacy.

Hence, while clinical data are limited, most (but not all) preclinical data suggest that NF- κ B could play a role in resistance of NSCLC to several types of agents.

14.2 Clock-related transcription factors

The circadian transcription factor Clock was overexpressed in cisplatin-resistant cells⁶²⁷ and Clock's target⁶²⁷ Activating Transcription Factor 4 (ATF4) was cisplatin-inducible at the transcriptional level⁶²⁸. In human lung cancer cell lines, cisplatin resistance correlated with expression of each of Clock⁶²⁷, ATF4⁶²⁸ and of another Clock target, HIV-1 Tat interacting protein⁶²⁹ (Tip60) (a histone acetyltransferase [HAT] gene that plays a role in chromatin remodeling, transcription, DNA repair, and signal transduction). Hyperacetylation of H3K14 and H4K16 was found in cisplatin-resistant cells⁶²⁹. Expression of two other HAT genes, HAT1 and MYST1 did not correlate with cisplatin resistance⁶²⁹. Downregulation of Clock and ATF4 augmented sensitivity of NSCLC cells to cisplatin and etoposide⁶²⁷, and knockdown of Tip60 expression downregulated expression of several DNA repair genes and rendered cells sensitive to cisplatin (but not to oxaliplatin, vincristine, and etoposide)⁶²⁹. Conversely, transfection with ATF4 decreased sensitivity to cisplatin, but not to vincristine⁶²⁸. ATF4 expression also correlated with expression of genes for glutathione metabolism⁶²⁷.

14.3 Zinc finger transcription factors

In NSCLC cells, siRNA inhibition of the zinc finger transcription factors TWIST⁶³⁰ and SNAIL⁶³¹ (which may play a role in epithelial-mesenchymal transition³⁵⁴, ⁶³¹ and in regulation of N-cadherin expression³⁵⁴) significantly augmented efficacy of cisplatin by inducing activation of the JNK/mitochondrial pathway⁶³⁰, ⁶³¹, while mRNA expression of the Kruppel-related zinc finger protein-1 (HKR1) transcription factor was induced by exposure to cisplatin⁶³². High expression levels of HKR1 in autopsy lung cancer tissues were associated with antemortem platinum drug administration⁶³².

14.4 Other transcription factors

The transcription factors HIF-1 and p53 have been discussed previously. Other transcription factors that have been associated with cisplatin resistance in NSCLC cell lines include signal transducer and activator of transcription (STAT)-3⁶³³, Thyroid Transcription Factor-1 (TTF-1) (when coactivated with the transcription factor NKX2-8)⁶³⁴, and a splice variant of the nuclear receptor protein/transcription factor peroxisome proliferator-activated receptor gamma (PPAR γ)1 ⁶³⁵. Exposure of a NSCLC cell line to cisplatin and etoposide led to decreased levels of E2F4 (which suppresses proliferation-associated genes) and to induction of E2F1 (which mediates cell proliferation)⁶³⁶. Cells in which the E2F4 gene was lacking or inhibited were more sensitive to apoptosis induced by cisplatin and etoposide, while E2F1-deficient cells were less sensitive⁶³⁶.

15.0 Cancer stem cells and resistance

A small proportion of cells present within tumors have stem cell properties, including self-renewal capability. Cancer stem cells are highly resistant to chemotherapy^{637–644}, and exposure to therapy selects for tumor cells with stem-cell properties⁶³⁹, ⁶⁴⁰, ⁶⁴³. While stem cells may have high expression of a variety of resistance factors⁶³⁷, ⁶³⁸, ⁶⁴², part of the resistance of stem cells has been attributed to some stem cell populations being quiescent, but still having the capacity to proliferate⁶³⁷, ⁶³⁸, ⁶⁴⁴. Such quiescent populations could explain the flattening of the chemotherapy dose-response curve seen clinically in NSCLC⁴.

Lung cancer cell populations with stem cell properties have been reported^{640–643}, ^{645–648}, and more than one distinct stem cell population may be present in a single cancer cell

line⁶⁴⁸. A population of CD133-positive (CD133+) cells expands during repair of lung damage, and CD133+ cells have been found in human lung cancers⁶³⁸. CD133 may normally play a role in the organization of cell membrane topology⁶⁴⁹. Lung cancer CD133+ cells generated long-term chemotherapy-resistant spheroids in vitro, indicating potential stem cell function⁶³⁸. CD133+ lung cancer cell lines were resistant to cisplatin^{642, 643}, etoposide⁶⁴², doxorubicin⁶⁴², paclitaxel⁶⁴² and radiotherapy⁶⁴², and CD133 expression correlated with clinical resistance to platinum regimens in patients with advanced NSCLC⁶⁴³. Resistance to cisplatin of CD133+ NSCLC cell lines and xenografts was reversed by siRNA against the stem cell transcription factor Oct4⁶⁴².

Lung cancer cells with increased aldehyde dehydrogenase (ALDH) activity also have stem cell properties^{646, 647}, and maintain longer telomeres than do cells with low ALDH levels⁶⁴⁷. In lung cancer cell lines, ALDH1 expression correlated with CD133 expression⁶⁴¹. ALDH is thought to suppress stem cell differentiation by processing retinoic aldehydes. Clinically, lung cancer expression of ALDH1 correlated with tumor stage and grade, and was associated with poor prognosis⁶⁴¹.

In breast cancers, cells that are CD44-positive but also CD24-negative are highly invasive⁶⁵⁰, have stem cell properties⁶⁵¹ and are chemotherapy-resistant⁶⁵¹. CD44 is a transmembrane glycoprotein that plays a role in cell adhesion and in angiogenesis⁶⁵², while CD24 is a mucin-like adhesion molecule⁶⁵³ that may recruit beta1 integrins into membrane lipid raft domains⁶⁵⁴. In resected NSCLC tumors, 36.4% had cells with a CD44+/ESA+/CD24- phenotype⁶⁵⁵.

Each of the Notch, Sonic Hedgehog and Wnt pathways may help maintain stem cell populations. For example, mammalian cells have 4 Notch receptors (Notch 1–4) and 5 membrane-bound Notch ligands (Delta-like1, 3 and 4, Jagged1 and 2)^{656, 657}. Binding of ligand to receptor on an adjacent cell results in a 2-step cleavage of Notch. The second cleavage step is catalyzed by γ -secretase⁶⁵⁷, and releases Notch intracellular domain (NICD). NICD then translocates to the nucleus where it interacts with the proteins CSL and Mastermind to promote transcription of HES and HEY family members, which then repress various tissue-specific transcription factors^{657, 658}. Notch cleavage may inhibit cellular differentiation, thereby promoting asymmetric cell division and maintenance of a stem cell phenotype in daughter cells that are in contact with cells bearing Notch ligands⁶⁵⁷. Notch function in inhibiting differentiation and in promoting stem cell renewal is enhanced by hypoxia through an effect of HIF-1 α and HIF-2 α ⁶⁵⁹. The Notch pathway also may play an important role in epithelial-mesenchymal transition (EMT)⁶⁶⁰.

A high proportion of NSCLC cell lines expressed Notch receptors, particularly Notch 2 and 3⁶⁶¹. Notch 3 expression^{661, 662} and expression of the Notch ligand Jag1⁶⁶² was also common clinically in resected lung cancer specimens, and expression of the activated NICD form of Notch 3 was noted in 41% of lung cancer cell lines⁶⁶¹. Notch-1 expression was substantially increased in lung cancer cell lines in the presence of hypoxia⁶⁶³. Expression of the Notch ligand Jagged1 and the Notch transcriptional target genes HES1 and HEY1 was also very common in lung cancer cell lines⁶⁶¹, the Notch targets HES-1 and HES-5 were elevated in lung tumors⁶⁶³, and ALDH-positive lung cancer stem cells had increased expression of Notch⁶⁶⁴, Notch ligands⁶⁴⁶ and Notch effectors⁶⁴⁶.

Since there is evidence that stem cells may play a major role in chemotherapy and radiotherapy resistance^{637–640, 643, 644}, and since Notch may be important in maintaining stem cell populations, there is interest in targeting Notch⁶⁶⁵. Inhibition of γ -secretase inhibits Notch activity by preventing the cleavage of Notch to NICD. γ -Secretase inhibitors were active in lung cancer cell lines and xenografts^{661, 666}, including in putative lung cancer

stem cells that overexpressed both ALDH and Notch receptors⁶⁶⁴, in a NSCLC cell line that overexpressed Notch 3⁶⁶⁷, and in lung cancer cell lines grown in hypoxic conditions that expressed Notch 1⁶⁶³. Because of their potential to target cancer stem cells, there is interest in assessing ability of γ -secretase inhibitors to modulate lung cancer resistance to chemotherapy, and early clinical trials are in progress.

With respect to other stem cell pathways, Wnt appeared to be active in only a small proportion of lung cancer cell lines in one study⁶⁶⁸, but Wnt activation was found in 50% of NSCLC tumors in another study⁶⁶⁹. Wnt1 was expressed in 40–50% of NSCLC clinical specimens⁶⁶⁹, 670, and its expression correlated with Ki-67 expression and with poor survival⁶⁷⁰. Similarly, Sonic Hedgehog (SHH) was detected in 84% of clinical NSCLC and SCLC tissue specimens⁶⁷¹. However, SHH pathway activation (as manifested by expression of its targets Gli1 and PTCH1) was only detected in 10.5% of clinical tumor specimens in one study⁶⁷¹, and Gli1 expression was uncommon in SCLC cell lines⁶⁷², but Gli1 was detected in 85% of clinical SCLC tumor samples in another study⁶⁷². Host genetic polymorphisms for the SHH pathway correlated with survival in advanced NSCLC treated with platinum-based chemotherapy regimens⁶⁷³.

Overall, available preclinical data support a role for cancer stem cells in resistance to chemotherapy, and limited clinical data are also supportive.

16.0 “Global” indicators of chemotherapy sensitivity and resistance

As noted above, there are several different factors that have been associated with resistance to chemotherapy in lung cancer, and it is difficult to predict clinically how expression of any one of these factors or any group of factors would translate into success or failure of a therapy. Hence, various “global” indicators have also been assessed that would take into consideration several factors simultaneously. We will describe in more detail below some examples to which we alluded briefly above:

16.1 Gene expression arrays

In resected NSCLC, significant associations were found between expression levels of dozens of genes and in vitro chemosensitivity for docetaxel, paclitaxel, irinotecan, cisplatin, gemcitabine, and vinorelbine²². When gene expression profiles were compared to chemosensitivity in a range of NSCLC cell lines, gemcitabine belonged to an isolated cluster, while taxanes, 5-FU, SN-38, and platinum were gathered together into one large cluster³⁶. In a gemcitabine-resistant line, approximately 18.8% of the total DNA elements had substantially altered level of expression compared to the sensitive parent by cDNA microarray, with differences seen in expression of oncogenes, tumor suppressor genes, cell cycle regulators, heat shock proteins, apoptotic and antiapoptotic factors, DNA transcription factors, DNA repair and recombination factors, signal transduction genes, protein translation genes, and many metabolic genes²¹. When expression of 1291 sensitivity-related genes were assessed using cDNA macroarrays, substantial differences were found between SCLC and NSCLC cell lines⁶⁷⁴.

Gene signatures were defined for response of NSCLC patients to cisplatin-based therapy, with a positive predictive value of 78% and a negative predictive value of 100%³⁴. There was an inverse correlation between the likelihood of response to cisplatin vs pemetrexed³⁴. In another study involving SCLC and NSCLC patients receiving platinum-based chemotherapy, there was a significant increase in the expression of nine genes in non-responders compared with responders²³. Allogeneic inflammatory factor, HLA-DR antigen associated invariant subunit and MHC class II HLA-DR- β precursor were significantly associated with chemotherapy resistance²³. One or more resistance genes were

overexpressed in 28% of SCLC tumors and in 66% of NSCLC tumors ($p=0.012$)²³. In patients with NSCLC treated with paclitaxel plus irinotecan, cDNA microarray analysis of peripheral blood samples for expression levels of 1176 genes revealed that the genes encoding protein phosphatase, IL-1 α and IgA were independent predictive factors for chemosensitivity, while the thyrotropin-releasing hormone receptor and alkylation repair genes were independent prognostic factors⁶⁷⁵.

Overall, gene expression arrays hold promise as tools for guiding therapy choice in patients, but substantial work remains to be done.

16.2 Chromosomal alterations

In a SCLC cell line, the process of rendering cells resistant by exposure to cisplatin and oxaliplatin caused similar chromosomal changes to emerge⁵. The resistant cell lines lost their resistant phenotype after 3 months of drug-free culture, and many (but not all) of the acquired chromosomal abnormalities were also lost at the same time, suggesting a link between the chromosomal changes and emergence of resistance⁵. Emergence of resistance to taxanes has been linked in a NSCLC cell line to amplification of resistance factors as a result of repeated chromosomal breakage-fusion-bridge cycles with telomere addition⁷.

With in vitro sensitivity testing of resected NSCLC tumor samples, aneuploid tumors tended to have increased resistance to etoposide and topotecan, while aneuploidy had less of an impact on resistance to gemcitabine, and no major impact with platinum⁶.

16.3 DNA hypermethylation

In a NSCLC cell line and in a xenograft model, pulse exposures to etoposide, doxorubicin, vinca alkaloids, cisplatin, hydroxyurea, 1- β -D-arabinofuranosylcytosine, 5-fluorouracil, 5-fluorodeoxyuridine, and methotrexate caused DNA hypomethylation at drug concentrations which produced mild DNA synthesis inhibition and which killed less than 50% of exposed cells, while marked drug-induced DNA hypermethylation was noted when the degree of DNA synthesis inhibition caused by the drug exceeded 90% and when drug exposure was sufficient to kill 90–100% of exposed cells⁸. Drug-induced DNA hypermethylation could be blocked by preexposure to hypomethylating agents administered at low concentrations⁸. Drug-induced DNA hypermethylation may be capable of creating drug-resistant phenotypes by inactivating genes the products of which are required for drug cytotoxicity, and may also contribute to resistance by potentiating the process of gene amplification⁸.

16.4 In vitro sensitivity testing

Several studies have assessed the ability of in vitro sensitivity testing to predict response of lung cancer to chemotherapy. In some studies of in vitro sensitivity testing involving patients with lung cancer^{10–13} and other malignancies¹⁰ using cisplatin^{10–13}, carboplatin¹³, vindesine¹³, vinorelbine¹³, docetaxel^{12, 13}, paclitaxel^{12, 13}, etoposide^{12, 13}, irinotecan^{12, 13}, gemcitabine^{12, 13}, mitomycin-C^{10–12}, 5-fluorouracil^{10, 12}, doxorubicin^{10–12}, mitoxantrone¹¹, and other agents¹¹, there were relatively frequent false positives (predicted sensitive but failed clinically, with true positive rates of up to 73%) but no false negatives (predicted resistant but succeeded clinically). In still other studies involving patients with chemonaive unresectable NSCLC assessed for in vitro sensitivity to platinum¹⁴, the positive/negative predictive values were 61.1% and 78.6% with a predictive accuracy of 68.8%¹⁴. The group predicted to be sensitive had better clinical response ($P=.036$), longer progression-free survival ($P=.060$), and longer overall survival ($P=.025$) than those predicted to be resistant¹⁴. Occasional studies did not find that in vitro sensitivity testing did any better than empiric therapy in NSCLC⁹.

With limited SCLC patients treated with 4 cycles of etoposide plus platinum plus radiotherapy followed by either 4 cycles of cyclophosphamide plus doxorubicin plus vincristine vs 4 cycles of therapy based on in vitro sensitivity testing, median survival was 19 months with standard therapy vs 38.5 months with therapy based on sensitivity testing, but other factors besides in vitro sensitivity testing may have contributed¹⁵. In other studies in SCLC patients treated with therapy selected by in vitro sensitivity testing either after 12 weeks of etoposide-platinum or at relapse, there was a trend towards a higher response rate than in patients with therapy selected empirically⁹.

Overall, the results of in vitro sensitivity testing suggest that it may be fairly good at predicting resistance, but somewhat less good at predicting sensitivity. Factors that operate against it being used routinely include the frequency of false positives, as well as the amount of fresh tissue required and the amount of time required to perform the tests.

17.0 Summary

There are numerous factors that directly or indirectly may contribute to lung cancer resistance to chemotherapy. Of the many factors that have been linked to resistance in preclinical systems, several have also been linked to resistance in the clinic, although conflicting clinical data are common. Issues that muddy the water clinically include the fact that in some instances it is difficult to determine whether a factor is primarily a prognostic factor (correlating with survival whether or not therapy is given) vs a predictive factor (correlating with benefit of the therapy being assessed). The use of chemotherapy agents in combination may also blunt the impact of a resistance factor, and the presence of co-morbid conditions and the administration of subsequent therapy may make it more difficult to discern the effect of a resistance factor on patient survival.

Factors for which there is now substantial clinical evidence of a link to SCLC resistance to chemotherapy include MRP (for platinum-based combination chemotherapy) and MDR1/P-gp (for non-platinum agents). SPECT MIBI and Tc-TF scanning appears to predict chemotherapy benefit in SCLC. In NSCLC, the strongest clinical evidence is for taxane resistance with elevated expression or mutation of class III β -tubulin (and possibly α tubulin), platinum resistance and expression of ERCC1, platinum resistance and BCRP expression, gemcitabine resistance and RRM1 expression, and resistance to several agents and COX-2 expression (although COX-2 inhibitors have had minimal impact on drug efficacy clinically). Tumors expressing high BRCA1 may have increased resistance to platinum but increased sensitivity to taxanes. Limited early clinical data suggest that chemotherapy resistance in NSCLC may also be increased with decreased expression of cyclin B1 or of the microtubule motor protein Eg5, or with increased expression of stem cell markers, ICAM, matrilysin, osteopontin, DDH, survivin, PCDGF, caveolin-1, p21WAF1/CIP1, or 14-3-3sigma, and that IGF-1R inhibitors may increase efficacy of chemotherapy, particularly in squamous cell carcinomas. Equivocal data (with some positive studies but other negative studies) suggest that NSCLC tumors with EGFR mutations (particularly exon 19 deletions) may have increased sensitivity to chemotherapy, while K-ras mutations and expression of GST-pi or RB may possibly confer resistance. While limited clinical data suggest that p53 mutations are associated with resistance to platinum-based therapies in NSCLC, data on p53 IHC positivity are equivocal, with some studies showing a link to resistance, some showing no correlation, and some instead unexpectedly showing increased sensitivity to therapy in p53 positive tumors. Some limited clinical studies suggested increased resistance with p27kip1 while others suggested increased sensitivity.

18.0 Future directions

Future studies might benefit from attempting to identify targets that must be present within tumor cells if a therapy is going to be of value, as well as identifying tumor cell, microenvironment and host factors that will render a tumor resistant despite the presence of the required target. Concurrent combined correlations of tumor expression of putative targets and resistance markers, in vitro tumor cell sensitivity to therapy, and clinical tumor shrinkage on therapy might help in this regard. Future studies of resistance modulating strategies also need to take into consideration the fact that such trials could potentially fail because a required therapy target is lacking (and hence, response could not occur even if resistance factors were neutralized), because host/microenvironment factors protect the tumor despite neutralization of the resistance factor, because several resistance factors are at play in addition to the one being targeted, or because the tumor does not express the resistance factor being targeted. We also need to do a better job of assessing not just which resistance factors are at play at initiation of therapy (leading to intrinsic resistance) but also which ones emerge after exposure to therapy, leading to acquired resistance. Since many factors may lead to broad cross-resistance to several agents, and since some of these cross-resistance factors either limit uptake of drug into tumor cells or else increase drug efflux, progress might also be helped by concentrating on new therapies that work at the outer cell membrane level and do not require entry into tumor cells as a prerequisite for efficacy.

Future studies also need to do a better job of differentiating whether a factor that is associated with outcome is just a prognostic factor (correlating with tumor cell growth rate or with relapse probability) or whether instead it is a predictive factor (correlating with ability of a chemotherapy agent to kill tumor cells). Factors that correlated with tumor shrinkage on therapy might be expected to be predictive, while factors correlating with tumor stability or survival outcomes could be either predictive or prognostic. Future studies might also benefit from assessing host genotype polymorphisms for targets and resistance factors at the same time that tumor mRNA and protein expression are assessed. The host genotype polymorphisms could impact half life or enzymatic activity of the target/resistance factor, and could help explain discrepancies between target/resistance factor mRNA expression, protein expression and therapy efficacy.

Overall, epithelial tumors in general and lung cancer in particular are likely to remain a major challenge for some time to come.

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Fig 1.

Cell kill by first order kinetics gives a straight line in sensitive cells. Deficiency of a factor needed for tumor cell killing ("saturable passive resistance") gives a terminal plateau on the dose-response curve, analogous to non-competitive inhibition of drug effect. Alteration or mutation of a factor ("non-saturable passive resistance") results in a decreased dose-response curve slope (analogous to decreased affinity of a drug for its receptor). Excess of a mutation factor ("active resistance") gives a shoulder on a dose-response curve, analogous to competitive inhibition of drug effect.

Fig. 2.

Drug effect may be decreased by a decrease in drug bioavailability, hepatic activation, penetration across vessel endothelium or interstitium, uptake across cell membranes, or intracellular activation, or by decrease in tumor blood flow or oxygenation.

Fig. 3.
Drug intracellular concentration may be decreased by efflux pumps, or drug may be detoxified by various factors.

Fig. 4. Drug-induced DNA damage may be repaired by various DNA repair pathways, or apoptosis may be triggered as a result of attempted repair of platinum-induced damage by the Mismatch Repair pathway. Drug effect can also be reduced due to increased, decreased or mutated target.

Fig. 5.

Drug resistance can also occur due to deficient or defective proapoptotic factors or due to antiapoptotic effects of growth factors, growth factor receptors, signaling pathways, various membrane components, chaperones, transcription factors and cell cycle factors. Tumor cells are most sensitive to platinum during G1 and to taxanes during G2.

Table 1

Classification of resistance

Classification Method	Types of resistance
Time of onset	Intrinsic (de novo) Acquired
Pharmacodynamic	Active (competitive inhibition) Non-saturable passive (decreased affinity) Saturable passive (non-competitive inhibition)
Kinetic	Quiescent Accelerated
Genetic	Mutation Epigenetic
Host vs tumor	Host factor Tumor factor
Cell involved	Tumor cell factor Microenvironment/stromal cell factor Abscopal effect of distant resistant tumor

Table 2

Resistance factors for which host gene polymorphisms correlate with resistance clinically

Platinum regimens:
MRP2
Glutathione-S-transferase- π
Other glutathione-related genes
ERCC1
Xeroderma pigmentosum C ^a
Xeroderma pigmentosum D ^a
Xeroderma pigmentosum G
XRCC1 ^a
BRCA1
NQO1
P53
Cyclin D1
SHH
Gemcitabine:
Deoxycytidine deaminase
Irinotecan (with cisplatin):
MRP2
Etoposide/vinorelbine (with cisplatin):
MDR1/P-glycoprotein ^b

^aData were negative or equivocal in some individual trials^bNo association with outcome in patients treated with cisplatin-docetaxel

Table 3

Tumor factors contributing to lung cancer resistance to chemotherapy

Factor	Agents affected preclinically	Clinical data support link to resistance	
		Yes	No
Decreased tumor blood flow:			
↓ Drug delivery	All?		Cisplatin combinations
↓ Oxygen delivery	Etoposide, paclitaxel (not cisplatin, topotecan)		
↑ sphingosine kinase 2 / sphingosine-1-phosphate	Etoposide		
↑ HIF-1α	Cisplatin, doxorubicin, paclitaxel		Cisplatin-gemcitabine
↑ VEGF			Cisplatin-gemcitabine
Alterations of tumor extracellular pH:			
↓ pH	Weak bases (doxorubicin, vinca alkaloids)		
↑ pH	Weak acids (platinums, alkylating agents)		
Decreased drug uptake:			
↑ Cell membrane rigidity/sphingomyelin/cholesterol	Platinums, etoposide, paclitaxel		
↓ Long chain/unsaturated fatty acids	Platinums		
↓ CTR1	Platinums		
↓ Multiple membrane transporters	Platinums		
↓ Na+, K+ ATPase/ ↑ thromboxane A2/ ↑ sorbitol	Platinums	Platinums ^a	
↑ Intracellular chloride or zinc	Platinums		
↓ Human equilibrative nucleoside transporter 1	Gemcitabine	Gemcitabine ^a	
↓ Folate transporters	Pemetrexed	Pemetrexed	
Increased drug efflux:			
↑ MRP/GSH-conjugate pump	Platinums ^a , anthracyclines, vincas, etoposide, taxanes, gemcitabine ^a	Multiple platinum regimens ^a ; vindesine + etoposide	
↑ MDR1/p-glycoprotein ^b	Anthracyclines, vincas, etoposide, taxanes	Multiple regimens ^a	
↓ Connexin 32	Vinorelbine		
↑ Breast cancer resistance protein		Platinum regimens	
↑ RLIP76/RALBP1	Vinorelbine, doxorubicin		
↑ Lung resistance protein ^c	Cisplatin ^a , etoposide ^a	Platinum regimens ^a	Taxanes, CAV, some cisplatin regimens
↑ P-type adenosine triphosphatase 7B	Cisplatin		
Increased drug detoxification:			
↑ Glutathione (GSH)	Cisplatin, etoposide ^a , anthracyclines ^a , vincas ^a , radiation, camptothecins, mitomycin, alkylating agents.		

Factor	Agents affected preclinically	Clinical data support link to resistance	
		Yes	No
	methotrexate		
↑ Glutamate-cysteine ligase	Cisplatin		
↑ GSH peroxidase/GSH reductase	Cisplatin		
↑ Glutathione-S-transferase- π	Cisplatin ^a	Platinum regimens ^a	Vinorelbine regimens
↑ Metallothioneins	Cisplatin, etoposide	Cisplatin-etoposide/CAV	
↑ Dihydrodiol dehydrogenase	Cisplatin, doxorubicin, taxanes ^a , vincas, melphalan		
↑ Thymidine & folate pools	Pemetrexed		
↑ Peroxiredoxin V	Doxorubicin, etoposide		
↑ Deoxycytidine deaminase	Gemcitabine		
Decreased drug activation or binding:			
↓ Deoxycytidine kinase activity	Gemcitabine		
↓ drug binding/ ↑ intracellular pH	Cisplatin		
Increased, decreased or altered target:			
↑ Ribonucleotide reductase M1	Gemcitabine	Gemcitabine regimens	Platinum + etoposide
↑ Ribonucleotide reductase M2		Gemcitabine + docetaxel	
↑ Folate pathway enzymes	Pemetrexed		
↓ Stathmin (oncoprotein 18)	Vincas		Cisplatin-vinorelbine ^g
↑/mutated III β tubulin (+/- α tubulin)	Taxanes, vincas ^a , cisplatin, doxorubicin, etoposide	Taxanes, cisplatin-vinorelbine ^a	
↑ histone deacetylase 6 (↑ tubulin stability)	Taxanes		
↓ /mutated Topoisomerase II- α	Etoposide, anthracyclines	Etoposide	
↑ Fragile histidine triad gene	Etoposide, camptothecins		
Increased DNA damage repair:			
↑ Topoisomerase II- α	Cisplatin, radiation, vincas	Cisplatin regimens	
↑ ERCC1	Platinums ^a	Platinum regimens ^a	Gemcitabine/docetaxel, gemcitabine/epirubicin
↑ Xeroderma Pigmentosum A	Platinums		
↑ Rad23A	Cisplatin		
↑ Ribonucleotide reductase M1	Gemcitabine, cisplatin	Gemcitabine regimens	Platinum + etoposide
↑ Rad51 (homologous DNA repair)	Platinums, etoposide		Cisplatin + gemcitabine
↑ DNA-dependent protein kinase	Etoposide		
↑ Hus1	Cisplatin		
↑ BRCA1	Platinums ^d	Cisplatin + gemcitabine ^a , cisplatin regimens	Gemcitabine + epirubicin or docetaxel ^d
↑ FANCD2			Platinum regimens
↑ apurinic/apyridinimic endonuclease	Cisplatin	Cisplatin regimens	
↑ Eme1 endonuclease	Cisplatin		
↑ PARP	Cisplatin, topotecan, temozolamide		

Factor	Agents affected preclinically	Clinical data support link to resistance	
		Yes	No
↓ High mobility group box 2	Cisplatin		
↓ Fragile histidine triad gene	Cisplatin		
↑ Thymidylate synthase	Platinums		
↑ Dihydropyrimidine dehydrogenase	Platinums		
Decreased apoptotic response:			
↓ DNA mismatch repair	Platinums	Platinum regimens ^a	
p53 mutation/ ↑ IHC expression	Cisplatin, etoposide, camptothecin, methotrexate, anthracyclines, radiation, taxanes ^a , others	Platinum regimens ^{a,c} , CAV	Taxanes or vincas without platinums
↓ p53-binding protein 2	Cisplatin, radiation		
↓ GML protein	Cisplatin	Cisplatin	
↓ Caspase-8 activity	Cisplatin, topotecan, radiation		
↓ Caspase-9 activity	Cisplatin		
↓ FUS1	Cisplatin		
↓ SAPK/c-Jun N-terminal kinase	Platinums, gemcitabine		
↓ Bak, Bad, Bid	Cisplatin, etoposide, radiation, Fas ligand		Vinorelbine
↓ Bax ^a	Cisplatin, etoposide, taxanes, doxorubicin		Cisplatin regimens, Vinorelbine/docetaxel
↓ Apoptosis signal transduction	Cisplatin, taxanes		
↓ ERK1/2 & MAPK/ERK kinase	Taxanes ^a , cisplatin ^a		
↓ p-ERK		Gemcitabine	Platinum regimens, taxane regimens
↓ βig-h3	Etoposide		
Increased Apoptosis Inhibitors:			
↑ Cyclooxygenase-2	Cisplatin, anthracycline, etoposide, vinca, taxane, gemcitabine	Carboplatin ^h , gemcitabine ^h , vinorelbine ⁱ , docetaxel	
↑ Bcl-2 ^{a,f}	Cisplatin, camptothecin, doxorubicin, etoposide, vincas		Platinum regimens, vincas, taxanes, etoposide regimens
↑ Bcl-xL	Cisplatin, gemcitabine, doxorubicin, vincas, taxanes, etoposide, others		Vinorelbine
↑ Mcl-1	Cisplatin, etoposide, taxanes, radiation		
↑ Survivin	Cisplatin, gemcitabine, taxanes	Cisplatin/etoposide	
↑ Livin	Etoposide		
↑ XIAP	Cisplatin, etoposide		
↑ IAPs	Gemcitabine		
↑ Telomerase	Cisplatin, docetaxel, etoposide		
↑ TRAIL decoy receptors 1 & 2	Doxorubicin, etoposide		
↑ Epidermal growth factor receptor (by IHC or gene copy number) ^j	Cisplatin ^a , doxorubicin, etoposide, vincas, taxanes,		Platinums, taxane, XRT, gemcitabine, vinorelbine

Factor	Agents affected preclinically	Clinical data support link to resistance	
		Yes	No
	camptothecin, pemetrexed, gemcitabine, others		
EGFR wild type vs exon 19 deletion		Platinum regimens	
↑ HER-2/neu (erbB-2, p185)	Cisplatin, etoposide, doxorubicin, taxanes, gemcitabine ^{a,e}		Platinum regimens ^a
↑ IGF-1R	Platinums, etoposide, taxanes	Carboplatin/paclitaxel	
↑ Attachment to ECM: integrins	Cisplatin, doxorubicin, taxanes, etoposide, others		
↑ Intercellular adhesion molecule		Cisplatin/paclitaxel	
↑ Osteopontin	Cisplatin	Carboplatin/paclitaxel	
↑ Stromal-cell-derived factor-1/CXCL12	Etoposide		
↑ Matrix metalloproteinase-7 (matrilysin)		Platinum regimens	
↑ Hyaluronan/CD44	Cisplatin		
↑ Growth hormone releasing hormone	Taxanes		
↓ c-Kit		Cisplatin + etoposide	
↑ PDGFR α	Cisplatin		
↑ Hepatocyte growth factor	Cisplatin		
↑ Fibroblast growth factor 2	Etoposide		
↑ PC cell-derived growth factor		Platinum regimens	
↑ PTEN/PI3K/Akt/mTOR pathway activation	Cisplatin, etoposide, taxanes, gemcitabine, others		Platinum regimens, taxanes
↑ p70S6K & S6 phosphorylation	Cisplatin		
K-ras mutation		Taxanes	Platinum regimens ^{a,j}
↑ ERK1/2	Etoposide, cisplatin ^a , taxanes ^a		
↑ MAPK phosphatase-1	Cisplatin		
↑ PKC- α	Platinums ^a , vincas, taxanes ^a , doxorubicin, others		Cisplatin + gemcitabine
↓ PKC- β	Cisplatin, etoposide		
↑ PKC- δ	Etoposide, cisplatin		
↑ PKC- ϵ	Etoposide, doxorubicin		
↑ PKC- η	Platinums ^a , vincas, taxanes ^a , doxorubicin, others		
↑ Caveolin-1/Caveolae organelles	Etoposide, paclitaxel	Gemcitabine/cisplatin, gemcitabine/epirubicin	
Altered membrane gangliosides	Cisplatin		
↑ Annexin IV	Taxanes		
↑ c-Src	Amrubicin		
↑ Heat shock protein 90	Taxanes		
↑ Heat shock protein 27		Vinorelbine ⁱ	

Factor	Agents affected preclinically	Clinical data support link to resistance	
		Yes	No
↑ Clusterin	Paclitaxel, gemcitabine		
↑ Nrf2/heme oxygenase-1	Platinums, etoposide, doxorubicin		
↓ / mutated Keap1	Platinums, etoposide, doxorubicin		
↑ Glucose-regulated protein 78	Etoposide		
Altered cell cycling:			
Cell cycle phase	Varies with drug		
Abnormal mitotic spindle checkpoint	Vinorelbine, taxanes		
↓ mitotic slippage/ ↓ aneuploidy	Taxanes		
↑ aneuploidy	Etoposide, topotecan, gemcitabine		
↓ CHK2 kinase	Cisplatin		
↑ HUS1	Cisplatin		
↑ RB/ ↓ pRB	Cisplatin, etoposide, taxanes, 5-FU	Cisplatin regimens ^a	
↑ 14-3-3ζ	Cisplatin		
↑ 14-3-3σ		Cisplatin + gemcitabine	
↑ p27Kip1		Cisplatin regimens ^a	
↑ P21WAF1/CIP1	Cisplatin, camptothecin, doxorubicin, etoposide	Platinum regimens	
↑ SKP2	Cisplatin, camptothecin, others		
↑ Cdc2/cdk1		Platinum regimens	
Cyclin D1 polymorphisms		Platinum regimens	
↓ Eg5		Cisplatin + antimetabolic agent	
↓ Cyclin B1		Platinums + antimetabolic agents	
Increased transcription factors:			
↑ NF-κB	Cisplatin, doxorubicin ^a , etoposide ^a , gemcitabine, taxanes		
↑ Clock	Cisplatin, etoposide		
↑ Activating Transcription Factor 4	Cisplatin, etoposide		
↑ HIV-1 Tat Interacting Protein 60	Cisplatin		
↑ TWIST	Cisplatin		
↑ SNAIL	Cisplatin		
↑ Kruppel-related zinc finger protein-1 (HKR-1)	Cisplatin	Cisplatin	
↑ STAT3	Cisplatin		
↑ Thyroid Transcription Factor-1	Cisplatin		
↑ NKX2-8	Cisplatin		
↑ PPARγ splice variant	Cisplatin		
↑ c-myc	Cisplatin		

Factor	Agents affected preclinically	Clinical data support link to resistance	
		Yes	No
↑ E2F4/ ↓ E2F1	Cisplatin, etoposide		
↑ Oct4	Cisplatin		
Stem cell markers and pathways			
↑ CD133	Cisplatin, etoposide, doxorubicin, paclitaxel	Platinum regimens	
SHH polymorphisms	Cisplatin, carboplatin	Platinum regimens	
“Global” factors			
Gene expression arrays	Cisplatin, taxanes, irinotecan, gemcitabine, vinorelbine	Cisplatin regimens, pemetrexed	
Chromosomal alterations	Platinums, taxanes, etoposide, topotecan		
DNA methylation			
In vitro sensitivity		Platinums, vincas, taxanes, topo I & II inhibitors, gemcitabine, cyclophosphamide	

^aData were not consistent, and were equivocal or negative in some studies

^bNo association preclinically with resistance to platinums; may sensitize to gemcitabine

^cNo association preclinically with resistance to anthracyclines, vinca alkaloids, bleomycin, irinotecan/SN-38

^dBRCA1 expression sensitized cells to antimicrotubule agents, and improved clinical outcome with gemcitabine + docetaxel

^eParadoxical increase in sensitivity to gemcitabine-cisplatin combination in one preclinical study

^fParadoxical increase in sensitivity to taxanes in some preclinical studies

^gEffect clinically was opposite to preclinical effect, with worse outcome in patients with tumors with high stathmin

^hParadoxical increase in efficacy for patients with tumors positive for p53 by IHC

ⁱTrend present