Cancer drug resistance: an evolving paradigm

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Abstract | Resistance to chemotherapy and molecularly targeted therapies is a major problem facing current cancer research. The mechanisms of resistance to 'classical' cytotoxic chemotherapeutics and to therapies that are designed to be selective for specific molecular targets share many features, such as alterations in the drug target, activation of prosurvival pathways and ineffective induction of cell death. With the increasing arsenal of anticancer agents, improving preclinical models and the advent of powerful high-throughput screening techniques, there are now unprecedented opportunities to understand and overcome drug resistance through the clinical assessment of rational therapeutic drug combinations and the use of predictive biomarkers to enable patient stratification.

Chemotherapy is one of the principal modes of treatment for cancer, but the effectiveness of chemotherapy is limited by drug resistance. Resistance to chemotherapeutics can be divided into two broad categories: intrinsic or acquired. Intrinsic resistance indicates that before receiving chemotherapy, resistance-mediating factors pre-exist in the bulk of tumour cells that make the therapy ineffective. Acquired drug resistance can develop during treatment of tumours that were initially sensitive and can be caused by mutations arising during treatment, as well as through various other adaptive responses, such as increased expression of the therapeutic target and activation of alternative compensatory signalling pathways[1]. Moreover, it is increasingly recognized that tumours can contain a high degree of molecular heterogeneity[2], thus drug resistance can arise through therapy-induced selection of a resistant minor subpopulation of cells that was present in the original tumour.

The use of modern genomic, proteomic and functional analytical techniques has resulted in a substantial increase in our ability to identify novel genes and signalling networks that are involved in determining the responsiveness of tumours to a particular drug treatment. Moreover, the use of high-throughput techniques in combination with bioinformatics and systems biology approaches has aided the interrogation of clinical samples and allowed the identification of molecular signatures and genotypes that predict responses to certain drugs. In addition, such approaches can identify novel therapeutic targets for overcoming or bypassing drug resistance. A diverse range of molecular mechanisms have been implicated in drug resistance; these include increased rates of drug efflux, alterations in drug metabolism and mutation of drug targets[1,3–5]. Tumours are highly adaptable, and the activation of survival signalling pathways and the inactivation of downstream death signalling pathways can also lead to drug resistance[6,7]. Epigenetic changes and the influence of the local tumour microenvironment have also been identified as important contributors to chemoresistance[8,9]. More recently, treatment failure in certain settings has been attributed to the presence of cancer stem cells (BOX 1), which are intrinsically highly resistant to many therapeutic approaches[10]. Moreover, the increasingly recognized molecular and genetic heterogeneity that is present in many tumours[2] is another major problem that can contribute substantially to resistance.

As our understanding of the molecular biology of cancer has advanced, drug development has shifted towards agents that target specific molecular alterations in tumours. These 'molecularly targeted therapies' have had varying degrees of success because a diverse range of resistance mechanisms have limited patient responses. Importantly, the mechanisms of resistance to cytotoxic chemotherapies and targeted drugs largely overlap, thus knowledge gained from earlier research into the mechanisms of resistance to cytotoxic drugs can be applied to help anticipate and elucidate mechanisms of resistance to emerging molecularly targeted agents.

Although toxicity to normal tissues and pharmacokinetic effects such as drug solubility, systemic distribution, metabolism and elimination are all important factors that can limit the amount of drug reaching the tumour

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Key points

- Tumour resistance to chemotherapy and molecularly targeted therapies limits the effectiveness of current cancer therapies.
- Toxicity to normal tissues limits the amount of drug that can be systemically administered, and pharmacokinetic effects (absorption, distribution, metabolism and elimination (ADME)) limit the amount of drug that reaches the tumour.
- At the level of the tumour, various resistance mechanisms can operate, such as increased drug efflux, mutations of the drug target, DNA damage repair, activation of alternative signalling pathways and evasion of cell death.
- Tumour resistance can be intrinsic (that is, present before treatment), or acquired during treatment by various therapy-induced adaptive responses.
- Tumours are heterogeneous; therefore, resistance can also arise by positive selection of a drug-resistant tumour subpopulation.
- High-throughput screening techniques and systems biology approaches have the power to identify novel mechanisms of drug resistance and molecular signatures and genotypes that predict tumour response.
- Increasingly, predictive biomarkers will be used clinically to stratify patients to receive specific therapeutics.
- Improved understanding of the molecular basis of resistance will inevitably lead to the clinical assessment of rational drug combinations in selected patient populations.

(FIG. 1), we do not discuss these further. Rather, this Review focuses on the mechanisms that enable the survival of cancer cells during drug treatment and on current research efforts to identify and overcome underlying mechanisms of resistance to both standard chemotherapeutic agents (TABLE 1) and molecularly targeted therapies (TABLE 2).

Antimetabolites
A class of drug that interferes with normal cellular metabolism by disrupting the function of a normal cellular metabolite. Examples that are used as anticancer therapies include 5-fluorouracil, methotrexate and pemetrexed.

Drug transport and metabolism

Drug efflux. Several cell membrane transporter proteins have been linked to resistance to commonly used chemotherapeutics by promoting drug efflux. Most notably, the ATP-binding cassette (ABC) transporter family of transmembrane proteins regulate the flux across the plasma membrane of multiple structurally and mechanistically unrelated chemotherapeutic agents. In total there are 49 members of this protein family, but only three have been studied extensively in relation to multidrug resistance (MDR). These are multi-drug resistance protein 1 (MDR1; also known as P-glycoprotein and ABCB1), MDR-associated protein 1 (MRP1; also known as ABCC1) and breast cancer resistance protein (BCRP; also known as ABCG2)[3]. All three have broad, overlapping substrate specificity and promote the elimination of various hydrophobic compounds, including major cancer chemotherapeutics such as taxanes, topoisomerase inhibitors and antimetabolites.

MDR1 was the first ABC transporter to be identified; it is a membrane-bound glycoprotein that is expressed in almost all tissues at low levels, but is found at much higher levels on the surface of epithelial cells that have excretory roles, such as those lining the colon, small intestine, pancreatic ductules, bile ductules and kidney proximal tubules[11,12]. MDR1 is overexpressed in many tumours (thus causing intrinsic drug resistance) and the expression of MDR1 can be induced by chemotherapy (thus also resulting in the acquired development of MDR)[13]. MDR1 overexpression has been associated with chemotherapy failure in many cancers, including kidney, colon and liver cancers, as well as leukaemias and lymphomas. More recently, the overexpression of MRP1 has also been correlated with chemoresistance in prostate, lung and breast cancer[14,15,16]. BCRP, which was the third major MDR drug efflux pump to be identified, has been associated with chemoresistance in breast cancer and leukaemia[17,18]. Recent reports have suggested that molecularly targeted therapies such as imatinib, erlotinib, sunitinib and nilotinib (TABLE 2) are also substrates for and modulators of MDR1 and BCRP[19]. Cancer stem cells, which are inherently drug resistant, also display higher levels of drug efflux proteins[20]. CD44, which is a cancer stem cell marker that exhibits strong negative correlations with patient survival[21], has been associated with expression of MDR proteins, most notably BCRP. However, as these cells persist after treatment with drugs that are not MDR substrates, it seems that efflux mechanisms may not be crucial to the drug-resistant phenotype displayed by cancer stem cells.

MDR1 inhibitors such as zosuquidar and tariquidar have high potency and specificity; however, results from clinical trials have been disappointing. Tariquidar showed limited activity in combination with an anthracycline or taxanes in a small cohort of women with stage III–IV breast carcinoma[22], whereas a Phase II study in breast cancer found no additional benefit in overall survival, progression-free survival or response rates when zosuquidar was co-administered with docetaxel[23]. Taken at face value, this suggests that the contribution of MDR proteins to clinical drug resistance may be less important than preclinical models have suggested; however, it is also possible that the ABC transporter family exhibits a high degree of functional redundancy. Indeed, one study of ABC transporters in the NCI60 cell line panel suggested a role in drug resistance for more than half of the family members[24]. Increased understanding of the molecular

Box 1 | **Cancer stem cells and drug resistance**

Two current models of carcinogenesis are the stochastic model, which proposes that every transformed cell within a tumour has tumorigenic potential, and the cancer stem cell (CSC) model, which proposes that only a small subset of cells can give rise to a new tumour. The CSC model has fundamental implications for cancer therapeutics and drug resistance. CSCs (or cancer cells with stem-cell-like properties) represent an important target population for anticancer therapeutics as their survival following therapy is highly likely to result in disease relapse.

Stem-cell-like cancer cells are believed to be highly resistant to conventional chemotherapies owing to various crucial features, including high expression of ATP-binding cassette (ABC) transporter proteins[144,145], aldehyde dehydrogenase (ALDH) activity[146], expression of anti-apoptotic proteins such as BCL-2 and BCL-X_L, enhanced DNA damage repair and activation of key prosurvival signalling molecules such as NOTCH and nuclear factor-κB (NF-κB)[147]. They are also relatively quiescent and therefore resistant to chemotherapies, which target rapidly dividing cells. The CSC model also has implications for the development of targeted therapies. Second-line resistance to imatinib has been associated with the persistence of leukaemic stem cells[148]; however, other studies suggest that long-term treatment with both imatinib and nilotinib may reduce the abundance of leukaemic stem cells in certain patients[149].

However, this area of research faces many challenges, and many questions remain regarding the abundance, characteristics and origins of these cells. For example, reliable CSC markers have not yet been established, and the stability of the CSC phenotype is being questioned[150]. The plasticity of tumours is such that more-differentiated tumour cells may revert to being stem-cell-like, indicating that the bulk of cells in a tumour must also be targeted in conjunction with any novel stem-cell-targeting agents.

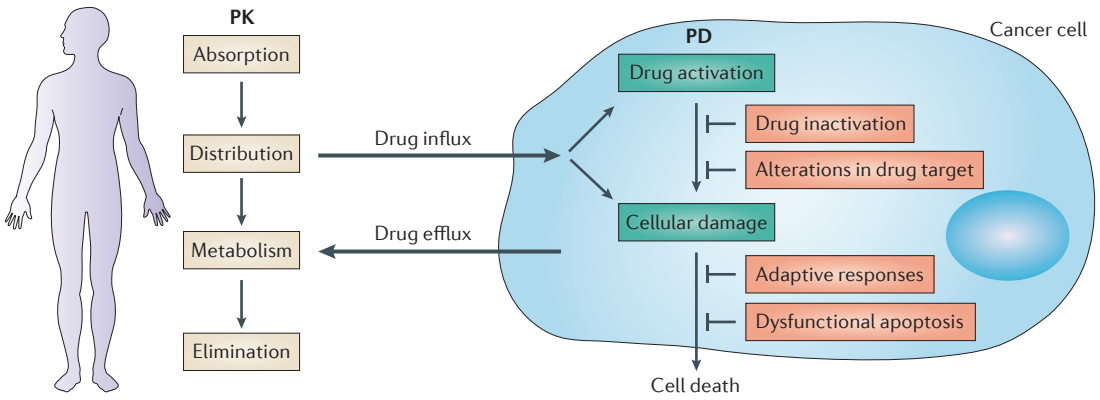

Figure 1 | **General principles of drug resistance.** Pharmacokinetic (PK) factors such as drug absorption, distribution, metabolism and elimination (ADME) limit the amount of a systemically administered drug that reaches the tumour. In the tumour, the effects of the drug on the cancer cell are collectively termed its pharmacodynamic (PD) properties. The anticancer activity of a drug can be limited by poor drug influx or excessive efflux; drug inactivation or lack of activation; alterations such as changes in expression levels of the drug target; activation of adaptive prosurvival responses; and a lack of cell death induction due to dysfunctional apoptosis, which is a hallmark of cancer.

features that make a drug a substrate for ABC transporters may allow the avoidance of such features to be factored into medicinal chemistry during the development of new anticancer drugs.

Drug activation and inactivation. Other upstream resistance mechanisms, which are relevant for both chemotherapeutics and molecularly targeted agents, are drug inactivation or a lack of drug activation. Such mechanisms are highly specific for each class of drug; for example, platinum drugs can be inactivated by the thiol glutathione[25], and the conversion of antimetabolites such as 5-fluorouracil (5-FU) and methotrexate to their most active forms does not occur when the relevant cellular enzyme activities are absent[26,27]. Capecitabine is a fluoropyrimidine prodrug that is converted into 5-FU by thymidine phosphorylase[28]. The gene encoding thymidine phosphorylase can be inactivated by methylation, thereby causing capecitabine resistance[29]. However, this epigenetic silencing is tumour-specific and can be reversed by treatment with inhibitors of DNA methyltransferases (DNMTs). Epigenetic silencing can also promote drug activity, as observed in the case of the topoisomerase I inhibitor irinotecan, which is inactivated by UDP glucuronosyltransferase 1 (UGT1A1); expression of *UGT1A1* is negatively regulated by DNA methylation of the promoter. Therefore, in this case, promoter methylation promotes irinotecan activity[30,31].

Alterations in drug targets

Drug response and resistance can be affected by alterations to the drug target, such as mutations or changes in expression level. Examples of this are thymidylate synthase inhibitors such as 5-FU and pemetrexed[32], which post-transcriptionally upregulate thymidylate synthase expression owing to the inhibition of a negative feedback loop in which substrate-free thymidylate synthase binds to and inhibits the translation of thymidylate synthase mRNA. Another example is the androgen receptor (AR), which is genomically amplified in ~30% of prostate cancers that have developed acquired resistance to standard androgen deprivation therapy using testosterone-lowering drugs such as leuprolide and AR antagonists such as bicalutamide[33]. In both cases, increased drug target expression reduces the effectiveness of inhibitors of these targets because more target molecules be must be inhibited to have a therapeutic effect.

Cancers are often highly dependent on specific oncogenic mutations that occur in kinases; however, the outgrowth of cells with gatekeeper residue mutations is a common mechanism of resistance to agents that target these oncogenic kinases. For example, high response rates to inhibitors of the epidermal growth factor receptor (EGFR) such as gefitinib and erlotinib in non-small-cell lung cancers (NSCLCs) with activating mutations in the EGFR tyrosine kinase domain (in-frame deletions in exon 19 and the L858R mutation in exon 21) have been reported[34]. However, most patients with initial responses acquire resistance within 1 year, and in 50% of such cases, a secondary EGFR-T790M gatekeeper mutation has been identified[35–37]. Chromosomal rearrangements and/or other genetic alterations, such as amplification and mutations in anaplastic lymphoma kinase (*ALK*) have been identified in anaplastic large-cell lymphoma and paediatric neuroblastoma, as well as in 4% of patients with NSCLC[38–41]. Tumour responses of ~60% to the tyrosine kinase inhibitor (TKI) crizotinib, which inhibits ALK and MET, have been observed in patients with ALK-positive NSCLC. However, most of these patients relapse within 1 year[42,43], and secondary mutations in the ALK tyrosine kinase domain or *ALK* fusion gene amplifications have been reported in these relapsing patients.

This type of target-associated resistance has also been observed for imatinib, which is a highly effective inhibitor of the BCR–ABL1 oncogenic kinase that causes chronic myeloid leukaemia (CML)[44]. The first mutation identified in patients with CML who relapsed on treatment with imatinib was in the gatekeeper residue of BCR–ABL1, T315. This single missense mutation in the kinase domain of BCR–ABL1

Gatekeeper residue
A conserved residue that lies at the opening of the ATP-binding pocket in many kinases. Mutations in these sites are frequently observed as a resistance mechanism to inhibitors of oncogenic kinases.

hinders imatinib binding while preserving the catalytic activity that is needed for the oncogenic function of BCR–ABL1 (REF. 45). Second-generation BCR–ABL1 inhibitors have been developed, three of which (nilotinib, dasatinib and bosutinib) have been approved for the treatment of patients who have developed imatinib resistance; these inhibitors are active against all of the common BCR–ABL1 mutants with the exception of T315I[46–48].

Drug resistance due to gatekeeper mutations in oncogenic kinases is clearly an important clinical challenge. A new BCR–ABL1 inhibitor, ponatinib, has shown promising efficacy in patients whose leukaemias harbour the T315I mutation and is also active against other BCR–ABL1 mutations[49–51]. Second-generation, irreversible EGFR TKIs — such as HKI-272, afatinib and PF00299804 — are currently being assessed in Phase I/II clinical trials. However, although these drugs are active in patients with NSCLCs that have the EGFR-T790M mutation, there are toxicity issues associated with the activity of these drugs against wild-type EGFR[52,53]. A recent study using cell-based screening methods has identified a novel class of inhibitors that do not inhibit wild-type EGFR but are active against the T790M-mutated form, thus these inhibitors may have fewer adverse side effects[54]. It will be interesting to determine whether the new generation of BCR–ABL1 and EGFR inhibitors encounter target-associated mechanisms of resistance that are similar to the previous generation.

DNA damage repair

Many chemotherapeutic drugs induce DNA damage either directly (for example, platinum-based drugs) or indirectly (for example, topoisomerase inhibitors). The cellular response to DNA damage is repair or cell death; therefore, the DNA damage repair capacity of cancer cells has a major influence on the effectiveness of DNA-damaging drugs. The role of DNA damage repair in drug resistance was recently reviewed in this journal[55], so we will only briefly discuss it here. DNA damage induces cell cycle arrest, which has evolved to allow cells time to repair the damage. In some cancers, the regulation of cell cycle arrest is disrupted owing to gain-of-function alterations to oncogenes and/or loss-of-function alterations to tumour suppressor genes. For example, mutation of p53, which has an important role in regulating numerous cell cycle checkpoints can disrupt DNA-damage-induced cell cycle arrest[56]. p53 is also involved in the induction of apoptosis, and its mutation is frequently associated with drug resistance[57].

Inhibiting DNA damage repair in cancer cells is an obvious therapeutic strategy to combine with DNA-damaging agents. Moreover, cancers frequently have a dysfunction in at least one DNA damage repair pathway, which can lead to complete dependence on an alternative repair pathway that is functionally redundant in normal cells and therefore can be inhibited to induce cancer-cell-specific death; this is the

Table 1 | **Summary of resistance mechanisms to some common chemotherapeutic agents**

Cytotoxic agent	Cancer type	Target	Resistance mechanism	Refs
Antimetabolites (for example, 5-FU, methotrexate, gemcitabine and cytarabine)	Breast cancer, colorectal cancer, pancreatic cancer, gastric cancer, head and neck cancer, ovarian cancer, lymphoma and leukaemia	Thymidylate synthase and DNA synthesis	Increased target expression (thymidylate synthase)	151
			MLH1 hypermethylation	152
			Activation of survival pathways (for example, ERBB signalling pathways)	101
			Increased expression of anti-apoptotic proteins (for example, FLIP, BCL-2 or MCL1)	90,75
Platinum compounds (for example, cisplatin and oxaliplatin)	Ovarian cancer, testicular cancer, sarcoma, lymphoma and small-cell lung carcinoma	DNA	Reduced cellular uptake	153
			Increased efflux	13
			Increased DNA repair	66–69
			MLH1 hypermethylation	64
Topoisomerase I inhibitors (for example, irinotecan)	Colorectal cancer and small-cell lung carcinoma	Topoisomerase I	Drug efflux	13
			Reduced target expression	25
			Topoisomerase I mutations	154
			Suppression of apoptosis	75
			Activation of survival pathways (for example, ERBB signalling pathways)	99
Topoisomerase II inhibitors (for example, doxorubicin and etoposide)	Kaposi's sarcoma, Ewing's sarcoma, lung cancer, testicular cancer, lymphoma, leukaemia and glioblastoma	Topoisomerase II	MDR1 overexpression	13
			Mutation or decreased expression of topoisomerase II	155
			Decreased apoptosis due to mutation of p53	59
Microtubule poisons (for example, paclitaxel and vinorelbine)	Lung cancer, ovarian cancer, breast cancer, head and neck cancer, Kaposi's sarcoma	Tubulin	Tubulin mutations	156–157
			MDR1 overexpression	13
			Chromosomal instability	71

5-FU, 5-fluorouracil; MDR1, multi-drug resistance protein 1.

Table 2 | **Summary of resistance mechanisms to some common molecularly targeted agents**

Targeted therapy	Cancer type	Target	Resistance mechanism	Refs
Imatinib	CML, ALL and GIST	BCR–ABL1, KIT and PDGFRα	Mutations of the target (for example,T315 in ABL1, T670I in KIT and T674I in PDGFRα)	41
			Elevated MDR1 expression	20–22
Dasatinib	ALL and CML	BCR–ABL1	T315 mutation in ABL1	43
Nilotinib	CML	BCR–ABL1	BCR–ABL1 upregulation	158,159
			T315 mutation in ABL1	42
Trastuzumab	ERBB2-positive breast cancer	ERBB2	PTEN loss	160
			Truncation of ERBB2	161
			Activating mutations of PIK3CA	162
			Activation of alternative signalling pathways (such as IGF1 and ERBB3)	163
Gefitinib	NSCLC	EGFR	EGFR kinase domain mutations (for example, T790M)	38–40
			Epithelial–mesenchymal transition	118
			Epigenetic mechanisms	164
			Increased ERBB family signalling or *MET* amplification	108,109
Cetuximab	Head and neck cancer and colorectal cancer	EGFR	KRAS mutation	165
			EGFR-S492R mutation inhibits cetuximab binding	166
			Increased ERBB family signalling	107
Vemurafenib	Melanoma	BRAF-V600E	Elevated BRAF-V600E expression	110
			Acquired mutations in KRAS, NRAS or MEK1	111–114
			Activation of EGFR, IGF1R and PDGFRβ pathways	115
Crizotinib	NSCLC	EML4–ALK	Secondary EML4–ALK mutations or rearrangement	45,46
			COT-mediated MAPK reactivation	112
			CD74–ROS1 rearrangement	167
Bortezomib	Multiple myeloma and mantle cell lymphoma	Proteasome	Mutation in the binding site for bortezomib	168
			Anti-apoptotic mechanisms	169
Bevacizumab	Colorectal cancer, NSCLC, glioblastoma and renal cell carcinoma	VEGF	Activation of alternative signalling pathways (such as IGF1R, PDGFR, FGFR or MET)	170
			Hypoxia-induced autophagy	171
			Induction of tumour dormancy or an increase in the cancer stem cell niche	172

ALL, acute lymphoblastic leukaemia; CML, chronic myeloid leukaemia; COT, cancer Osaka thyroid oncogene (also known as MAP3K8); EGFR, epidermal growth factor receptor; EML4–ALK, a fusion of echinoderm microtubule-associated protein-like 4 and anaplastic lymphoma kinase; FGFR, fibroblast growth factor receptor; GIST, gastrointestinal stromal tumour; IGF1, insulin-like growth factor 1; IGF1R, IGF1 receptor; MDR1, multi-drug resistance 1; NSCLC, non-small-cell lung cancer; PDGFR, platelet-derived growth factor receptor; PIK3CA, PI3K catalytic subunit-α; VEGF, vascular endothelial growth factor.

concept of synthetic lethality, which is described in detail elsewhere[58]. Thus, molecularly targeted agents that inhibit components of the DNA damage response machinery have been developed, such as inhibitors of the single-strand-break DNA repair enzyme poly(ADP–ribose) polymerase 1 (PARP1), which have been shown to exhibit synthetic lethality in breast and ovarian tumours harbouring mutations in the *BRCA1* or *BRCA2* genes[59]. *BRCA1*- or *BRCA2*-mutant cells are sensitive to treatment with PARP1 inhibitors owing to their impaired homologous recombination DNA repair pathways, which normally compensate for loss of PARP1 activity. However, resistance has been reported in *BRCA2*-mutant tumours treated with PARP inhibitors due to in-frame deletions in *BRCA2* that partially restore its DNA repair function, thereby allowing these cells to survive treatment[60,61].

The mismatch repair (MMR) system is crucial for maintaining genomic integrity, and mutations in MMR genes such as *MLH1* and *MSH2* can lead to the microsatellite instability (MSI) phenotype. Moreover, MMR deficiency has been linked to the resistance to various cytotoxic chemotherapies; for example, hypermethylation of *MLH1* has been reported to cause resistance to cisplatin and carboplatin[62]. Conversely, a synthetic lethal interaction between MSH2-targeted short interfering RNA (siRNA) and methotrexate was identified in MMR-deficient cancer cells; methotrexate caused the accumulation of oxidative lesions such as 8-oxoguanine (8-oxoG) in MSH2-deficient cells, which resulted in a loss of viability through apoptosis[63]. This has led to an on-going Phase II clinical trial with methotrexate in patients with MSH2-deficient metastatic colorectal cancer using measurement of 8-oxoG lesions as a biomarker

Mismatch repair
(MMR). A mechanism that corrects base–base mismatches, or insertion and deletion mismatches that are caused by DNA polymerase errors during DNA replication.

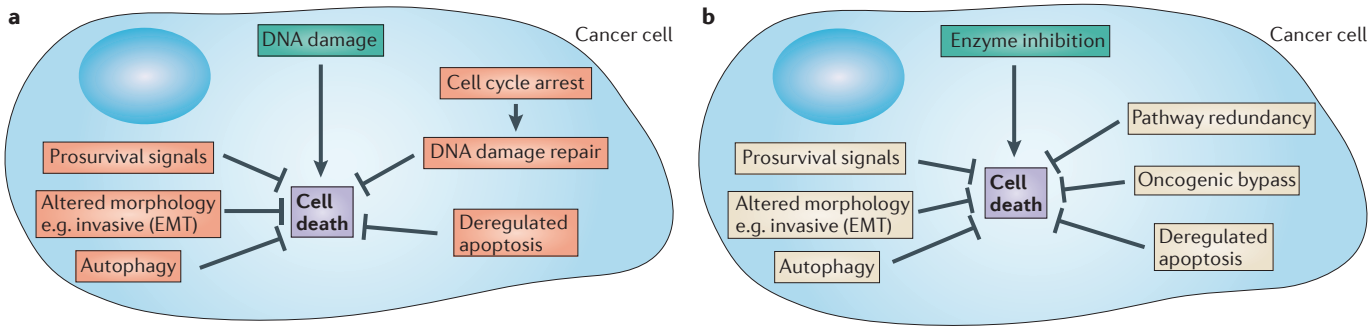

Figure 2 | **Summary of downstream factors that influence drug resistance. a** | The DNA damage induced by agents such as cisplatin cause cell cycle arrest, which may give the cancer cell time to repair the damage, resulting in drug resistance. Dysfunctional apoptosis can reduce the efficiency with which drug-induced DNA damage is linked to cell death. Cytotoxic drugs frequently activate prosurvival adaptive responses such as the activation of prosurvival signals, alterations in morphology (such as an epithelial–mesenchymal transition (EMT)) and autophagy. **b** | Similar mechanisms limit the effectiveness of targeted therapies that inhibit specific cellular enzymes and receptors. Additional drug resistance mechanisms that are highly relevant for these molecularly targeted agents include pathway redundancy and oncogenic bypass (also known as kinome reprogramming), all of which allow the cancer cell to survive the effects of target enzyme inhibition.

(ClinicalTrials.gov identifier NCT00952016). Efficient nucleotide-excision repair (NER) is required for the repair of DNA damage caused by many DNA-damaging drugs, such as platinum-based drugs[64]. One of the crucial components of the NER pathway is excision repair cross-complementing 1 (ERCC1). High expression of ERCC1 has been linked with poor responses to chemotherapy in numerous cancer types, including NSCLC, gastric cancer and ovarian cancer[65,66]. Notably, testicular cancers, which are very sensitive to cisplatin treatment, have very low levels of ERCC1 (REF. 67).

Genomic instability is a hallmark of cancer and can lead to increased tumour heterogeneity and resistance to both chemotherapies and molecularly targeted therapies. Chromosomal instability (CIN), which comprises changes in chromosome number and structure, is the most common form of genomic instability. Preclinical studies have indicated a role for CIN in both acquired and intrinsic resistance to certain chemotherapeutics such as taxanes[68,69]. A recent study has highlighted the importance of CIN in drug resistance by demonstrating that the overexpression of CIN genes (that is, genes that are involved in maintaining the genomic integrity of the cell) was associated with poor survival in myeloma and in seven other cancers that were examined[70,71]. Of these genes, a strong association was found for *NEK2*, which encodes a serine/threonine mitotic kinase that has roles in spindle formation and chromosome segregation[70,71]. In addition, silencing of NEK2 *in vitro* and *in vivo* inhibited cell growth and decreased resistance to the targeted proteasome inhibitor bortezomib. The mechanism of NEK2-mediated drug resistance was further demonstrated to be through AKT–mediated upregulation of ABC transporters.

Downstream resistance mechanisms

After sufficient active drug has accumulated and inhibited its cellular target or targets, the outcome of treatment is dependent on how the cancer cell responds. This is equally true for both classical cytotoxic drugs (FIG. 2a) and the newer molecularly targeted agents (FIG. 2b). Ideally, drug-induced damage should be tightly coupled to the induction of cell death. However, numerous intrinsic adaptive responses can be triggered that promote cancer cell survival. In addition, as part of the process of transformation, the pathways that regulate cell death by apoptosis frequently become dysfunctional; this is one of the classic hallmarks of cancer[72].

Deregulation of apoptosis. There is accumulating evidence that although resistance to apoptosis is a hallmark of cancer and can cause resistance to drug treatment, cancer cells are typically 'addicted' to a fairly small number of anti-apoptotic proteins for their survival[73], providing a strong rationale for targeting these proteins therapeutically. Most prominent among these are the anti-apoptotic BCL-2 family members, inhibitor of apoptosis proteins (IAPs) and the caspase 8 inhibitor FLIP (FIG. 3). Mutations, amplifications, chromosomal translocations and overexpression of the genes encoding these proteins have been associated with various malignancies and linked to resistance to chemotherapy and targeted therapies. Moreover, these genes are transcriptional targets for prosurvival transcription factors such as nuclear factor-κB (NF-κB) and signal transducer and activator of transcription 3 (STAT3). During tumorigenesis, these transcription factors are activated by oncogenic mutations in kinases that regulate upstream prosurvival signalling pathways.

The role of BCL-2 family members in regulating responses to chemotherapy has been extensively studied. Initial studies showed that the overexpression of BCL-2 renders leukaemic cells and mouse thymocytes resistant to cytotoxic chemotherapeutic agents[74,75]. This suggests that despite diverse mechanisms of action, cytotoxic drugs all signal to a common pathway of cell death. This pathway involves mitochondrial outer membrane permeabilization (MOMP) and can be blocked

Nucleotide-excision repair (NER). A mechanism of repair for DNA damage caused by crosslinking of DNA bases. It is particularly important for resistance to platinum-based chemotherapeutics.

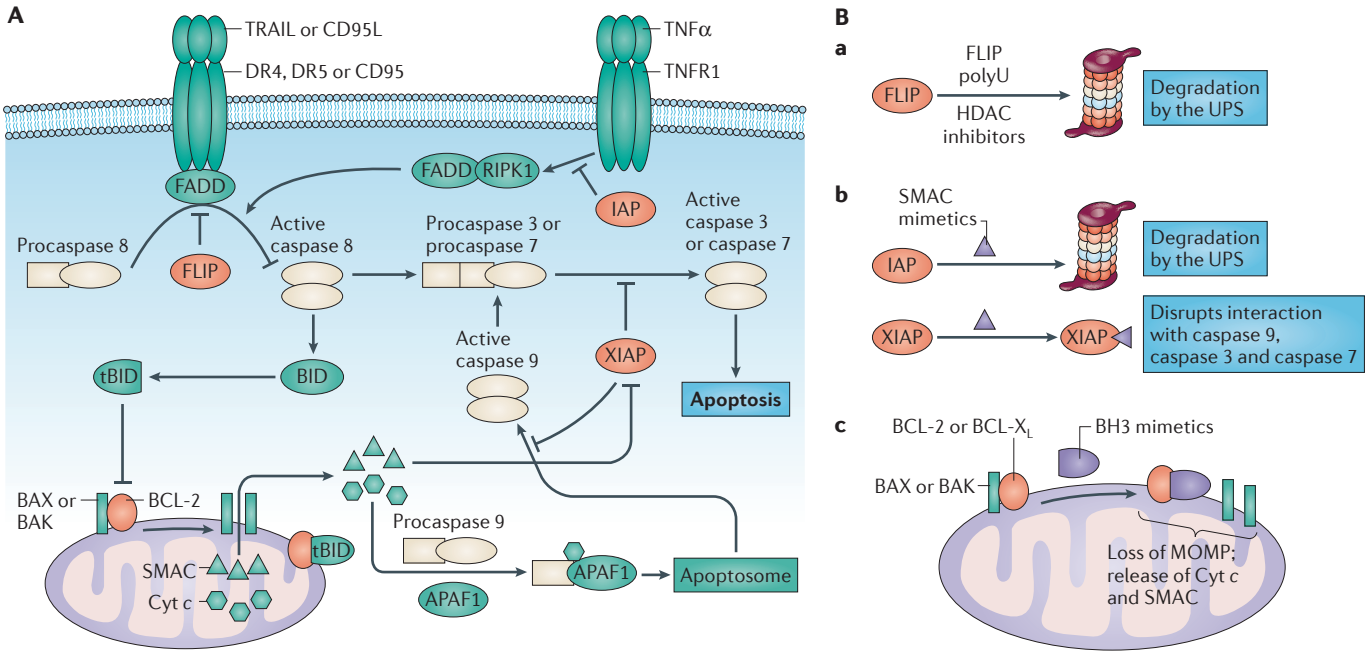

Figure 3 | **Apoptosis signalling and therapeutic targeting. A** | Overview of apoptosis signalling. The death receptors CD95 (also known as FAS), death receptor 4 (DR4), DR5 and tumour necrosis factor receptor 1 (TNFR1) can all induce apoptosis when bound by their ligands. For example, when TNF-related apoptosis-inducing ligand (TRAIL) binds to DR4 or DR5, the receptors recruit FAS-associated protein with death domain (FADD). The resulting complex, termed the death-inducing signalling complex (DISC), recruits procaspase 8 monomers, which are then activated by homodimerization-induced cleavage to form caspase 8. Dimerization of procaspase 8 at the DISC is inhibited by FLIP. Mitochondria-mediated apoptosis is controlled by the BCL-2 family of pro- and anti-apoptotic proteins. When pro-apoptotic BAX and BAK oligomerize, they form pores in the outer mitochondrial membrane that allow the release into the cytoplasm of cytochrome *c* (Cyt *c*), second mitochondria-derived activator of caspases (SMAC) and other pro-apoptotic factors. BAX and BAK oligomerization is controlled by anti-apoptotic BCL-2 proteins, including BCL-2 and BCL-X_L. BID, which is a BH3-only protein, can also be cleaved by caspase 8 to form truncated BID (tBID), which translocates to the mitochondria to promote oligomerization of BAX and BAK. Cytochrome *c* forms a complex with apoptotic protease-activating factor 1 (APAF1) and procaspase 9 that is termed the apoptosome, in which procaspase 9 dimerizes and becomes activated. Activation of initiator caspases 8 and 9 results in the activation of downstream executioner caspases 3 and 7, which selectively cleave a range of proteins that bring about the morphological characteristics of apoptosis. Activation of caspases 3, 7 and 9 is inhibited by inhibitor of apoptosis proteins (IAPs), particularly XIAP. XIAP itself is antagonized by SMAC released from the mitochondria. IAP1 and IAP2 promote the ubiquitination of receptor-interacting serine/threonine protein kinase 1 (RIPK1) at TNFR1 'complex 1', and this leads to downstream activation of nuclear factor-κB (NF-κB) and MAPK pathways. In the absence of IAP1 and IAP2, RIPK1 is deubiquitylated and forms a second complex, 'complex 2', containing FADD that can recruit and activate procaspase 8. **B** | Targeting anti-apoptotic proteins. Histone deacetylase (HDAC) inhibitors can trigger the rapid degradation of FLIP by the ubiquitin–proteasome system (UPS) (**a**). Targeted agents that mimic the activity of SMAC ('SMAC mimetics') inhibit XIAP and also trigger the rapid degradation of IAP1 and IAP2. By inhibiting XIAP, SMAC mimetics derepress caspases 9, 3 and 7, and by promoting the degradation of IAP1 and IAP2, they induce formation of the caspase 8-activating complex 2 (**b**). BH3-mimetic drugs such as ABT-737 and ABT-263 have been developed that antagonize anti-apoptotic BCL-2 proteins, thereby triggering BAX and BAK oligomerization, mitochondrial outer membrane permeabilization (MOMP) and release of apoptogenic factors from the mitochondria (**c**). CD95L, CD95 ligand.

BH3 profiling
A functional assay that can be used to measure how close a cell is to committing to apoptosis. It involves measuring the mitochondrial response to peptides derived from the BH3 domain of BCL-2 family members.

Mitochondrial priming
A measurable property that determines the proximity of a cell to the apoptotic threshold based on its BH3 profile.

by BCL-2. Various other BCL-2 family proteins have since been demonstrated to have roles in regulating chemotherapy-induced apoptosis. These include the anti-apoptotic BCL-2 family members (such as BCL-X_L and MCL1) and pro-apoptotic family members (such as BAX, BAD and BAK, as well as various BH3-only proteins that can antagonize the anti-apoptotic BCL-2 family members)[76–78]. It is the interplay between members of this family that is critical in determining the fate of the cell by either inhibiting or facilitating MOMP induction[79]. In a recent study, a crucial role for BCL-2 family proteins in determining whether patients respond to conventional chemotherapy was demonstrated. Using a method called BH3 profiling, which measures how 'primed' a cell is to committing to apoptosis, they were able to show that the level of mitochondrial priming correlated with clinical response to chemotherapy across a range of malignancies[80]. This indicates that BCL-2 family proteins have a pivotal role in dictating cell fate following chemotherapy treatment. It remains to be seen whether BH3 profiling will also predict responses to targeted therapies.

The BH3-only protein BIM has been identified as a central player in both imatinib-induced apoptosis in CML and also in gefitinib- and erlotinib-induced apoptosis in EGFR-mutated NSCLC[81–83]. Increased BIM expression is induced following the inhibition of AKT

and ERK that occurs in response to these TKIs. More recently, BIM levels have been demonstrated to predict clinical responsiveness to inhibitors of EGFR, ERBB2 (also known as HER2) or PI3K[84]. Moreover, a germline deletion in the gene encoding BIM has been identified in an East Asian population of patients and has been significantly associated with intrinsic resistance to TKI therapies in both chronic-phase CML and EGFR-mutant lung cancer[85].

Recently, substantial progress has been made in generating pharmacological inhibitors of anti-apoptotic BCL-2 family members as anticancer therapies. The best studied and most successful of these is ABT-737 and its orally bioavailable form ABT-263 (also known as navitoclax), which mimic the action of BH3-only BCL-2 family members by antagonizing the anti-apoptotic function of BCL-2, BCL-X_L and BCL-W and promoting the pro-apoptotic function of BAX and BAK[86] (FIG. 3). Preclinical data indicate that ABT-737 and ABT-263 exhibit cytotoxicity when combined with chemotherapies or radiation[87] and display single-agent effectiveness against various tumour types[87,88]. However, resistance mechanisms limit the effectiveness of these agents, most notably due to another anti-apoptotic BCL-2 family member, MCL1. ABT-737 binds weakly to MCL1, and resistance has been observed in cells that express MCL1 (REFS 86,88,89), whereas silencing of MCL1 has been shown to restore sensitivity to ABT-737 (REF. 90). MCL1 is clearly an important determinant of resistance to ABT-737 and is also an important mediator of resistance to cytotoxic chemotherapeutics.

Various strategies aimed at targeting MCL1 are being investigated; some of these are aimed at exploiting a feature of MCL1 that is not shared with other anti-apoptotic BCL-2 family members: it has an extremely short half-life due to its degradation by the ubiquitin–proteasome system. Thus, enzymes that are involved in regulating MCL1 degradation may prove to be useful therapeutic targets[91,92]. Obatoclax, which is a synthetic derivative of prodiginines, was initially thought to be a pan-BCL-2 inhibitor and was discovered in a high-throughput screen of natural compounds that inhibit protein–protein interactions in the BCL-2 family[93]. Obatoclax has high affinity for all anti-apoptotic members of the BCL-2 family, including MCL1; however, it is not entirely dependent on BAX and BAK to induce apoptosis, indicating that obatoclax may also affect other pathways to induce apoptosis[94]. More recently, MCL1-selective inhibitors have been developed using the same fragment-based design approach that was used to develop ABT-737 (REF. 95). This powerful medicinal chemistry approach has the potential to deliver clinically relevant small molecules for difficult drug targets, such as protein–protein interactions, and therefore substantially widens the scope of proteins that can be considered 'druggable'.

Recombinant forms of tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) and agonistic antibodies that recognize either death receptor 4 (DR4; also known as TRAILR1) or DR5 (also known as TRAILR2) have been developed. These pro-apoptotic receptor agonists have demonstrated antitumour activity *in vitro* and in xenograft models; however, the results of clinical trials of both recombinant TRAIL and TRAIL-receptor-targeted agonistic antibodies as monotherapies have been disappointing. Despite a lack of clinical activity as monotherapies (at least in unselected patient populations), combinations of TRAIL receptor agonists with both chemotherapeutics and targeted therapies — such as paclitaxel, carboplatin, bevacizumab, BCL-2 antagonists, histone deacetylase (HDAC) inhibitors and various kinase inhibitors — are currently being evaluated in preclinical and clinical trials and are showing promise[96,97]. Numerous drugs that have been shown to restore TRAIL sensitivity do so by decreasing the expression of the caspase 8 inhibitor FLIP, which is not only a major regulator of the pathway to apoptosis that is triggered by binding of death ligands to death receptors, but also of apoptosis that is induced by various chemotherapeutic agents[98]. Importantly, normal cells do not seem to be dependent on FLIP for survival to the same extent as cancer cells[99]. As such, inhibition of FLIP constitutes a promising therapeutic strategy for the treatment of numerous cancer types. At present, there are no specific inhibitors for FLIP; however, HDAC inhibitors seem to be highly effective at downregulating the expression of this short-lived protein through transcriptional and post-transcriptional mechanisms[100,101] (FIG. 3).

The role of IAPs in blocking both apoptosis and a programmed form of necrosis termed necroptosis has led to intense investigation into the use of small molecule inhibitors of IAPs based on a tetrapeptide motif (AVPI) that is present in the endogenous IAP antagonist, second mitochondria-derived activator of caspases (SMAC). Increased expression of IAPs has been associated with chemoresistance and poor outcome in patients with cancer[102]. SMAC-mimetic drugs act by inhibiting XIAP — which otherwise can inhibit caspases 3, 7 and 9 — and by inducing the degradation of IAP1 (also known as BIRC3) and IAP2 (also known as BIRC2) through the ubiquitin–proteasome system, leading to the formation of a caspase 8-activating complex (FIG. 3). SMAC mimetic drugs are therefore unique agents that can promote the activation of caspases 3, 7, 8 and 9 and have been shown to sensitize various tumour types to treatment with chemotherapy or TRAIL both *in vitro* and *in vivo*[103]. Furthermore, some SMAC mimetics are now being evaluated in clinical trials.

Autophagy. Autophagy is a lysosomal degradation pathway that degrades cellular organelles and proteins in order to maintain cellular biosynthesis and viability during metabolic stresses such as nutrient deprivation. The role of autophagy in cancer is paradoxical as it functions both as a tumour suppressor pathway that inhibits tumour initiation and also as a drug resistance mechanism by facilitating cancer cell survival during metabolic stresses caused by anticancer agents[104]. Indeed, many anticancer therapies, both chemotherapy and targeted therapies, have been shown to activate autophagic pathways. The ability of autophagy to promote cell survival during metabolic stress suggests that it may promote resistance to cytotoxic therapy, and several studies have been carried out that demonstrate this. For example, treatment with an inhibitor of autophagy (chloroquine)

Prodiginines
Bioactive secondary metabolites that are produced by bacteria and that have immunosuppressant, anticancer and antimalarial activities.

Fragment-based design
An NMR-based approach that identifies small organic molecules that bind to adjacent sites in a target molecule with relatively low affinity. Linking of two molecules that bind to adjacent sites then generates high-affinity ligands or inhibitors.

enhanced tumour regression in response to alkylating agents in a mouse model of lymphoma[105], and hydroxychloroquine was found to sensitize human cancer cells to cancer therapy[106].

Resistance-promoting adaptive responses

Activation of prosurvival signalling. Numerous studies have reported the activation of EGFR as a resistance mechanism to various chemotherapies[107–110]. Accordingly, EGFR-targeted therapies have been shown to sensitize various tumour types to agents such as 5-FU, irinotecan, paclitaxel and TRAIL *in vitro* and/or *in vivo*[108–110]. Moreover, some clinical trials have shown the benefit of the addition of EGFR-targeted therapies to irinotecan-based chemotherapy in *KRAS*-wild-type colorectal cancer and have led to approvals from the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for EGFR-targeted agents in this genetic subtype of colorectal cancer. However, *KRAS*-mutant colorectal cancer is unresponsive to EGFR inhibitors because oncogenic KRAS is not dependent on upstream activation by EGFR; this is an example of 'oncogenic bypass' (see below).

ADAM (a disintegrin and metalloproteinase) enzymes are zinc-dependent, membrane-associated metalloproteinases that cleave and thereby activate the ligands for various growth factor receptor tyrosine kinases (RTKs)[111,112]. Studies from our group have shown that chemotherapy-induced activation of EGFR occurs as a result of an acute ADAM17-dependent adaptive resistance mechanism[110,113]. Moreover, inhibition of ADAM17 results in synergistic inhibition of tumour growth when combined with chemotherapy in several cancer models[113,114]. As ADAM17 regulates the shedding of ligands that activate numerous growth factor receptors, inhibiting its activity may have a more profound therapeutic effect than blocking individual growth factor receptors.

Oncogenic bypass and pathway redundancy. Although inhibiting prosurvival signals can increase sensitivity to chemotherapeutics and can exploit tumour addiction to specific gain-of-function mutations, the molecularly targeted agents that are used to block these pathways are themselves subject to various adaptive resistance mechanisms. For example, numerous reports have identified ERBB3 (also known as HER3) and downstream signalling through the PI3K–AKT pathway as an important mechanism of adaptive resistance to EGFR-targeted therapies *in vitro* and *in vivo*[115,116]. This mechanism has been termed 'oncogenic bypass' or 'kinome reprogramming' because the primary drug target remains unaltered and continues to be inhibited, but an alternative kinase becomes activated owing either to an adaptive feedback loop or a genetic mutation that is selected for during treatment; this is emerging as a major mechanism of resistance to the newer molecularly targeted agents (FIG. 4). Amplification of *MET*, which encodes a protein that drives ERBB3-dependent activation of PI3K, has been found to cause resistance to EGFR inhibitors in approximately 20% of patients with oncogenic-EGFR-driven lung cancer[117]. This is another example of oncogenic bypass as MET can compensate for EGFR blockade by activating the downstream effectors of EGFR signalling.

The serine/threonine kinase BRAF, which is the kinase immediately downstream of KRAS, is itself frequently activated by mutation in numerous cancers, particularly melanoma. However, unlike KRAS, for which there are currently no direct inhibitors, some inhibitors such as vemurafenib have been developed that target oncogenic BRAF, specifically the most commonly mutated form, BRAF-V600E. Although clinical response rates to vemurafenib in $BRAF^{V600E}$-mutated melanoma is high (~50%), secondary resistance invariably develops[118]. A range of compensatory resistance mechanisms has been identified, including acute adaptive responses (such as the activation of alternative RAF isoforms) and selection of tumour cells with acquired mutations in genes such as *KRAS, NRAS* and *MEK1* (REFS 119–122). In contrast to *BRAF*-driven melanoma, BRAF inhibitors are less effective in *BRAF*-mutant colorectal cancer; this seems to be due to the activation of an EGFR–AKT signalling axis that results in the intrinsic resistance of this tumour type to BRAF inhibitors[123].This is a good example of how tissue type can influence resistance mechanisms to the same targeted agent in cancers harbouring identical oncogenic mutations.

Epithelial–mesenchymal transition (EMT). Epithelial cells can undergo transition to a mesenchymal phenotype, during which they lose their polarized organization and tight cell–cell junctions and undergo changes in cell shape to develop a fibroblast-like morphology that is associated with increased motility and invasive capacity. This change in cellular phenotype is driven by various transcription factors that regulate the expression of proteins that are involved in cell polarity, cell-to-cell contact, cytoskeletal structure and extracellular matrix (ECM) degradation. Recent studies have demonstrated a link between chemotherapy and targeted therapy resistance and the EMT phenotype. For example, resistance to EGFR inhibitors was observed in cell lines undergoing EMT[124,125]. In the clinic, EMT was also observed in tumour samples from patients with NSCLC who developed resistance to EGFR inhibitors[126,127]. In addition, two recent studies have identified the RTK AXL as a potential therapeutic target for overcoming EGFR inhibitor resistance that is associated with development of the mesenchymal phenotype[128,129]. Moreover, a recent study using a large-scale siRNA screen to discover the determinants of response to ALK and EGFR inhibitors identified MED12, which is a component of the Mediator transcription complex that is mutated in cancers. MED12 loss was shown to induce an EMT-like phenotype through the activation of transforming growth factor-β receptor (TGFβR) signalling, and this change was associated with drug resistance. Notably, inhibition of TGFβR signalling was able to restore drug responsiveness in MED12-depleted cells. This study suggests that EMT arising during the development of drug resistance may be counteracted by using a TGFβR antagonist[130].

Another recent study used gene expression profiling of a large panel of NSCLC cell lines to define a signature consisting of 76 genes for which expression most closely correlated with several established markers of EMT, including

Mediator transcription complex
A large (1.2 MDa) multiprotein complex of up to 30 subunits that regulates transcription from a diverse set of RNA polymerase II-controlled promoters.

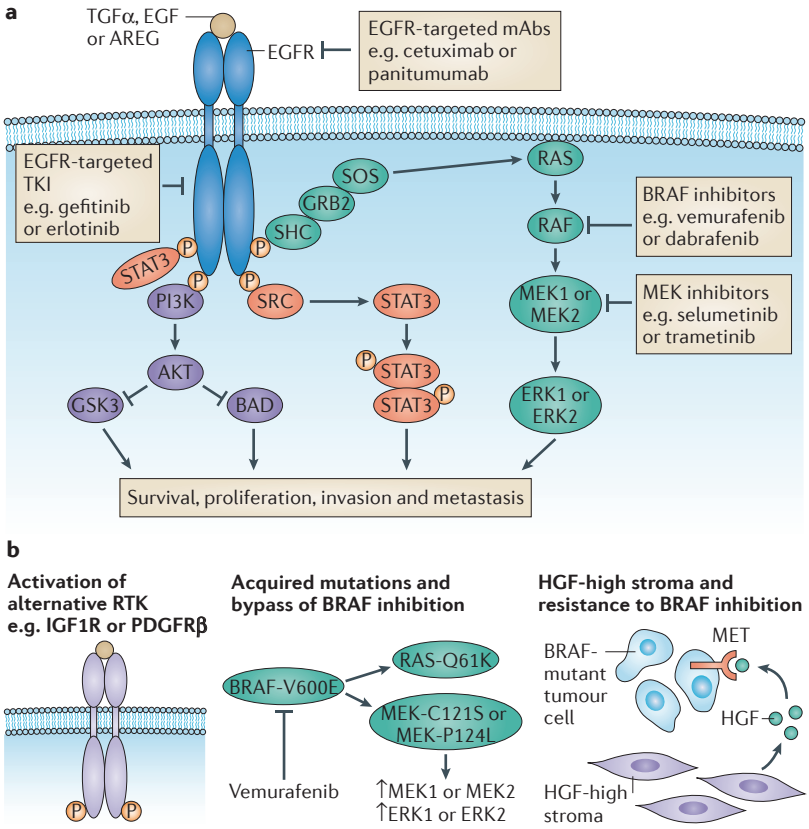

Figure 4 | **Mechanisms of resistance to molecularly targeted therapies as exemplified by EGFR, RAF and MEK inhibitors.** **a** | Binding of ligands such as transforming growth factor-α (TGFα), epidermal growth factor (EGF) and amphiregulin (AREG) to the EGF receptor (EGFR) promotes the activation of downstream prosurvival signalling pathways. These include the RAS–RAF–MEK–ERK, PI3K–AKT, SRC and Janus kinase (JAK)–signal transducer and activator of transcription 3 (STAT3) pathways. Activation of these pathways promotes survival, proliferation, invasion and metastasis. Inhibition of EGFR — using monoclonal antibodies (mAbs) to block receptor dimerization or small molecule tyrosine kinase inhibitors (TKIs) — is a clinically relevant strategy for blocking EGFR signalling. **b** | However, there are various resistance mechanisms such as oncogenic bypass, which involves the activation of alternative receptor tyrosine kinases (RTKs) (for example, insulin-like growth factor 1 receptor (IGF1R) or platelet-derived growth factor receptor-β (PDGFRβ)) (left panel). Inhibition of MEK1 or MEK2 kinases in oncogenic-KRAS-driven cancers, or inhibition of the BRAF kinase in BRAF-driven cancers, are other important clinical approaches. However, there are also multiple resistance mechanisms to these targeted therapies, such as acquired mutations in pathway-relevant kinases (middle panel) and the activation of RTKs by stromal-derived growth factors (right panel). GSK3, glycogen synthase kinase 3; HGF, hepatocyte growth factor.

E-cadherin and vimentin. This gene expression classifier reliably clustered the NSCLC cell lines into either an epithelial or mesenchymal group[128]. The authors also showed that the EMT gene expression signature could be used as a predictive biomarker of resistance to the EGFR inhibitor erlotinib and to inhibitors of PI3K–AKT–mTOR signalling in a panel of NSCLC cell lines that were derived from treatment-naive patients.

Tumour microenvironment

In solid tumours, the microenvironment consists of the ECM, cancer-associated fibroblasts, immune and inflammatory cells and blood vessels[131,132]. In haematological malignancies the microenvironment is composed of bone marrow stromal cells, bone marrow endothelial cells, osteoclasts, osteoblasts, macrophages and T cells among others[133,134]. The protection provided by the microenvironment provides refuge for cancer cells from cytotoxic agents, thus allowing them to evade apoptosis and to develop acquired resistance leading to disease relapse. Microenvironment-mediated resistance to both chemotherapy and targeted therapies has been recently reviewed[135], so we will only discuss it briefly in this article.

Integrins. Integrins are cell surface adhesion molecules that connect cells to the ECM[136]. Expression of integrins can be altered in tumour cells, and higher expression is associated with increased cancer cell survival and drug resistance[137]. Recent findings show that integrin-mediated adhesion to the ECM can modify responses to chemotherapeutic agents by various mechanisms, including inhibition of apoptosis and alterations in drug targets[138]. Integrins modulate many signalling pathways, including the PI3K–AKT, ERK and NF-κB pathways that promote cell survival and drug resistance[139], thus implying that they may also be important factors in resistance to kinase-targeted agents. This has been demonstrated in ERBB2-positive metastatic breast cancer in which β1-integrin expression levels were identified as an independent prognostic biomarker of the response to the ERBB2-targeted antibody trastuzumab[140].

Cytokines and growth factors. Autocrine, paracrine and endocrine activation of oncogenic signalling by soluble factors such as cytokines and growth factors can have key roles in resistance to both chemotherapy and molecularly targeted therapies by maintaining the activation of various survival signalling pathways. In one study, a murine model of Burkitt's lymphoma was used to demonstrate how paracrine factors in the tumour microenvironment can modulate lymphoma cell survival following chemotherapy treatment. Both interleukin 6 (IL-6) and tissue inhibitor of metalloproteinases 1 (TIMP1) were released in the thymus in response to doxorubicin treatment, leading to the establishment of what the authors term a 'chemoresistant niche', which can in turn lead to survival of residual lymphoma cells and ultimately patient relapse[141]. A recent study[142] used a cell line panel derived from various cancer types to assess the effects of different growth factors on sensitivity to kinase inhibitors. Hepatocyte growth factor (HGF), fibroblast growth factor (FGF) and neuregulin 1 (NRG1) were all shown to cause drug resistance by reactivating either or both of the PI3K–AKT and MEK–ERK pathways. Inhibition of their corresponding RTKs was able to overcome the growth-factor-mediated drug resistance, but was ineffective as a monotherapy. This type of ligand-mediated therapeutic resistance has been reported in preclinical models, including HGF-induced resistance to vemurafenib in *BRAF*^{V600E} melanoma models and to the ERBB2 inhibitor lapatinib in *ERBB2*-amplified breast cancer cell lines. Clinically, circulating levels of HGF before treatment have been correlated with worse progression-free and overall survival in patients with *BRAF*^{V600E} melanoma who were treated with vemurafenib.

Orthogonal therapies
Two therapies are considered orthogonal if they target a cancer in two different ways such that a resistance mechanism for the first therapy is unlikely to suppress the activity of the second therapy and vice versa.

A recent study used co-culture experiments to assess how stromal cells affect the sensitivity of human cancer cell lines to various anticancer drugs[143]. The panel of stromal cell lines used were derived from human bone marrow stroma, mammary fibroblasts, cancer-associated fibroblasts (from both the breast and lung), skin and umbilical epithelium, as well as murine adipocytes and fetal fibroblasts. They were co-cultured with NSCLC, breast cancer, pancreatic cancer, colorectal cancer, head and neck squamous cell carcinoma, melanoma, and gastrointestinal stromal tumour (GIST) cell lines. The authors then focused on the BRAF inhibitor PLX4720 and showed that *BRAF*^{V600E} melanoma cells became resistant to this drug when co-cultured with fibroblasts, and this was then shown to be due to the presence of HGF in the co-culture medium. Moreover, the presence of HGF-positive tumour-associated stromal cells correlated with a poorer clinical response of *BRAF*^{V600E} melanoma to BRAF inhibitors. Notably however, melanoma cell lines could be resensitized to BRAF inhibition *in vitro* by inhibiting either HGF or its receptor, MET[143].

Conclusions

Despite the daunting range of resistance mechanisms and the complexities caused by tumour heterogeneity and microenvironment interactions, we should not lose sight of the fact that chemotherapeutics and molecularly targeted therapies are effective in many disease settings, significantly prolonging patients' lives or, in some cases, producing cures. The current challenge is to learn from experiences with traditional cytotoxic drugs and the first wave of molecularly targeted agents to use the increasing arsenal of anticancer therapies in the most effective ways. Rational drug combinations are often proposed on the basis of *in vitro* and *in vivo* synergy between agents; however, hitting the same pathway at multiple points may in some cases provide a relatively simple 'escape route' for the tumour. Orthogonal therapies that target completely independent pathways may therefore sometimes be a better option as the avenues for the development of tumour drug resistance may be more limited. Most importantly, we need to be able to stratify patients according to whether they are likely to respond to a particular drug or drug combination. The use of powerful high-throughput techniques such as microarray profiling and next-generation sequencing provide an abundance of data that can be used to identify potential predictive biomarkers for patient stratification. However, whether cell lines are the best platform for identifying clinically meaningful biomarkers and evaluating drug combinations is a matter of debate, given the impact of the microenvironment on drug resistance. Although cell lines are often a good starting point, improved *in vitro* and *in vivo* models, such as patient-derived xenografts, that more closely model tumour–stroma interactions are clearly needed to more accurately assess drug resistance, evaluate potential drug combinations and determine the therapeutic value of predictive biomarkers. Subsequently, such preclinical studies need to be tested in the clinic, which will require the design of 'smart' trials incorporating state-of-the-art molecular pathology techniques.

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Competing interests statement
P.G.J. declares competing financial interests: see Web version for details. The other authors declare no competing financial interests.

DATABASES
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5-fluorouracil | ABT-263 | afatinib | bevacizumab | bicalutamide | bortezomib | bosutinib | capecitabine | carboplatin | cetuximab | cisplatin | crizotinib | cytarabine | dabrafenib | dasatinib | docetaxel | doxorubicin | erlotinib | etoposide | gefitinib | gemcitabine | hydroxychloroquine | imatinib | irinotecan | lapatinib | leuprolide | methotrexate | nilotinib | obatoclax | oxaliplatin | paclitaxel | panitumumab | pemetrexed | ponatinib | selumetinib | sunitinib | tariquidar | trametinib | trastuzumab | vemurafenib | vinorelbine | zosuquidar
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