Understanding and targeting resistance mechanisms in NSCLC

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Abstract | The expanding spectrum of both established and candidate oncogenic driver mutations identified in non-small-cell lung cancer (NSCLC), coupled with the increasing number of clinically available signal transduction pathway inhibitors targeting these driver mutations, offers a tremendous opportunity to enhance patient outcomes. Despite these molecular advances, advanced-stage NSCLC remains largely incurable due to therapeutic resistance. In this Review, we discuss alterations in the targeted oncogene ('on-target' resistance) and in other downstream and parallel pathways ('off-target' resistance) leading to resistance to targeted therapies in NSCLC, and we provide an overview of the current understanding of the bidirectional interactions with the tumour microenvironment that promote therapeutic resistance. We highlight common mechanistic themes underpinning resistance to targeted therapies that are shared by NSCLC subtypes, including those with oncogenic alterations in epidermal growth factor receptor (EGFR), anaplastic lymphoma kinase (ALK), ROS1 proto-oncogene receptor tyrosine kinase (ROS1), serine/threonine-protein kinase b-raf (BRAF) and other less established oncoproteins. Finally, we discuss how understanding these themes can inform therapeutic strategies, including combination therapy approaches, and overcome the challenge of tumour heterogeneity.

Intrinsic resistance
Tumour cell resistance to therapy due to baseline characteristics present before therapy exposure.

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Lung cancer is the leading cause of cancer-related mortality worldwide, and non-small-cell lung cancer (NSCLC) represents the major histological subtype of the disease[1]. Improved understanding of the molecular changes that drive tumour progression has revolutionized the clinical management of NSCLC. Almost two-thirds of patients with NSCLC harbour an oncogenic driver mutation, approximately half of whom have a therapeutically targetable lesion, which expands treatment options and leads to improvements in survival and safety compared with conventional chemotherapy[2]. Activating genetic mutations or fusions in the epidermal growth factor receptor (EGFR; also known as ERBB1), anaplastic lymphoma kinase (ALK), ROS1 proto-oncogene receptor tyrosine kinase (ROS1) and serine/threonine-protein kinase b-raf (BRAF) are now targets for kinase-inhibitor therapy in NSCLC, and additional targeted therapies are currently under evaluation in other oncogenic driver subtypes of NSCLC[3–6] (FIG. 1).

Although treatment with a targeted therapy improves outcomes in patients with NSCLC, responses to these agents are generally incomplete and temporary. Resistance to targeted agents can be sub-classified as intrinsic resistance, adaptive resistance and acquired resistance[7]. Some tumours exhibit intrinsic resistance and fail to respond to initial treatment; this intrinsic resistance might be related to driver mutations that are insensitive to therapy, as with *EGFR* exon 20 insertions, which are typically insensitive to currently available EGFR tyrosine kinase inhibitors (TKIs)[8], or to the baseline presence of other alterations such as the germline *BIM* (also known as *BCL2L11*) deletion polymorphism or activation of nuclear factor-κB (NF-κB)[9], which each impair the apoptotic response to EGFR TKI therapy[10]. In other patients, despite a partial response to therapy, adaptive resistance occurs when the tumour cells undergo early adaptive changes that permit their ongoing survival and persistence following therapy[7]. Acquired resistance likely arises from a combination of selection for pre-existing genetic alterations within an initially heterogeneous tumour cell population and from the acquisition of new alterations under the selective pressure imposed by therapy[7]. Importantly, there is biological overlap in the events that drive these types of therapeutic resistance, which exist on a continuum. The current understanding of the mechanisms of resistance stems from multidisciplinary studies that have incorporated both preclinical models and clinical samples (BOX 1).

Resistance mechanisms can be classified as 'on-target' or 'off-target'. On-target resistance occurs when the primary target of the drug is altered, limiting the drug's ability to inhibit the activity of its target. Off-target resistance occurs through the activation of collateral signalling

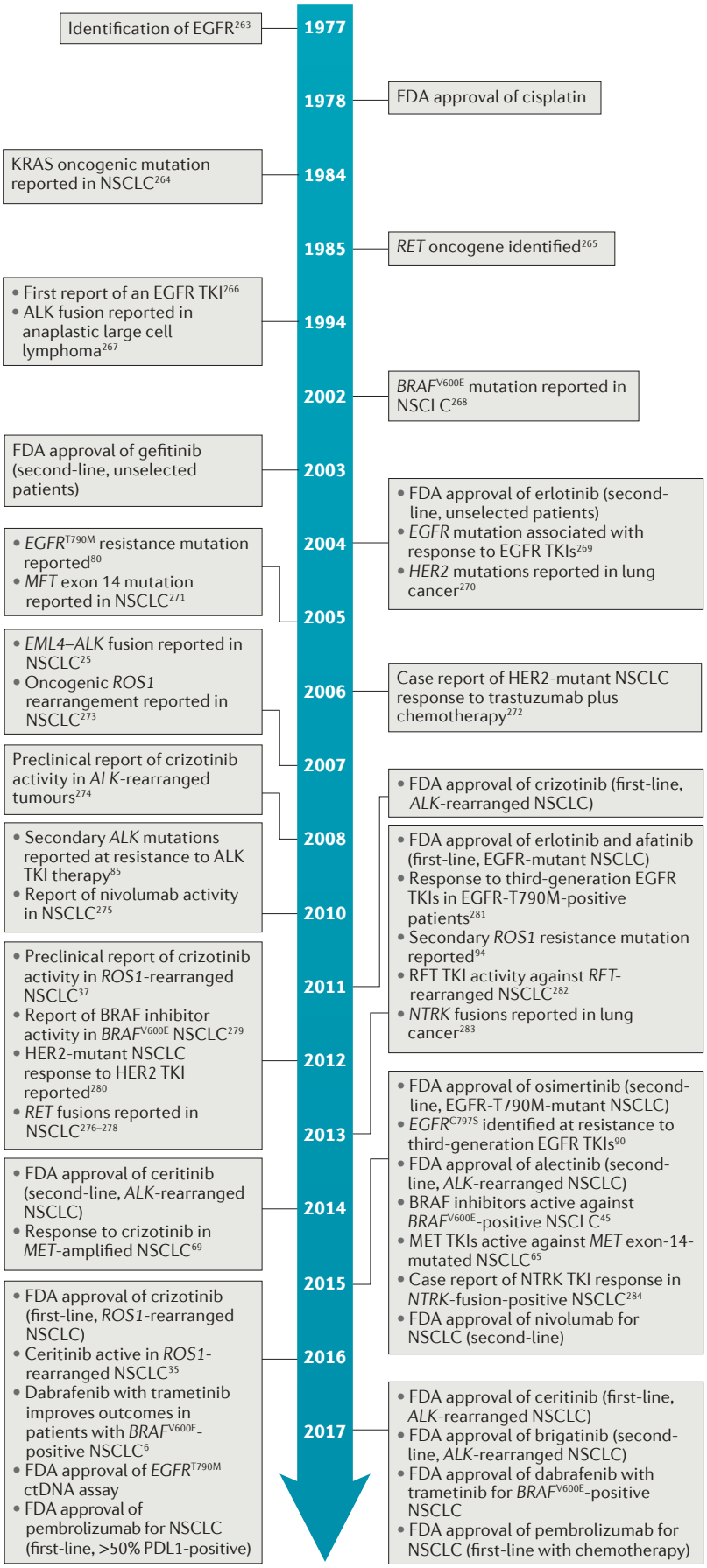


Figure 1 | **Milestones in targeted therapy for NSCLC.** ALK, anaplastic lymphoma kinase; BRAF, serine/threonine-protein kinase b-raf; ctDNA, circulating tumour DNA; EGFR, epidermal growth factor receptor; EML4, echinoderm microtubule-associated protein-like 4; FDA, US Food and Drug Administration; HER2, human epidermal growth factor receptor 2; MET, hepatocyte growth factor receptor; NSCLC, non-small-cell lung cancer; NTRK, neurotrophic tyrosine kinase; PDL1, programmed cell death ligand 1; RET, proto-oncogene tyrosine-protein kinase receptor Ret; ROS1, ROS1 proto-oncogene receptor tyrosine kinase; TKI, tyrosine kinase inhibitor.

events that are parallel to, or downstream of, signalling by the driver oncoprotein. These collateral signalling events bypass the requirement of the driver oncoprotein for cell survival and growth. In addition, histological transformations and interaction with the tumour microenvironment (TME) can be associated with resistance[11,12]. Further challenges beyond the tumour cell and the TME include overcoming barriers that limit effective drug delivery to central nervous system (CNS) metastases (BOX 2) and alterations in drug exposure due to differences in drug absorption[13].

In this Review, we examine the current understanding of resistance mechanisms to targeted therapies in oncogene-driven NSCLC and highlight therapeutic strategies to circumvent them. Such strategies include the development of inhibitors with a higher potency against their intended target and greater activity against on-target resistance mutations and the use of combination therapies incorporating inhibitors of parallel or downstream signalling pathways mediating off-target resistance. We also examine the current understanding of tumour heterogeneity in NSCLC, including challenges in measuring heterogeneity and implications for the design of novel therapeutic strategies.

Targeting oncogenic drivers in NSCLC

Oncogenic EGFR mutations, *ALK* and *ROS1* fusions and BRAF mutations are all the target of US Food and Drug Administration (FDA)-approved medications for treating NSCLC. In addition, there are other oncogenic drivers that have been reported in NSCLC, including but not limited to KRAS mutations, selected hepatocyte growth factor receptor (MET, also known as HGFR) alterations and human epidermal growth factor receptor 2 (HER2, also known as ERBB2) mutations, which may be amenable to treatment with targeted therapies.

Oncogenic EGFR mutations

Somatic activating mutations in EGFR are the most common driver mutations for which targeted therapies in NSCLC are available, occurring in ~16% of patients with advanced lung adenocarcinoma[14]. Four FDA-approved EGFR TKIs are currently in clinical use, with response rates of ~50–80%, including the first-generation non-covalent inhibitors erlotinib and gefitinib, the second-generation covalent inhibitor afatinib and the more recently approved third-generation, wild-type-sparing, mutant EGFR-specific TKI osimertinib[3,15,16].

Adaptive resistance
Dynamic changes in tumour cell signalling occurring during treatment with targeted therapy that promote therapy resistance.

Acquired resistance
New molecular alterations leading to the development of targeted therapy resistance after an initial period of drug sensitivity.

Tyrosine kinase inhibitors
A class of small-molecule inhibitors that antagonize receptor tyrosine kinase signalling.

Non-covalent inhibitors
Inhibitors that bind to a target protein in a non-covalent, reversible manner.

Covalent inhibitor
An inhibitor that binds to a target protein via irreversible, covalent bonds.

The efficacy of EGFR TKI therapy varies among specific activating mutations. The activating *EGFR* exon 19 deletions and the *EGFR*^{L858R} mutation in exon 21 account for the vast majority (85–90%) of all EGFR mutations in NSCLC, and tumours harbouring these alterations show high rates of response to EGFR TKIs[17,18]. These constitutively active mutant EGFR oncoproteins signal through the MAPK, PI3K–AKT and Janus kinase (JAK)–signal transducer and activator of transcription (STAT) signalling pathways to promote oncogenesis[19]. Conversely, ~4% of *EGFR* mutations are exon 20 insertions; these mutations do not impact the affinity of EGFR for ATP, and a response to EGFR TKIs is uncommon in tumours bearing these mutations[8,20]. An EGFR TKI targeted against exon 20 insertions is in early-phase trials[21]. The EGFR-T790M mutation, typically found in tumours with acquired resistance to first-generation and second-generation EGFR TKIs, has been reported at baseline in ~0.5% patients and is associated with intrinsic resistance to these EGFR TKIs[22]. The challenge of predicting response to EGFR TKI therapy is highlighted by the identification of rare EGFR mutations, such as EGFR-G719X (where X is any other amino acid) and EGFR-L861X, for which the rate of response to EGFR TKI therapy is uncertain[23,24]. Mechanisms of resistance to EGFR TKIs are discussed later in this Review and are summarized in FIG. 2.

Oncogenic ALK *gene rearrangements*

Oncogenic *ALK* gene rearrangements, which fuse the intact ALK kinase domain to N-terminal fusion partners, occur in ~1–7% of patients with NSCLC[25,26]. The resulting overexpression and ligand-independent activation of ALK is at least partially determined by the nature of the fusion partner[27]. Although echinoderm microtubule-associated protein-like 4 (EML4) is the most common ALK fusion partner in NSCLC, multiple other fusion partners have been reported[28]. Four ALK inhibitors are FDA-approved for use in treating NSCLC — crizotinib, ceritinib, alectinib and brigatinib. Crizotinib, a first-generation ALK inhibitor, also functions as a ROS1 and MET TKI[4]. Compared with crizotinib, the second-generation ALK inhibitors ceritinib, alectinib and brigatinib demonstrate increased potencies for ALK inhibition and improved CNS penetration and activity against multiple secondary ALK mutations that confer resistance to crizotinib[29–35]. Alectinib is now the preferred first-line ALK TKI for treating patients with *ALK*-rearranged NSCLC, and it resulted in improved outcomes in the ALEX trial[36]. Although ceritinib and brigatinib also inhibit ROS1, alectinib instead inhibits proto-oncogene tyrosine-protein kinase receptor Ret (RET), giving these agents differing spectrums of activity against other oncogenic drivers[32,34,35]. Mechanisms of resistance to ALK TKIs are summarized in FIG. 3 and are discussed later in this Review.

Oncogenic ROS1 *gene rearrangements*

ROS1 gene rearrangements occur in ~1–2% of patients with NSCLC[37]. These fusions pair the intact ROS1 kinase domain with a wide range of partners, the most common of which is CD74, to promote constitutive ROS1 kinase activity[37]. As there is structural homology between the ALK and ROS1 kinase domains, cross-inhibition with current therapies targeted against these kinases can occur[38]. Crizotinib, although initially approved for the treatment of *ALK*-rearranged NSCLC, is also approved for the treatment of *ROS1*-rearranged NSCLC, in which it showed an objective response rate (ORR) of 72% and a median progression-free survival (PFS) of 19.2 months[5]. As would be expected, the mechanisms of resistance in *ROS1*-rearranged NSCLC overlap with those in *ALK*-rearranged NSCLC (FIG. 3).

Oncogenic BRAF mutations

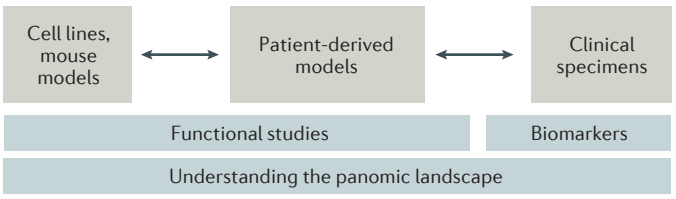
Somatic mutations in the *BRAF* gene occur in 3–8% of lung adenocarcinomas[39,40], ~50% of which are the *BRAF*^{V600E} mutation[41]. Other common *BRAF* mutations include the *BRAF*$^{G469A/V}$ and *BRAF*^{D594G} mutations, occurring in 35% and 6% of patients with *BRAF*-mutant NSCLC, respectively[42]. BRAF-V600E mutations induce constitutive BRAF activation in its monomeric form, activating downstream MEK–ERK signalling[43]. Although the BRAF-V600-specific inhibitors vemurafenib and dabrafenib have clinical activity as a monotherapy[44,45], the addition of a MEK inhibitor further improves outcomes, and the combination of dabrafenib and trametinib was FDA-approved in 2016 for treating BRAF-V600E-positive NSCLC[6]. As ~50% of BRAF-mutated NSCLC tumours harbour non-BRAF-V600E mutations, there is a clinical need for BRAF inhibitors with activity against such mutations. Similar to wild-type RAF proteins, these less common BRAF mutants signal

Box 1 | Approaches to studying mechanisms of resistance

General strategies to understand mechanisms of resistance to targeted therapies include the use of preclinical models and clinical approaches utilizing patient specimens (see the figure below). Novel bioinformatics techniques allow for the global identification of genomic, transcriptomic, proteomic and metabolomic alterations — a 'panomic' approach — to understand how tumour cell phenotype and behaviour influence resistance to therapy.

Highly sensitive sequencing techniques now permit unbiased genomic and transcriptomic analysis to identify alterations that are relevant to therapeutic resistance. Phosphoproteomic assays provide a global assessment of pathway activation. Functional genetic and pharmacological screens offer a rapid assessment of promising, novel targets. These techniques together permit the identification of novel targets as mediators of therapeutic resistance.

Incorporating tissue collection into clinical trial protocols is essential to the development of biomarkers as tools to predict the probability of therapeutic efficacy, to provide a mechanistic understanding of resistance via global assays of cellular status and for the generation of patient-derived research models (cell lines, xenografts and organoids) for more detailed study and functional validation[245]. Several clinical trials are evaluating the use of expanded assessments for potential oncogenic driver mutations at baseline as a form of biomarker-driven therapy, including the BATTLE[245] and MATCH[246] trials.



Box 2 | **Resistance in central nervous system metastases**

The blood–brain barrier presents an additional challenge to the delivery of targeted therapies to the central nervous system (CNS), reducing drug concentrations in the cerebrospinal fluid (CSF) and/or brain parenchyma and therefore increasing the risk of tumour resistance. More than 50% of living patients with metastatic epidermal growth factor receptor (EGFR)-altered or anaplastic lymphoma kinase (ALK)-altered non-small-cell lung cancer (NSCLC) will develop brain metastases within five years of diagnosis[247]. Passive diffusion across the blood–brain barrier is limited to small, lipophilic molecules. Drug efflux transporters, including P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP), further reduce intracellular levels of substrate drugs in the CNS[248]. The Table below lists the CNS penetration characteristics of approved targeted agents for *EGFR*-mutant and *ALK*-rearranged NSCLC.

Drug name	CNS penetration characteristics
***EGFR*-targeted therapies**	
Erlotinib	2.8–5.1% CSF penetration[249,250]
Gefitinib	1.1–1.3% CSF penetration[250,251]
Afatinib	0.70–1.65% CSF penetration[252,253]*
Osimertinib	0.39 brain-to-plasma partition ratio[254]‡
***ALK*-targeted therapies**	
Crizotinib	0.26% CSF penetration[255]§
Ceritinib	15% brain-to-blood exposure ratio[256]‖
Alectinib	63–94% CNS penetration[257]‖
Brigatinib	Data not reported

*Single patient case report in the setting of CNS response to alectinib. ‡In a mouse model. §Single patient case report in the setting of progression of CNS disease on crizotinib therapy. ‖In a rat model.

Beyond local therapy, approaches to improve the activity of targeted therapies against CNS metastases include increasing systemic drug dosing to produce higher drug concentrations in the CNS[258,259]; the use of higher-potency inhibitors that require lower concentrations in the CNS for activity[260]; the design of inhibitors with improved CNS penetration by increasing their lipophilicity or by minimizing their eligibility as a drug efflux substrate[32]; and drug use in combination with agents that either disrupt the blood–brain barrier[261] or inhibit the activity of drug efflux pumps[262].

as dimers and are relatively resistant to current inhibitors targeting the BRAF-V600E oncoprotein compared with BRAF-V600E mutants[43]. Several new RAF inhibitors with activity against dimerized RAF forms and with a reduced affinity for wild-type BRAF are in development[46,47]. Downstream MEK inhibitor monotherapy is an alternative strategy that might be effective against RAF homodimer-induced and RAF heterodimer-induced activation of MEK–ERK signalling[48].

Other oncogenic drivers

There is an expanding spectrum of identified oncogenic driver alterations in NSCLC (FIG. 4), ranging from the common, but difficult to target, KRAS mutations to the less common, but more readily targeted, MET and HER2 mutations. Additional oncogenic drivers for which therapeutic strategies are being developed include *RET* rearrangements, neurotrophic tyrosine kinase (*NTRK*) fusions and the loss of neurofibromin 1 (*NF1*).

KRAS. Activating KRAS mutations, the most common of the oncogenic driver mutations, occur in ~20–30% of patients with NSCLC[26]. To date, efforts to target KRAS have been unsuccessful, including a lack of improved survival with downstream MEK inhibitor treatment in KRAS-mutant advanced NSCLC, despite initial promising results in early-phase trials[49]. Bypass pathway activation (for example, activation of PI3K or fibroblast growth factor receptor 1 (FGFR1)) might explain the limited activity of MEK inhibitors in this setting, and one possible strategy to overcome this resistance is the combination of MEK inhibition with PI3K or FGFR1 inhibition[50–52]. Additionally, activation of the Hippo pathway effector yes-associated protein 1 (YAP1) promoted resistance to MEK inhibition in preclinical models of KRAS-mutant NSCLC, suggesting that YAP1 inhibition is a potential polytherapy strategy to enhance the response to MEK–ERK blockade[53].

Another new strategy for the treatment of KRAS-mutant NSCLC involves the exploitation of targets that exhibit synthetic lethality when inhibited in combination with inhibitors of mutant KRAS signalling. Potential targets for this strategy including cyclin-dependent kinase 4/6 (CDK4/6), either alone or in combination with MEK inhibitors[54,55], and a phase III trial of the CDK4/6 inhibitor abemaciclib in KRAS-mutant NSCLC is ongoing[56]. Direct inhibitors of KRAS-G12C, the most common KRAS mutation[57], are also in preclinical development. These approaches to direct KRAS inhibition include the development of agents that target the GTP binding pocket of KRAS and/or the process of nucleotide exchange[58–60].

MET alterations. *MET* exon-14-skipping mutations are found in ~3% of lung adenocarcinomas[26,61]. Reported mutations are variable[61], but they share the common outcome of *MET* exon 14 loss, which contains inhibitory elements that antagonize MET kinase activation and promote MET degradation[62,63]. Clinical responses to MET inhibitors, including crizotinib and cabozantinib, have been reported in up to two-thirds of patients with a *MET* exon 14 mutation in one study[64,65]. *MET* amplification has also been reported in ~1–4% of patients with NSCLC[66,67]. Those patients with high-level *MET* amplification that is distinct from that seen in chromosomal polysomy — defined by a gain in *MET* copy number relative to the centromere of chromosome 7 and measured by fluorescent *in situ* hybridization (FISH) — might derive benefit from MET TKI therapy[68]. In one study, the response rate to crizotinib was 50% for patients with NSCLC and high-level *MET* amplification, with less frequent responses (0–20%) seen at lower levels of *MET* amplification[69]. Multiple clinical trials are underway to evaluate MET TKIs in both *MET* exon-14-mutated and *MET*-amplified NSCLC.

HER2 mutations. Somatic *HER2* mutations occur in ~2% of lung adenocarcinomas, 96% of which are kinase-activating exon-20-insertion mutations[70,71]. In a series of nine patients with HER2-mutated advanced NSCLC, a 67% response rate to the HER2-targeted monoclonal antibody trastuzumab in combination with chemotherapy and a 33% response rate to afatinib, a HER2 TKI with activity against EGFR, were reported[72].

Synthetic lethality
Induction of tumour cell death upon simultaneous inhibition of two signalling pathways, the individual loss of which does not lead to cell death.

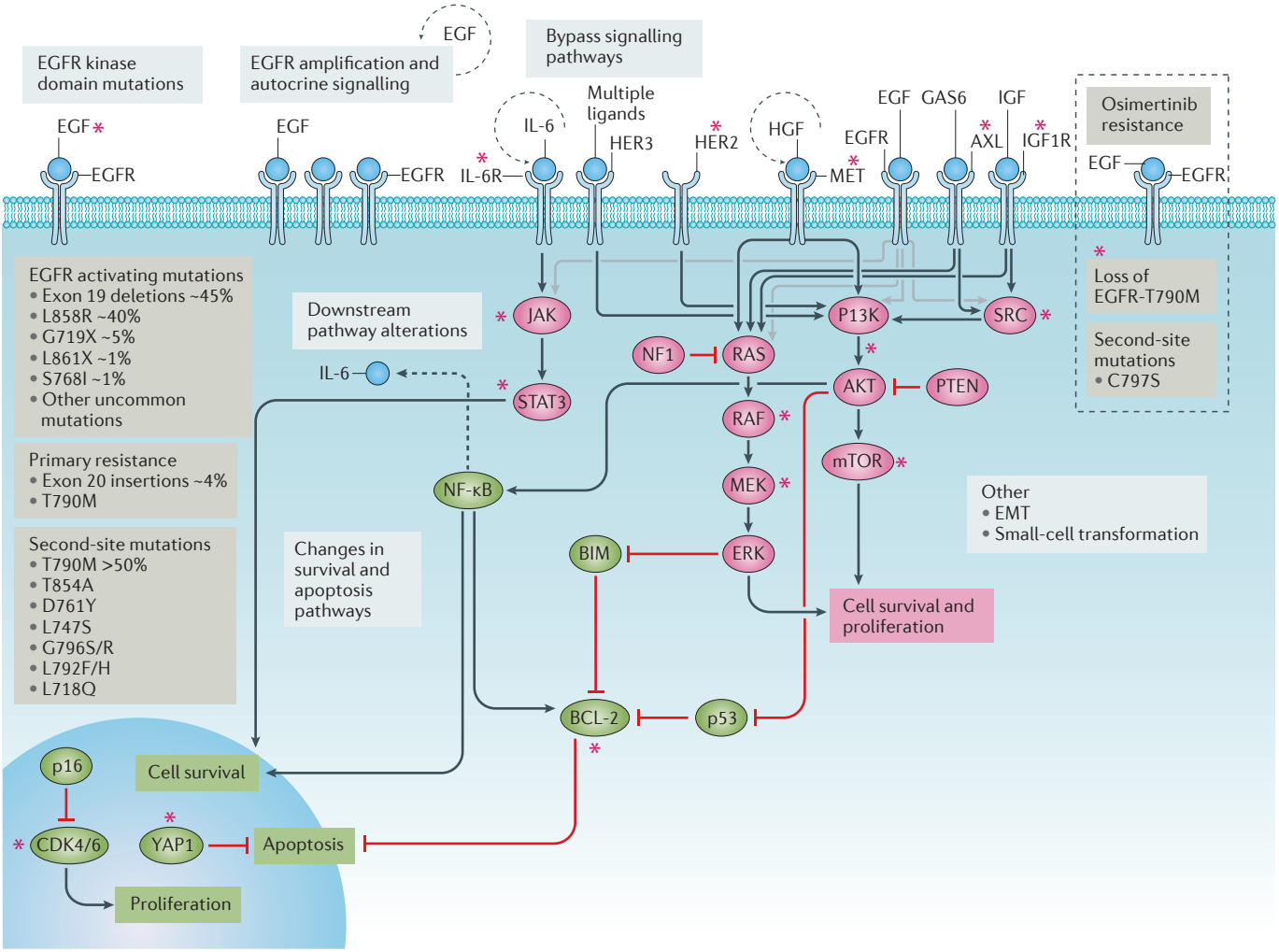


Figure 2 | **Signalling pathways driving resistance to EGFR TKIs in NSCLC.** Wild-type epidermal growth factor receptor (EGFR; also known as ERBB1) homodimerizes and heterodimerizes with other ERBB family members (including human epidermal growth factor receptor 2 (HER2) and HER3) upon ligand binding, leading to the activation of downstream pathways (pink ovals and box) that mediate cell survival and proliferation, including the PI3K–AKT, Janus kinase (JAK)–signal transducer and activator of transcription (STAT) and MAPK pathways[285]. Oncogenic activating mutations in EGFR[286], which most commonly occur in the tyrosine kinase domain, induce constitutive activation of EGFR and downstream signalling, independent of ligand binding. In non-small-cell lung cancer (NSCLC), resistance mechanisms to EGFR tyrosine kinase inhibitors (TKIs) at the level of the individual tumour cell include EGFR TKI-insensitive EGFR-activating mutations and second-site EGFR kinase domain mutations; *EGFR* gene amplification and autocrine epidermal growth factor (EGF) signalling; activation of bypass (black arrows) or downstream (grey arrows) signalling pathways, including activation by autocrine growth factor and/or cytokine signalling via cognate receptors (hepatocyte growth factor receptor (MET), AXL receptor tyrosine kinase (AXL), insulin-like growth factor 1 receptor (IGF1R), interleukin-6 receptor (IL-6R), HER2 and HER3); molecular changes to promote proliferation, cell survival and inhibition of apoptosis (green ovals and boxes); and histological transformations. Collectively, these resistance mechanisms reveal multiple potential targets for the treatment of EGFR TKI-resistant tumours (indicated with red asterisks). Although similar downstream pathways are active at resistance to each generation of EGFR TKI, distinct second-site mutations in EGFR occur with the use of first-generation and second-generation EGFR TKIs compared with third-generation EGFR TKIs such as osimertinib (highlighted in the dotted box). CDK4/6, cyclin-dependent kinase 4/6; EMT, epithelial-to-mesenchymal transition; HGF, hepatocyte growth factor; IL-6, interleukin-6; NF1, neurofibromin 1; NF-κB, nuclear factor-κB; SRC, proto-oncogene tyrosine-protein kinase Src; YAP1, yes-associated protein 1.

In a retrospective study of 101 patients with advanced NSCLC, the ORR for patients with HER2 mutations who received trastuzumab in combination with chemotherapy was 50.9%, compared with 43.5% in those who received chemotherapy alone[73]. However, lower response rates (7.4–12%) have also been reported in patients who received HER2 TKI monotherapy[73,74]. Specific characteristics of the underlying HER2 mutation, such as the presence of a glycine at position 770, might alter the sensitivity to treatment with HER2 TKIs and therefore might serve as a predictive biomarker to select patients who are more likely to respond to therapy[75].

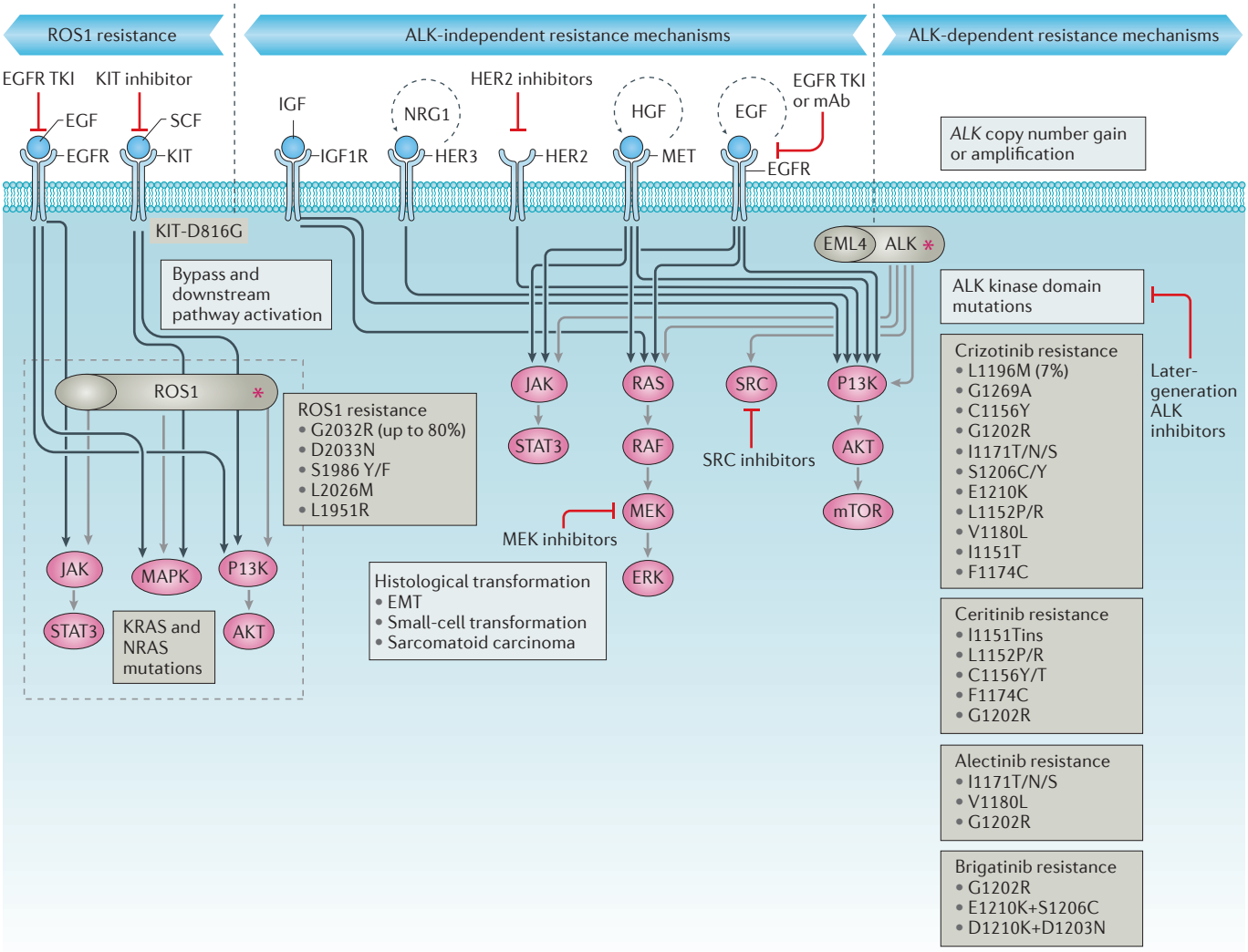


Figure 3 | **Signalling pathways in resistance to ALK and ROS1 TKIs in NSCLC.** Resistance to anaplastic lymphoma kinase (ALK) tyrosine kinase inhibitors (TKIs) in non-small-cell lung cancer (NSCLC) can be divided into ALK-independent and ALK-dependent mechanisms. ALK-independent resistance mechanisms include activation of bypass (black arrows) and downstream (grey arrows) pathways by growth factor and/or cytokine receptor signalling (insulin-like growth factor 1 receptor (IGF1R), human epidermal growth factor receptor 3 (HER3), HER2, hepatocyte growth factor receptor (MET), epidermal growth factor receptor (EGFR)) and aberrant downstream pathway activation, as well as histological transformations. ALK-dependent resistance mechanisms include *ALK* amplification and/or copy number gain and *ALK* kinase domain mutations. These resistance mechanisms can be targeted using higher potency, second-generation ALK TKIs (for ALK-dependent resistance) or with agents that target other pathways in addition to ALK (for ALK-independent resistance). Owing to substantial structural homology between the ROS1 proto-oncogene receptor tyrosine kinase (ROS1) and ALK kinase domains, resistance to ROS1 and ALK TKIs share similar mechanisms, including ROS1 kinase domain mutations and the activation of bypass and downstream signalling through oncogenic mutations (*RAS* mutations) or growth factor receptor signalling (EGFR, KIT). EGF; epidermal growth factor; EML4, echinoderm microtubule-associated protein-like 4; EMT, epithelial-to-mesenchymal transition; HGF, hepatocyte growth factor; JAK, Janus kinase; mAb, monoclonal antibody; NRG1, neuregulin 1; SCF, stem cell factor; SRC, proto-oncogene tyrosine-protein kinase Src; STAT, signal transducer and activator of transcription.

On-target resistance

Secondary alterations in the targeted oncogene can include either a second-site mutation that promotes TKI resistance or, less commonly, the amplification or loss of the targeted oncogene. Although the number and variability of reported second-site mutations differ both with the targeted oncogene and the specific TKI therapy, there are common themes based on shared structural and functional characteristics.

Second-site mutations

Resistance can occur via a secondary mutation (second-site mutations) in the drug target that interferes with inhibition by the targeted therapy. Kinase domains share structural components, including the ATP binding site flanked by an N-terminal lobe, containing the αC helix, and a C-terminal lobe, containing an activation loop, which is critical for kinase catalytic activity[76,77]. Although certain functionally important residues are

Activation loop
A structural component of receptor tyrosine kinases that is important for the regulation of catalytic activity.

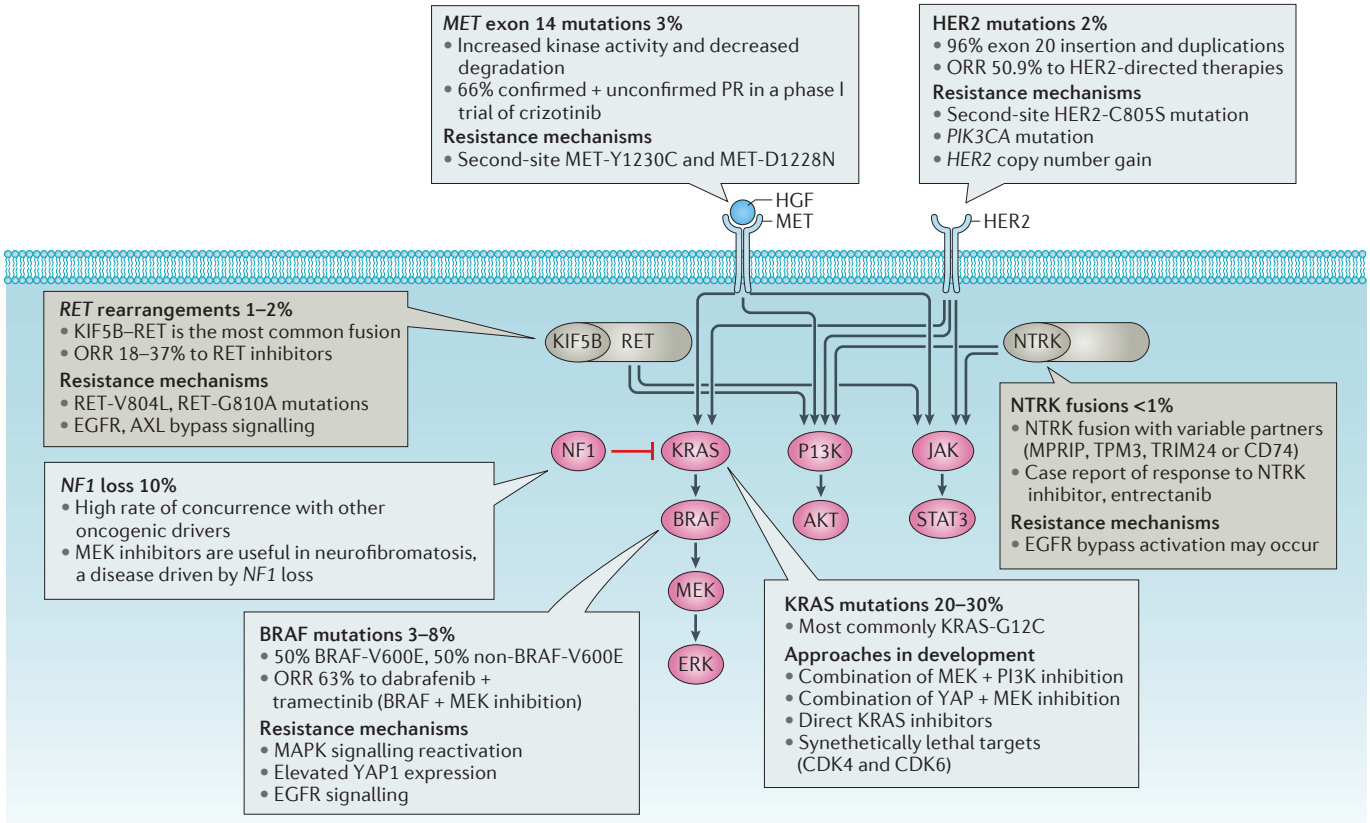


Figure 4 | **Other oncogenic drivers in NSCLC.** Multiple novel oncogenic drivers have been identified in non-small-cell lung cancer (NSCLC) that might be amenable to therapeutic targeting or, in the case of serine/threonine-protein kinase b-raf (BRAF), are newly established targets for FDA-approved therapies. These include BRAF mutations, hepatocyte growth factor receptor (*MET*) exon 14 mutations, proto-oncogene tyrosine-protein kinase receptor Ret (*RET*) and neurotrophic tyrosine kinase (*NTRK*) rearrangements, human epidermal growth factor receptor 2 (HER2) mutations, KRAS mutations and neurofibromin 1 (*NF1*) loss. *HER2* exon 20 mutations, which are analogous to the exon 20 mutations in epidermal growth factor receptor (EGFR)[75,287], comprise the majority of the *HER2* mutations in NSCLC. These *HER2* exon 20 mutations are associated with improved outcomes upon treatment with HER2-targeted therapies compared with outcomes following chemotherapy[73,288]. Response to HER2-targeted agents might be improved by the addition of a PI3K inhibitor, consistent with reports of mutations in *PIK3CA* (which encodes the PI3K catalytic subunit alpha) mediating resistance to HER2-targeted therapies[72,136]. In addition to the most common RET fusion protein, kinesin family member 5B (KIF5B)–RET[276,289,290], *RET* rearrangements result in multiple fusion proteins that lead to varying degrees of RET activation[278,291–294]. Although existing RET tyrosine kinase inhibitors (TKIs) have activity in NSCLC, responses are limited[290] compared with responses to other targeted therapies in the clinic. Predicted RET gatekeeper mutations RET-V804L and RET-G810A can confer RET TKI resistance *in vitro*, as can EGFR and AXL receptor tyrosine kinase (AXL) bypass signalling[87,161,295]. Although rare, neurotrophic tyrosine kinase 1 (*NTRK1*) gene fusions occur with multiple partners, including myosin phosphatase Rho-interacting protein (MPRIP), tropomyosin alpha-3 chain (TPM3), transcription intermediary factor 1α (TRIM24) or CD74 (REFS 283,296), and response to treatment with an NTRK inhibitor has been reported in a patient with *NTRK* fusion-positive NSCLC[284]. Although efforts to design therapeutics against mutations in the GTPase KRAS have thus far been unsuccessful[49], novel approaches are in development. Possible strategies include the combination of MEK and PI3K pathway inhibitors, which has shown preliminary clinical efficacy but also clinical toxicity[50,297], or the co-inhibition of yes-associated protein 1 (YAP1) and the MAPK pathway[53], reminiscent of approaches to treating EGFR-mutant and BRAF-mutant NSCLC. Direct KRAS inhibitors are in development that target the most common oncoprotein, KRAS-G12C[58–60]. In the setting of a KRAS mutation, a novel strategy under evaluation in an ongoing clinical trial[56] includes exploitation of synthetically lethal targets such as cyclin-dependent kinase 4/6 (CDK4/6)[55], either alone or in combination with MEK inhibitors[54]. *NF1* inactivation by somatic mutation or copy number loss is common, although often concurrent with other known oncogenic drivers[298]. Extrapolating from the activity of MEK inhibitors in neurofibromatosis, a disease driven by *NF1* inactivation, MEK inhibition might be a viable strategy for treating NSCLC associated with the loss of *NF1* (REF. 299). Similar to EGFR and anaplastic lymphoma kinase (ALK) inhibitors, resistance mechanisms to therapies targeted against these emerging drivers have been reported, including second-site mutations and bypass pathway activation[72,87,165,216,300]. *BRAF* mutations, ~50% of which are the $BRAF^{V600E}$ mutation, occur in ~3–8% of lung adenocarcinomas[41,42], for which the combination of BRAF and MEK inhibitors are now FDA-approved. Reported resistance mechanisms include increased bypass EGFR signalling[117], reactivation of downstream MAPK signalling[117], and increased *YAP1* expression[53]. *MET* exon 14 mutations and high-level *MET* amplification can also serve as oncogenic driver mutations in NSCLC and may respond to MET TKIs like crizotinib[65]. Second-site MET mutations have been reported at resistance to type I MET TKIs, which retain sensitivity to type II MET TKIs[105–109,301]. JAK, Janus kinase; ORR, objective response rate; PR, partial response; STAT, signal transducer and activator of transcription.

predisposed towards resistance mutations in different oncogenic backgrounds, the type of on-target mutation that occurs reflects the binding characteristics of the TKI used and the degree of drug exposure against the target in a particular tumour cell. This confluence of factors is likely a contributor to the intertumoural and intratumoural heterogeneity of resistance mechanisms to targeted therapies in NSCLC.

Gatekeeper mutations. The prototypical mutation leading to EGFR TKI resistance in NSCLC is the EGFR-T790M mutation, which occurs at a conserved 'gatekeeper' threonine residue within the ATP binding pocket and is found in ≥50% of patients with acquired resistance to early-generation EGFR TKIs[78–80]. Although initially thought to act via steric hindrance with TKI binding[81], the EGFR-T790M mutation might confer resistance by altering kinase ATP affinity[82]. Preclinical studies suggest that acquired resistance can occur via both the *de novo* acquisition of the EGFR-T790M mutation and expansion of small, pre-existing EGFR-T790M-positive subclones under the selective pressure of TKI therapy[83,84]. Analogous gatekeeper mutations leading to TKI resistance have now been reported in ALK (ALK-L1196M)[85] and ROS1 (ROS1-L2026M)[86]. Although the spectrum of reported ALK mutations is more variable than those seen at resistance to either EGFR or ROS1 TKIs, there is some predominance of the ALK-L1196M gatekeeper mutation, which sterically hinders TKI binding and was found to occur in 7% of cases at resistance to early-generation ALK inhibitors in one case series[30]. Similarly, preclinical studies in *RET*-rearranged NSCLC have identified RET-V804L as the gatekeeper mutation responsible for resistance to cabozantinib and reported ponatinib as the most active RET TKI in the setting of this secondary mutation[87].

Covalent binding site mutations. Although third-generation EGFR TKIs overcome the EGFR-T790M resistance mutation through tight covalent binding to the ATP binding pocket[88], their use is associated with novel second-site mutations that confer resistance. The most reported resistance mutation in response to osimertinib is EGFR-C797S, which occurs at the covalent binding site for osimertinib[89,90]. An analogous mutation, HER2-C805S, has been reported at resistance to HER2 TKI therapy in HER2-mutated NSCLC[75].

Solvent-front mutations. Solvent-front mutations, which occur at kinase residues exposed to solvent, are another site of on-target resistance mutations that occur across the spectrum of EGFR-mutant, *ALK*-rearranged and *ROS1*-rearranged lung cancer, and they limit TKI binding via steric hindrance[91]. The solvent-front mutations EGFR-G796S and EGFR-G796R have been reported at resistance to third-generation EGFR TKI therapy. As with the EGFR-L718Q mutation, the EGFR-G796S/R mutations occur in residues that form hydrophobic regions, which usually surround the aromatic ring of osimertinib during binding, thus altering osimertinib binding affinity[91].

Steric hindrance
Interference with protein binding due to physical interference related to protein structure.

The G1202R, D1203N and S1206 solvent-front mutations in ALK have been seen at resistance to crizotinib[30,92,93]. The ALK-G1202R mutation, which confers resistance to all currently approved ALK TKIs, is seen in only ~2% of patients at resistance to early-generation ALK TKIs, but it is the most common mutation (21–43% of cases) seen at resistance to later-generation ALK TKIs[30,92]. The ALK-G1202R mutation remains a challenge that limits the ability to continue treatment with currently approved ALK-directed therapy. Lorlatinib, a third-generation ALK TKI currently in phase III trials, has activity against the ALK-G1202R mutation and might be a future treatment option[30]. The ROS1-G2032R solvent-front mutation, which is structurally analogous to the ALK-G1202R mutation, appears to be similarly challenging to overcome as it is highly potent[94]. It is the most common mutation conferring resistance to crizotinib in ROS1-driven tumours, comprising 80% of observed ROS1 mutations in one small case series[95]. The similar ROS1-D2033N mutation, located at the ATP binding site, alters electrostatic interactions with crizotinib to confer resistance and is analogous to the ALK-D1203N mutation[96].

Although solvent-front mutations have not been reported in *NTRK*-rearranged NSCLC, a solvent-front NTRK-G595R mutation has been reported in a patient with *NTRK*-rearranged colorectal cancer at resistance to a TRK inhibitor. This mutant is analogous to the ALK-G1202R, ROS1-G2032R and EGFR-G796A/R mutations[97].

Other second-site mutations. Other mutations in functionally important residues within the kinase domain can also promote resistance by interfering with TKI binding or by altering ATP affinity. These include less frequently observed mutations that confer resistance to EGFR TKIs located at the ATP binding site (EGFR-T854A), at or near the αC helix (EGFR-D761Y, EGFR-L747S) and in the hinge region (EGFR-L792F/H)[91,98–100].

In *ALK*-rearranged NSCLC, there is greater variability in mutations conferring resistance to crizotinib than has been observed with later-generation ALK TKIs. In addition to the mutations already discussed, the ALK-G1269A ATP binding pocket mutation sterically hinders drug binding[101]. ALK mutations near the αC helix (ALK-1151T insertion, ALK-F1174C, ALK-L1152R and ALK-C1156Y) do not directly interact with TKI binding and likely cause resistance via conformational changes that alter kinase activity, a known function of the αC helix domain[29,85,93,102].

Additional resistance mutations are seen during treatment with later-generation ALK inhibitors. For example, the ALK-I1171T mutation, which is the second most common mutation conferring resistance to alectinib, distorts the αC helix, altering the position of a residue that is involved in alectinib binding[103]. The ALK-V1180L mutation is located in the ATP binding pocket and results in steric hindrance, which also interferes with alectinib binding[103].

Although a smaller spectrum of similar ROS1 mutations have been reported to confer resistance to crizotinib, many ROS1 mutations are analogous to reported

ALK mutations owing to the structural homology between the ALK and ROS1 tyrosine kinase domains. For example, the ROS1-S1986Y/F mutations inhibit crizotinib binding by altering the position of the αC helix, analogous to effect of the ALK-C1156Y mutation[104]. Compared with ALK mutations, this narrower spectrum of ROS1 resistance mutations might reflect the greater potency of crizotinib as a ROS1 TKI.

Second-site mutations in the MET activation loop, MET-D1228N and MET-Y1230C, have been reported to confer resistance to crizotinib, a MET type I TKI, in MET exon-14-mutated NSCLC[105,106]. These mutations disrupt π stacking interactions involved in type I MET TKI binding. As they are less dependent on π stacking, the activities of MET type II TKIs were not limited by these mutations in preclinical studies[107–109]. A similar second-site MET-D1228V mutation has been reported at resistance to the type I MET TKI savolitinib in a patient with EGFR-mutated NSCLC and secondary *MET* amplification, who then responded to the type II MET TKI cabozantinib[108]. The MET-Y1248H and MET-D1246N mutations have also been reported in patients receiving type I MET TKIs for the treatment of secondary *MET* amplification and were also associated with a response to type II MET TKI treatment *in vivo*[110]. These findings suggest that the use of type II MET TKIs is a viable approach in multiple settings for patients with MET alterations who progress on initial type I MET TKI therapy.

Compound mutations. The serial acquisition of multiple resistance mutations within the oncogenic driver, as a result of treatment with different generations of TKIs, can produce on-target resistance to therapy that is challenging to manage. Triple-mutant tumour cells, bearing the original oncogenic EGFR-activating mutation and both the EGFR-T790M and EGFR-C797S mutations, can be resistant to all clinically available EGFR inhibitors, particularly when these mutations are located on the same allele[111]. Both brigatinib, a dual EGFR and ALK kinase inhibitor approved for treating *ALK*-rearranged NSCLC, and EAI045, a novel fourth-generation EGFR TKI currently in development, were found to be active against triple-mutant NSCLC in preclinical models when combined with the monoclonal anti-EGFR antibody cetuximab[112,113]. Similarly, the accumulation of multiple ALK resistance mutations during the course of serial therapy with multiple ALK inhibitors presents a therapeutic challenge. Although a second resistance mutation has been occasionally reported to restore sensitivity to prior generations of ALK TKI therapy[114], the more typical outcome is additive, compound resistance.

As compound resistance mutations develop within the oncogenic target, rational selection of subsequent lines of therapy based on the mutational profile becomes more important. This is already standard clinical practice for the most common EGFR resistance mutations (EGFR-T790M). Improved understanding of the individual spectrum of activity of each ALK TKI against the various ALK resistance mutations now makes this approach a more feasible option for patients with other resistance mutations as well. Expanded access to biomarker-focused methodologies such as circulating tumour DNA (ctDNA) assays for mutational analysis and mutational testing at biopsy of progressive disease will also facilitate implementation of this strategy.

Oncogene amplification or loss

Alterations other than second-site mutations at the targeted oncogenic driver can lead to reactivation of oncogenic signalling and therapeutic resistance. Loss of the *EGFR*^{T790M} mutation and wild-type *EGFR* amplification have been reported at resistance to third-generation EGFR TKIs[90,115]. Similarly, *ALK* copy number gain and amplification mediate resistance to crizotinib[101]. This resistance can be overcome by using a higher-dose crizotinib treatment and has not been reported at resistance to more potent ALK inhibitors[116]. A truncated, RAF-inhibitor-insensitive form of BRAF-V600E promotes acquired resistance to BRAF inhibitor treatment in NSCLC preclinical models, which was reversed by addition of a MEK inhibitor[117]. In patients with HER2-mutated NSCLC, *HER2* copy number gain also confers resistance to HER2-targeted therapy[72].

Off-target resistance

Tumour cell alterations conferring resistance to targeted therapies may also occur in proteins other than the targeted oncoprotein. These off-target alterations activate signalling pathways downstream or in parallel to the targeted oncoprotein, sustaining oncogenic signalling and therefore favouring tumour cell survival and growth despite the effective inhibition of the original oncogenic driver protein.

Downstream signalling pathways

Mutational activation of downstream signalling pathway components can bypass the dependence on the upstream, blockaded oncoprotein in a manner that is often conserved across the oncogene and targeted inhibitor landscape of NSCLC.

MAPK pathway. In EGFR-mutated tumours, MAPK pathway reactivation occurs at multiple points in the signalling pathway. Resistance to early-generation EGFR TKIs can occur via the acquisition of a BRAF mutation (BRAF-G469A or BRAF-V600E), which was seen in 1% of tumour samples from EGFR TKI-resistant patients in one series[118], or through loss of the *NF1* gene, a negative regulator of RAS[119]. Similarly, MAPK signalling activation via the BRAF-V600E oncoprotein, activating NRAS mutations and *NRAS* or *KRAS* copy number gain, can occur at acquired resistance to third-generation EGFR TKIs[120–122]. In preclinical studies, this resistance to third-generation EGFR TKIs was shown to be reversed, and more importantly prevented, by combined MEK and EGFR inhibitor treatment[120,123]. Clinical trials testing a MEK inhibitor in combination with EGFR TKIs are underway (TABLE 1).

MAPK pathway activation, through mechanisms including the downregulation of the ERK-specific phosphatase dual-specificity protein phosphatase 6

Type I TKI
ATP-competitive small-molecule TKIs that bind at the ATP binding site while in the active kinase conformation.

Type II TKIs
Small-molecule TKIs that bind at and near the ATP binding site in the inactive kinase conformation.

Table 1 | **Selected clinical NSCLC trials evaluating combinations of targeted therapies to address resistance mechanisms**

Drug regimen	Phase	Patient population	Results	Clinicaltrials.gov identifier*
EGFR TKI + MEK inhibitor				
Osimertinib + savolitinib or selumetinib	Ib	EGFRm, prior EGFR TKI	Activity at preliminary analysis (abstract)[302]	NCT02143466
Erlotinib + MEK162	I/Ib	KRASm or EGFRm	Ongoing	NCT01859026
Gefitinib + selumetinib	I/II	EGFRm, prior EGFR TKI	Ongoing	NCT02025114
EGFR TKI + PI3K–mTOR pathway inhibitor				
Erlotinib + XL765 (dual PI3K and mTOR inhibitor)	I	Solid tumours	Poorly tolerated[303]	NCT00777699
Gefitinib + BKM120	Ib	EGFR overexpression or *PIK3CA* mutation, prior EGFR TKI	PFS 2.8 months[304]	NCT01570296
Gefitinib + everolimus (mTOR inhibitor)	I/II	Unselected	13% PR[305]	NCT00096486
Erlotinib + BKM120	II	EGFRm, prior response to an EGFR TKI	Ongoing	NCT01487265
EGFR TKI + JAK–STAT inhibitor				
Afatinib + dasatinib	I	Molecular or clinical suggestion of EGFRm, prior EGFR TKI or EGFR-T790M^{+}	Ongoing	NCT01999985
Afatinib + ruxolitinib	I	Molecularly unselected	40% PR, 86.7% DCR[306]	NCT02145637
Erlotinib + ruxolitinib	I/II	EGFRm, prior erlotinib	5% PR[143]	NCT02155465
Osimertinib + INCB039110	I/II	EGFRm, EGFR-T790M^{+}, prior EGFR TKI	Ongoing	NCT02917993
EGFR TKI + SRC inhibitor				
Erlotinib + dasatinib	I	Molecularly unselected	7% PR, 63% DCR[307]	NCT00444015
Osimertinib + dasatinib	I/II	EGFRm	Ongoing	NCT02954523
EGFR TKI + MET inhibitor				
Erlotinib + cabozantinib	Ib/II	EGFRm, prior erlotinib	ORR 0% for combination arm[308]	NCT00596648
EGF816 + capmatinib	I/II	EGFRm	Ongoing	NCT02335944
Gefitinib + capmatinib	II	EGFRm, *MET*-amplified, prior EGFR TKI	15% PR (abstract)[154]	NCT01610336
Erlotinib + cabozantinib	II	Wild-type EGFR	PFS 4.7 (combination) versus 1.8 months (erlotinib)[309]	NCT01708954
Erlotinib + tivantinib	II	Unselected, no prior EGFR TKI	PR 10% (combination) versus 7% (erlotinib)[153]	NCT00777309
Erlotinib + onartuzumab (anti-MET mAb)	II	Molecularly unselected	No effect in unselected patients PFS 2.9 versus 1.5 months in MET+ patients[310]	NCT00854308
EGFR TKI + AXL inhibitor				
Erlotinib + BGB324	I/II	Molecularly unselected	Ongoing	NCT02424617
EGFR TKI + anti-HER3 mAb				
Erlotinib + patritumab	Ib/II	Molecularly unselected	In HRG (HER3 ligand)-high population: PFs 3 versus 1.4 months (abstract)[311]	NCT01211483
Erlotinib + patritumab	III	Wild-type EGFR	Results pending	NCT02134015
EGFR TKI + anti-VEGF mAbs and/or TKIs				
Erlotinib + bevacizumab	II	EGFRm, EGFR-T790M$^{+/-}$	EGFR-T790M^{+}; PFS 16 months EGFR-T790M^{-}; PFS 10.5 months[312]	NCT01562028
Osimertinib + bevacizumab	II	EGFRm, EGFR-T790M^{+}, prior EGFR TKI	Ongoing	NCT03133546
Erlotinib + bevacizumab	III	EGFRm	Ongoing	NCT02633189
Erlotinib + ramucirumab	III	EGFRm	Ongoing	NCT02411448
Gefitinib + apatinib	III	EGFRm	Ongoing	NCT02824458

Table 1 (cont.) | **Selected clinical NSCLC trials evaluating combinations of targeted therapies to address resistance mechanisms**

Drug regimen	Phase	Patient population	Results	Clinicaltrials.gov identifier*
EGFR TKI + anti-EGFR mAb				
Afatinib + necitumumab	I	EGFRm, prior EGFR TKI	Ongoing	NCT03054038
Osimertinib + necitumumab	I	EGFRm, prior EGFR TKI	Ongoing	NCT02496663
EGFR + pro-apoptotic therapy				
Osimertinib + navitoclax	Ib	EGFRm, second-line; EGFR-T790M^{+} in dose expansion portion	Ongoing	NCT02520778
EGFR TKI + HDAC inhibitor				
Erlotinib + belinostat	I	Molecularly unselected	Results pending	NCT01188707
Erlotinib + vorinostat	I/II	EGFRm, prior EGFR TKI	TTP 8 weeks[313]	NCT00503971
Gefitinib + vorinostat	I/II	Molecularly unselected	No improvement in PFS[314]	NCT01027676
Erlotinib + SNDX-275	II	Progression on erlotinib	Results pending	NCT00750698
EGFR TKI + other				
Erlotinib + dalotuzumab (anti-IGF1R mAb)	II	Molecularly unselected	No improvement in PFS[315]	NCT00654420
Afatinib + xentuzumab (anti-IGF1R mAb)	Ib	EGFRm, prior EGFR TKI	Ongoing	NCT02191891
Osimertinib + INK128 (mTORC1/2 inhibitor)	I	EGFRm, prior EGFR TKI, EGFR-T790M^{-} in expansion phase	Ongoing	NCT02503722
Gefitinib + olaparib (PARP inhibitor)	I/II	EGFRm	Results pending	NCT01513174
ALK inhibitor combinations				
Crizotinib + dacomitinib (HER2 inhibitor)	I	Prior response to EGFR TKI in expansion phase	Excess toxicity[316]	NCT01121575
Crizotinib + ganetespib (HSP90 inhibitor)	I	*ALK*-rearranged	67% PR[317]	NCT01579994
Ceritinib + everolimus (mTOR inhibitor)	I/Ib	ALK+ NSCLC in dose expansion phase, prior ALK TKI	Ongoing	NCT02321501
Ceritinib + luminespib (HSP90 inhibitor)	Ib	ALK+, prior ALK TKI	Results pending	NCT01772797
Alectinib + bevacizumab (anti-VEGF mAb)	I/II	ALK+	Ongoing	NCT02521051
Crizotinib + onalespib (HSP90 inhibitor)	I/II	ALK+	No increase in PFS[318]	NCT01712217
Ceritinib + trametinib (MEK inhibitor)	I/II	ALK+; with or without prior ALK TKI	Ongoing	NCT03087448
Alectinib + cobimetinib	Ib/II	ALK+; s/p progression on prior alectinib	Ongoing	NCT03202940
Ceritinib + ribociclib (CDK 4/6 inhibitor)	I/II	ALK+	Ongoing	NCT02292550

ALK+, *ALK*-rearranged; AXL, AXL receptor tyrosine kinase; CDK, cyclin-dependent kinase; DCR, disease control rate; EGFRm, epidermal growth factor receptor activating mutation; HDAC, histone deacetylase; HER, human epidermal growth factor receptor; HRG, heregulin; HSP, heat shock protein; IGF1R, insulin-like growth factor 1 receptor; KRASm, KRAS activating mutation; mAb, monoclonal antibody; MET+, MET activating mutation; ORR, objective response rate; PARP, poly(ADP-ribose) polymerase; PFS, progression-free survival; PR, partial response; s/p, status post; SRC, proto-oncogene tyrosine-protein kinase Src; TKI, tyrosine kinase inhibitor; TTP, time to progression; VEGF, vascular endothelial growth factor. *Further details for trials with NCT numbers can be accessed at the clinicaltrials.gov website.

(DUSP6) or *KRAS* amplification, was also shown to be critical for resistance to ALK TKIs in *ALK*-rearranged NSCLCs[124]. Targeting downstream MAPK signalling through the addition of a MEK inhibitor to ALK TKI therapy improved both the initial depth and duration of the response to treatment *in vitro* and *in vivo* in NSCLC models[124]. An activating MEK1 mutation has also been reported at resistance to ALK TKIs and was associated with response to a MEK inhibitor in another patient-derived NSCLC model[125]. Phase I and II studies evaluating the use of combined ALK and MEK inhibitors are ongoing (TABLE 1). Similarly, KRAS and NRAS mutations have been reported at crizotinib resistance in *ROS1*-rearranged NSCLC cells[126].

PI3K–AKT pathway. The survival of EGFR-mutant cell lines is also supported by downstream PI3K–AKT–mTOR signalling[127]. Mutations in *PIK3CA* (which encodes the PI3K catalytic subunit alpha), which were identified in ~4% of patients at baseline, or the loss of PTEN, a negative regulator of PI3K signalling, both predicted a poor response to EGFR TKI therapy in NSCLC patients[128–130] and induced resistance to EGFR TKI therapy in cell lines[130,131]. The addition of a PI3K inhibitor increased gefitinib sensitivity in cell lines and xenograft models[132,133]. Downstream of PI3K–AKT, increased mTOR expression was associated with EGFR TKI resistance in clinical samples[134], and the addition of the mTOR inhibitor rapamycin slowed the progression of EGFR-mutant lung

tumours in mouse models[135]. Clinically, inhibitors of PI3K–AKT–mTOR signalling in combination with EGFR TKIs have shown mixed evidence of efficacy and tolerability (TABLE 1). A *PIK3CA* mutation has also been reported in patient samples at resistance to HER2-targeted therapy in *HER2*-mutant NSCLC, and response to combined HER2 TKI and mTOR inhibitor therapy has been reported[136]. Interestingly, PI3K–AKT pathway gene mutations have not been extensively reported to cause TKI resistance in *ALK*-rearranged and *ROS1*-rearranged NSCLC, suggesting a less dominant role for this mode of PI3K–mTOR pathway activation in these subtypes.

JAK–STAT pathway. JAK–STAT3 signalling can occur as an early, adaptive response to EGFR TKI treatment in *EGFR*-mutant NSCLCs, in some cases arising downstream of NF-κB activation[137]. In preclinical NSCLC models, the addition of JAK or STAT3 inhibitors to EGFR TKI therapy improved response[137–140]. Autocrine interleukin-6 (IL-6) signalling by tumour cells increases JAK–STAT3 activity, and the addition of a neutralizing antibody against IL-6 inhibited tumour growth in mouse models[141,142]. However, in an early-phase trial, there was a response rate of only 5% to the combination of the JAK inhibitor ruxolitinib with erlotinib in patients who progressed on prior erlotinib treatment, suggesting the inability of this combination to reverse established resistance[143]. Upfront JAK and/or STAT3 inhibitor and EGFR TKI co-treatment might be necessary for therapeutic efficacy given the early, adaptive activation of JAK–STAT3 signalling observed in response to EGFR TKI treatment in preclinical models[137,144]. Accordingly, the JAK inhibitor INCB39110 is being tested in combination with osimertinib in patients with the EGFR-T790M mutation (TABLE 1). JAK–STAT3 signalling has not yet emerged as a prominent driver of resistance in *ALK*-rearranged and *ROS1*-rearranged NSCLCs, again suggesting the importance of context specificity in pathway dependencies across the oncogene landscape of NSCLC.

SRC activation. Proto-oncogene tyrosine-protein kinase Src (SRC) is an intracellular tyrosine kinase implicated in cell survival and differentiation, and it operates downstream of several receptor tyrosine kinases (RTKs), including EGFR[145]. SRC activation was reported in EGFR TKI-resistant NSCLC cell lines, and the SRC inhibitor dasatinib was active in EGFR TKI-resistant cell lines[146,147]. In a phase II trial, the combination of dasatinib and erlotinib was well tolerated, with early signs of clinical efficacy in patients with an activating EGFR mutation[148]. As an example of conservation of function in resistance, the SRC pathway has also been identified as a mechanism of resistance to ALK TKIs *in vitro*, including in patient-derived cell culture models, which were responsive to the addition of a SRC inhibitor[125,149].

Parallel bypass signalling pathways

The activation of parallel signalling pathways via other RTKs can activate signalling pathways required for cell proliferation and survival, thus bypassing inhibition of the original targeted oncogenic driver[120].

MET is a transmembrane RTK that is activated through the binding of its ligand, hepatocyte growth factor (HGF), and promotes MAPK and PI3K–AKT–mTOR signalling[150]. *MET* amplification occurs in 5–20% of patients with NSCLC who progress on EGFR TKI therapy[78,127]. In an EGFR TKI-resistant cell line with acquired *MET* amplification, the addition of a MET inhibitor restored the response to EGFR TKI treatment[151]. Although the combination of EGFR and MET inhibitors has shown poor response rates in initial trials, these trials were not targeted towards patients with *MET*-amplified tumours who are most likely to derive benefit from this combination[152,153]. A phase Ib trial of gefitinib combined with the MET inhibitor capmatinib in NSCLC patients with *MET* amplification and resistance to prior EGFR TKI therapy showed a response rate of 15% at the preliminary efficacy assessment[154]. Additional trials evaluating combined EGFR and MET inhibitors are ongoing (TABLE 1). Transcriptional upregulation of *MET* and/or *HGF* has also been associated with resistance to MET-sparing ALK TKIs in *ALK*-rearranged NSCLC[12,155], demonstrating potential conservation of function across NSCLC genetic subtypes.

AXL receptor tyrosine kinase (AXL) is an RTK that activates MAPK, PI3K–AKT and NF-κB signalling to promote tumour cell survival and metastasis[156]. Expression of AXL and its ligand, growth arrest-specific protein 6 (GAS6), are increased in samples from patients with *EGFR*-mutant NSCLC obtained at resistance to EGFR TKIs[157], and AXL activation promoted resistance to EGFR TKIs in preclinical models, which was reversed by treatment with an AXL TKI[157,158]. The AXL TKI BGB324 is being evaluated in combination with erlotinib in an ongoing phase I/II study[159]. AXL overexpression has also been implicated as a mechanism of resistance to ALK and RET TKIs in NSCLC driven by ALK and RET, respectively[160,161].

As another example of conservation of function, EGFR signalling can serve as a bypass signalling pathway in *ALK*-rearranged and *ROS1*-rearranged tumours with TKI resistance. In one study, 44% of tumour samples obtained at progression after therapy with the ALK inhibitor crizotinib showed increased EGFR activation relative to baseline samples[93]. EGFR activation was reported in *ALK*-rearranged NSCLC cell lines resistant to ALK TKIs, and responses to these agents were improved by the addition of an EGFR TKI[162]. As with resistance to ALK TKIs, EGFR bypass activation can confer resistance in *ROS1*-rearranged NSCLC cell lines, which is reversed by co-treatment with ROS1 and EGFR inhibitors[163–165]. Autocrine upregulation of EGFR ligands, including EGF, has been reported at resistance to the inhibition of multiple oncogenic kinases, including in *ALK*-rearranged and *RET*-rearranged NSCLC[12,166–168,216].

Similar to EGFR, HER2 and HER3 (also known as ERBB3) are members of the ERBB family, and they stimulate the PI3K–AKT and MAPK pathways[169]. In *EGFR*-mutant cell lines with acquired EGFR TKI resistance, the expression of mutant *EGFR* was lost, and a gain of oncogenic addiction to *HER2* and *HER3* was observed, thus alleviating addiction to EGFR signalling

and sensitizing the cells to combined EGFR and HER2 inhibitor treatment[170]. *HER2* gene amplification (as measured by FISH) and HER2 overexpression (as measured by immunohistochemistry (IHC)) provide alternative measures of HER2 expression and might be relevant biomarkers for the selection of patients for HER2-directed therapy[171]. Amplification of *HER2* has been reported in 12% of tumour samples obtained from patients at resistance to EGFR TKI therapy[172]. HER2 and HER3 activation, potentially due to autocrine ligand signalling via the EGFR ligand EGF and the HER3 ligand neuregulin 1, has also been reported at ALK TKI resistance in samples obtained from patients and in preclinical models[162,173,174]. Clinical responses to treatment with HER2-targeted therapies in patients with baseline HER2 overexpression have shown limited overall responses, primarily in patients with high levels of HER2 overexpression (IHC score of 3+) or *HER2* amplification detected by FISH[175–178]. These findings suggest that this strategy will be difficult to employ in the setting of acquired HER2 overexpression following treatment with EGFR TKIs in *EGFR*-mutant NSCLC; however, the potential for clinical efficacy in this setting remains to be explored.

Other bypass signalling pathways have been implicated in EGFR TKI resistance, including upregulation of both FGFR1 and its ligand FGF2 (REFS 115,179) and RTK ephrin type-A receptor 1 (EPHA1) upregulation[180]. Additionally, insulin-like growth factor 1 receptor (IGF1R) activation has been reported in preclinical models at resistance to both ALK and EGFR TKIs[181,182]. *KIT* amplification has also been reported at crizotinib resistance[93], and an activating KIT mutation (KIT-D816G) was reported in a *ROS1*-rearranged tumour at resistance to crizotinib[183]. The KIT-D816G mutation is analogous to the MET-D1228V resistance mutation seen with MET inhibitor therapy[108].

Additional resistance mechanisms

Alterations in signalling pathways regulating cell survival and apoptosis, histological and phenotypic transformations, epigenetic changes that favour the development of drug-tolerant tumour cell populations, and bidirectional interactions with the TME can alter tumour cell susceptibility to the inhibition of target oncoproteins.

Survival and anti-apoptotic pathways

A response to TKI therapy requires the induction of apoptosis upon inhibition of the oncogenic target. Therefore, alterations in cell signalling pathways that control cell survival and apoptosis can alter the sensitivity to TKIs. The pro-apoptotic protein BIM (also known as BCL2L11), which inhibits BCL-2, is necessary for the effective induction of apoptosis in response to EGFR TKIs[184]. Patients with germline *BIM*-deletion polymorphisms are relatively resistant to both EGFR and ALK TKIs compared with patients without these polymorphisms[10,185]. This resistant phenotype has been shown to be overcome in preclinical models by the addition of BH3-mimetic drugs, which are small-molecule inhibitors of the anti-apoptotic proteins BCL-2 and BCL-XL[10], or by addition of the histone deacetylase (HDAC) inhibitor vorinostat, which increases BIM expression[186]. In patients without germline *BIM* deletions, low levels of *BIM* expression at baseline or after exposure to EGFR TKIs was correlated with reduced PFS and overall survival (OS) during EGFR TKI treatment[187]. BH3-mimetics such as navitoclax have been shown to increase apoptosis in response to erlotinib *in vitro,* and this agent is now being evaluated in a phase Ib study in combination with osimertinib[188]. Interestingly, both *BCL2* and *BCLXL* are NF-κB target genes, suggesting a common molecular network underlying different esistance mechanisms.

NF-κB is a transcription factor that regulates cell proliferation, apoptosis and inflammation, and its activation has been associated with resistance to multiple EGFR TKIs[9,137]. In a patient-derived xenograft model and additional cellular models, NF-κB was activated acutely following EGFR TKI treatment, and it promoted JAK–STAT3 pathway activation via NF-κB-mediated overexpression of IL-6 and consequent autocrine signalling[137]. This JAK–STAT3 activation and associated resistance to EGFR TKIs was overcome by the addition of a direct NF-κB inhibitor, PBS-1086. The enhancement of the initial magnitude and duration of the response to an EGFR TKI combined with NF-κB inhibition in preclinical models exemplifies the potential clinical utility of upfront combination therapy. In addition, AKT activation can promote NF-κB activation, demonstrating the molecular connections between signalling pathways that mediate EGFR TKI resistance[189]. NF-κB signalling has yet to emerge as a major mediator of resistance to other targeted therapies in NSCLC, illustrating the relevance of context specificity in the pathways mediating resistance.

YAP1 is a transcriptional co-activator that serves as a Hippo pathway effector through its interaction with transcription factors that promote cell proliferation and inhibit apoptosis[190]. High *YAP1* expression was associated with resistance to EGFR TKIs in preclinical models and with poor survival in a cohort of patients with NSCLC[191,192]. This resistance to EGFR TKIs could be reversed in cell lines by the addition of verteporfin, a small-molecule inhibitor of YAP1 that is in clinical use as a photosensitizer[192,193]. Co-activation of STAT3 and YAP1 has also been implicated in promoting tumour cell survival upon treatment with EGFR TKIs, and the co-inhibition of EGFR, STAT3 and SRC–YAP1 signalling demonstrated a synergistic effect that was more effective than the use of single-agent EGFR TKIs in cell lines[139]. A genetic screen also identified the activation of *YAP1* as a mediator of resistance to BRAF inhibitors in *BRAF*-mutant NSCLC cells, and YAP1 inhibition improved the response to BRAF and MEK inhibitors in this setting[53]. Interestingly, the EGFR ligand amphiregulin has been shown to be secreted in response to YAP1 activation[194]. Thus, YAP1 might function to promote RAF and/or MEK inhibitor resistance, in part via autocrine activation of EGFR signalling, extending the themes of signalling crosstalk and functional conservation among the mechanisms of resistance and across NSCLC genetic subtypes.

Alterations in cell cycle proteins, including the loss of the CDK inhibitor p16 (encoded by *CDKN2A*), have also been correlated with primary resistance to EGFR TKIs in patients with NSCLC[195]. Moreover, in EGFR TKI-resistant preclinical models, treatment with a CDK4/6 inhibitor improved the response to EGFR TKI treatment[196].

Histological transformation

The transformation of tumours from an epithelial to a small-cell lung cancer (SCLC) histology is seen in a subset of patients with NSCLC and acquired EGFR or ALK TKI resistance[78,197]. This histological transformation was associated with RB loss in all tested tumour samples obtained from patients in one series, which was necessary, but not sufficient, to induce resistance. SCLC histological transformation was also associated with the loss of EGFR expression and an improved response to treatment with a BCL-XL inhibitor compared with EGFR TKI-resistant cell lines without SCLC transformation[198]. Similarly, transformation to sarcomatoid carcinoma has been reported at resistance to crizotinib in *ALK*-rearranged tumours[199].

Epithelial-to-mesenchymal transition (EMT) is another phenotypic change seen at resistance to both EGFR and ALK TKI therapy[78,200], manifesting as a series of cellular alterations favouring a more invasive, mesenchymal phenotype. Markers of a mesenchymal phenotype — for example, low levels of the epithelial marker E-cadherin (encoded by *CDH1*) and increased levels of the mesenchymal marker vimentin — have been reported in samples from patients at acquired resistance to EGFR TKIs[78] and in NSCLC cell lines with acquired resistance to EGFR and ALK TKIs[201,202]. Elevated levels of transforming growth factor-β (TGFβ), a cytokine associated with inflammation, have been reported to promote EMT in NSCLC cell lines resistant to EGFR TKI therapy via the promotion of IL-6 secretion[203]. In addition, the transcription factor zinc-finger E-box-binding homeobox 1 (ZEB1) promotes EMT through, for example, the HDAC-mediated suppression of *CDH1* expression[202]. Increased *ZEB1* expression and has been reported to be both induced by EGFR TKI exposure[204] and associated with resistance to EGFR TKI therapy in NSCLC cell lines[202], which could be reversed by the inhibition of *ZEB1* expression[205].

Other gene expression changes associated with resistance to EGFR TKIs have also been associated with the promotion of a mesenchymal phenotype, including increased *SRC* and *AXL* expression, again demonstrating molecular crosstalk among different features associated with EGFR TKI resistance[158,206]. Targeting signalling pathways associated with EMT, for example with SRC inhibitors[207], HDAC inhibitors[202] or inhibitors of IL-6 signalling[208], could restore sensitivity to EGFR TKIs in preclinical studies.

Epigenetic resistance mechanisms

Epigenetic alterations are associated with EGFR TKI resistance and can be acquired during initial EGFR TKI treatment to induce a drug-tolerant state and therefore resistance. For example, HDAC activity promoted the survival of an EGFR TKI-tolerant cell population[209], and the combination of an EGFR TKI and the HDAC inhibitor panobinostat increased the response to therapy *in vitro*[210]. Early-phase studies testing HDAC inhibitors in combination with EGFR TKIs are underway (TABLE 1).

The tumour microenvironment

Dynamic interactions between tumour cells and stromal components within the TME influence the response to TKI therapy and highlight the connections and redundancies within the molecular and histological phenotypes underlying resistance (FIG. 5).

Co-culture with cancer-associated fibroblasts (CAFs) can induce both EMT and resistance to EGFR TKIs in NSCLC cells *in vitro*[211,212]. The secretion of multiple paracrine-acting factors from CAFs, including HGF, promoted ERK activation and consequent EGFR TKI resistance in NSCLC tumour cells, and co-treatment with HGF-targeted agents restored sensitivity to EGFR TKIs[213,214]. In turn, lung tumour cells can recruit fibroblasts through the induction of migration *in vitro* and have been found to colocalize with fibroblasts in patient-derived NSCLC tumour specimens[214]. CAFs can also secrete the AXL ligand GAS6 in response to cytotoxic therapies[215], which can subsequently promote EMT[158]. In *ALK*-rearranged NSCLC, secretion of the EGFR ligands EGF, TGFα, and heparin-binding EGF-like growth factor (HB-EGF) by endothelial cells and the secretion of HGF by CAFs induced EGFR-dependent and MET-dependent bypass signalling, leading to resistance to ALK TKIs[12]. Similarly, exposure to exogenous EGF or to EGF-secreting endothelial cells caused resistance to RET inhibitors in a *RET*-rearranged cell line, which was responsive to treatment with EGFR-targeted therapy[216]. CAFs might also promote the expression of anti-apoptotic genes in tumour cells, such as *BCL2*, which have been associated with TKI resistance in other studies[217].

Other interactions between NSCLC cells and the stroma have been implicated in resistance to EGFR TKIs. Low levels of SerpinB2 — a serine protease inhibitor that inhibits extracellular matrix (ECM) degradation — have been associated with poor prognosis and resistance to the EGFR TKI gefitinib *in vitro*, which was reversed by treatment with a SerpinB2-inducing agent[218]. Increased levels of N-cadherin and integrin β1, which are mediators of tumour adhesion to the ECM, have both been associated with EGFR TKI resistance[146,219] via activation of the PI3K–AKT pathway[132]. Induction of the chemokine receptor CXC-chemokine receptor 4 (CXCR4) in tumour cells, which binds to CXC-chemokine ligand 12 (CXCL12; also known as SDF1), a factor known to be expressed in the lung microenvironment, has been reported to promote tumour cell proliferation and EGFR TKI resistance in published and preliminary studies[220,221].

Hypoxia within the TME activates hypoxia-inducible factor 1α (HIF1α) (REF. 222) and promotes EGFR TKI resistance by activating EGFR signalling via autocrine TGFα signalling and promoting cancer stem cell features

Sarcomatoid carcinoma
Pulmonary sarcomatoid carcinoma is an uncommon and aggressive poorly differentiated form of NSCLC.

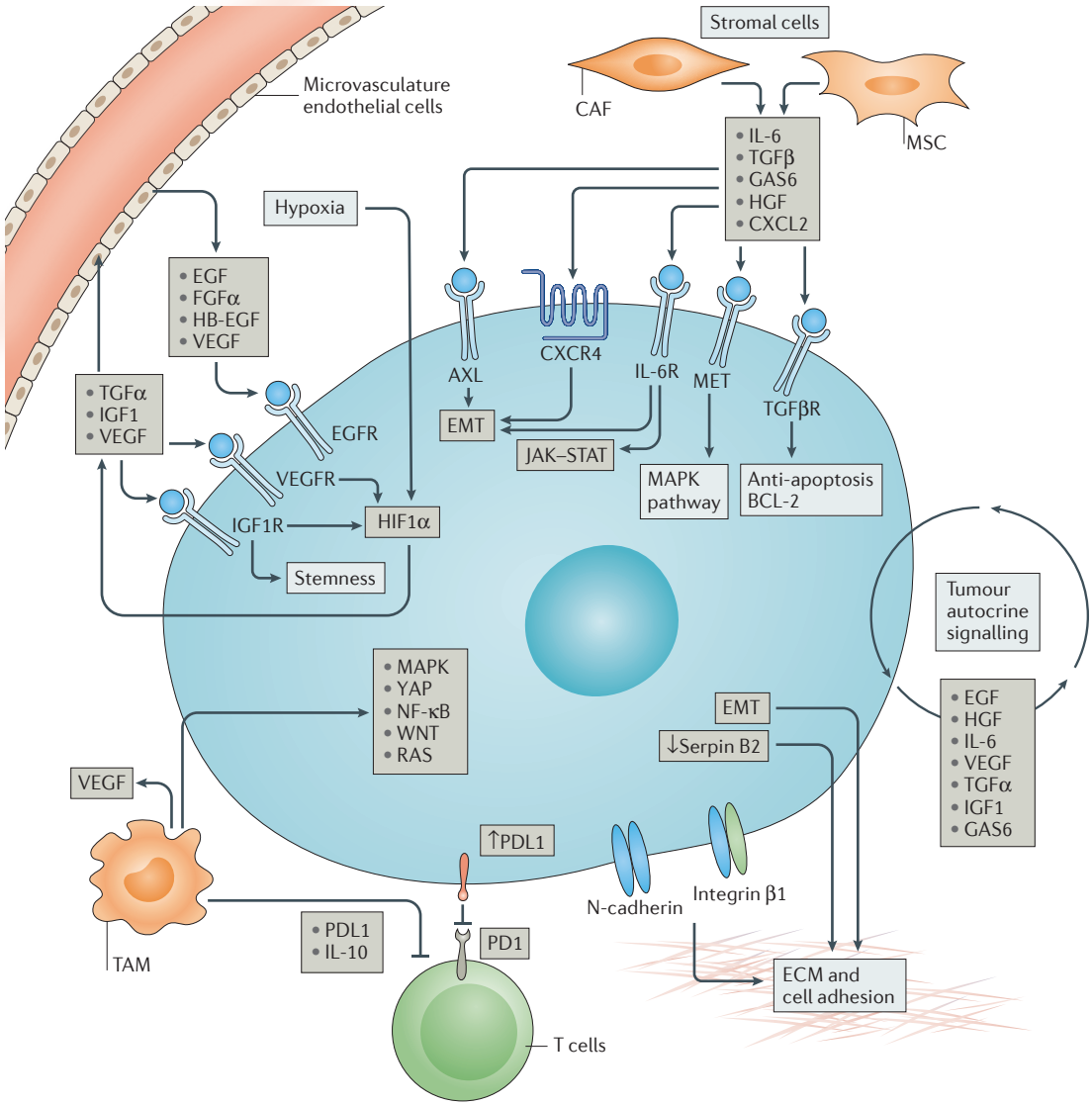


Figure 5 | **The tumour microenvironment and resistance to targeted inhibitors.** Bidirectional interaction occurs between tumour cells and resident cell types within the tumour microenvironment (TME). Tumour cells secrete growth factors and cytokines that attract and modulate the behaviour of both stromal cells and immune cells. In addition, tumour-derived factors such as interleukin-6 (IL-6), growth arrest-specific protein 6 (GAS6), hepatocyte growth factor (HGF) and epidermal growth factor (EGF) can promote resistance to targeted therapies through autocrine signalling. In turn, the interaction between tumour cells and the TME influences the tumour cell response to targeted therapy. These tumour–TME interactions include alterations in cell–cell adhesion via increased expression of N-cadherin and integrin β1 and increased extracellular matrix (ECM) degradation through the loss of Serpin B2 expression, an inhibitor of the plasminogen activation system. Cancer-associated fibroblasts (CAFs) and mesenchymal stem cells (MSCs) within the stroma secrete factors that promote resistance to EGF receptor (EGFR) tyrosine kinase inhibitors (TKIs) via activation of CXC-chemokine receptor 4 (CXCR4), IL-6 receptor (IL-6R), hepatocyte growth factor receptor (MET), AXL receptor tyrosine kinase (AXL) and transforming growth factor-β receptor (TGFβR), which in turn promote epithelial-to-mesenchymal transition (EMT), cell survival through MAPK and JAK–STAT pathways, and the inhibition of apoptosis through BCL-2 activity. Immune cells within the TME, including tumour-associated macrophages (TAMs) and T cells, produce factors that influence diverse pathways, including the MAPK, PI3K, Hippo–yes-associated protein (YAP1), TGFβ, nuclear factor-κB (NF-κB), WNT and RAS pathways. The upregulation of programmed cell death 1 ligand 1 (PDL1) expression in tumour cells following TKI therapy and the expression of anti-inflammatory cytokines by TAMs might also contribute to an immunosuppressive TME by inhibiting T cell-mediated antitumour cytotoxicity. Lastly, exposure to a hypoxic TME can activate hypoxia-inducible factor 1α (HIF1α) in tumour cells, leading to autocrine signalling via transforming growth factor-α (TGFα), which promotes resistance to TKI therapy, vascular endothelial growth factor (VEGF), which stimulates angiogenesis, and insulin-like growth factor 1 (IGF1), which can promote stem cell-like characteristics (stemness). Similarly, VEGF produced by microvasculature endothelial cells and TAMs can alter tumour cell characteristics and further promote angiogenesis. CXCL2, CXC-chemokine ligand 2; FGFα, fibroblast growth factor α; HB-EGF, heparin-binding EGF-like growth factor; IGF1R, IGF1 receptor; JAK, Janus kinase; PD1, programmed cell death protein 1; STAT, signal transducer and activator of transcription; VEGFR, VEGF receptor.

through IGF1R activation[223,224]. In *ALK*-rearranged NSCLC cells, the induction of EMT in response to hypoxia leads to ALK TKI resistance[225]. In addition, local secretion of vascular endothelial growth factor (VEGF) in response to hypoxia promotes angiogenesis and also acts in a feedforward manner to promote both VEGF and VEGF receptor (VEGFR) expression in tumour cells[226]. The combination of EGFR TKIs with VEGF-targeted inhibitors or monoclonal antibodies is under clinical investigation (TABLE 1).

Crosstalk between tumour cells and tumour-associated macrophages (TAMs) within the TME has been implicated in tumour cell survival in response to EGFR TKIs. In patients with advanced EGFR-mutant NSCLC who were treated with EGFR TKI therapy, increased levels of TAM infiltration within the TME at baseline correlated with poor PFS and reduced OS[227]. In a mouse model of NSCLC, computational modelling of RNA expression within tumour and stromal cell populations identified macrophage-derived factors as activating multiple tumour cell signalling pathways implicated in resistance to EGFR inhibitors, including the MAPK, PI3K, YAP, NF-κB, WNT and RAS pathways[228].

In addition, the upregulation of the immune checkpoint gene encoding programmed cell death 1 ligand 1 (PDL1) can occur in cells with activating EGFR mutations or *ALK*-rearrangements, creating a TME that is less permissive of T cell-mediated antitumour cytotoxicity[229,230]. However, checkpoint inhibitor therapies targeting PDL1 (or programmed cell death protein 1 (PD1)) have not shown strong clinical efficacy in patients with EGFR-mutated and *ALK*-rearranged NSCLC, with an ORR of only 3.6% reported in one series[231]. This poor efficacy may reflect the low immunogenicity of tumours that have less genomic complexity in the setting of a dependence on a particular oncogenic driver mutation, a notion that is supported by the reported low level of $CD8^+$ T cell infiltration in tumour samples from these patients[231]. The extent to which the TME contributes to resistance to targeted therapy in NSCLC is an understudied area that warrants increased investigation, particularly as new therapies that modulate immune and stromal cells in the TME continue to emerge. An important challenge is to understand whether there is potential for therapeutic synergy between oncoprotein inhibitors and immunomodulatory agents and, if so, in which NSCLC molecular subtypes.

Heterogeneity and clinical challenges

The heterogeneity of tumour evolution, both over time within a tumour and spatially between different primary and metastatic sites, raises the question of how to optimally define the molecular status of a tumour and of how to best incorporate the understanding of this heterogeneity into treatment strategies.

Tumour heterogeneity

The preponderance of intratumoural heterogeneity was highlighted by a study in which whole-exome sequencing revealed subclonal oncogenic alterations in 75% of early-stage surgically resected NSCLC tumours[232]. Similarly, the heterogeneity of tumour evolution over time has been described in both advanced EGFR-driven and ALK-driven NSCLC[30,233], and increased baseline heterogeneity has been correlated with a shorter duration of response to EGFR TKI therapy[233]. The extent to which selection for pre-existing (intrinsic) versus *de novo* (acquired) resistance mutations occurs in tumour cells during targeted therapy remains an important and open question in the field. From an evolutionary perspective, prioritizing therapies that block the more truncal resistance mutations might impede subclonal genetic diversification and branched evolution. Alternatively, the convergent evolution of pathways that are recurrently activated in the context of resistance might reduce the challenge of genetic heterogeneity to a more limited set of targetable pathways.

Liquid biopsies

The heterogeneity of potentially targetable lesions raises challenges in designing personalized treatment regimens, as a single biopsy might not capture the full spectrum of molecular changes and resistance mechanisms. Measurement of ctDNA offers a noninvasive complement to tumour biopsy for the assessment of mutational status, which may provide an integrative view of molecular alterations that are not readily captured by individual tissue biopsies[234]. The noninvasive nature of ctDNA monitoring can also permit serial monitoring for emerging resistance mechanisms. Rising frequencies of $EGFR^{T790M}$ detected by ctDNA have been observed before the onset of clinical resistance to EGFR TKIs[235,236], as early as 344 days before clinical progression in one study[235]. In another study, ctDNA profiles were established in earlier-stage NSCLC before definitive resection could predict subsequent relapse via the detection of re-emerging subclones[237]. The implications of these findings for the optimal selection of therapy, particularly before radiographic progression, remain to be determined.

Residual disease

For patients with NSCLC who receive treatment with a targeted therapy, achieving a complete response to therapy is rare. The residual disease contains persisting tumour cells, which might be clonally derived from a small resistant subpopulation present at baseline and/or through the induction of adaptive changes within the tumour cells in response to TKI therapy[83]. These persisting cells have the capability to acquire additional resistance mechanisms *in vitro* and ultimately give rise to resistant, progressive disease[238]. Systemic or local ablative therapy targeting these persisting tumour cells might eliminate this reservoir of resistant cells and improve the response to therapy. In a clinical trial, the addition of local therapy to residual lesions following either chemotherapy or EGFR-targeted or ALK-targeted therapy improved PFS[239]. For patients with a more extensive disease, knowledge of the pathways underlying the survival of persisting cells is necessary to design systemic therapeutic strategies. These reported changes include NF-κB activation[137], reduced pro-apoptotic signalling[83]

Intratumoural heterogeneity
Variation in tumour cell genomic and phenotypic characteristics within a given tumour.

Convergent evolution
The independent development of alterations within the same signalling pathways among different tumour cell clones during the course of tumour cell evolution.

Radiographic progression
Tumour enlargement and/or new lesion development that are visible on radiographic studies and meet specific criteria.

Residual disease
Persistent tumour burden despite disease stabilization and/or an objective response to antineoplastic therapy.

Oligoprogressive disease
Isolated growth of malignant lesions despite continued control of overall tumour disease burden.

and epigenetic alterations that diminish tumour cell apoptosis in response to the inhibition of oncogene signalling[209]. As residual disease sites are rarely biopsied during the course of standard of care therapy, research protocols that permit biopsy of these sites are necessary for this purpose and might be complemented by ctDNA analysis.

Polytherapy strategies

The increased number of therapeutic options for treating oncogene-driven NSCLC has raised the question of how to best sequence and combine these agents. In published and preliminary studies, the first-line use of later-generation TKIs has demonstrated improved outcomes in both EGFR-mutant and *ALK*-rearranged NSCLC[36,240,241]. Whether improvements in PFS will translate to improved OS with later-generation TKIs compared with the sequential use of the various generations of TKIs remains to be established — if so, the tolerability of later-generation TKIs in the first-line setting might support an initial period of monotherapy before the initiation of combination therapy approaches at the emergence of resistance.

Alternatively, the design of upfront combinatorial treatment regimens to pre-emptively constrain the emergence of common mechanisms of resistance has been shown to improve the depth and duration of the response to EGFR-targeted and ALK-targeted therapy in preclinical models[120,124,137]. An open question is whether pharmacological blockade of upstream bypass pathways or of downstream signalling pathway components will be superior to forestall resistance when combined with the inhibition of a driver oncoprotein. In *EGFR*-mutant cell lines, the addition of a MEK inhibitor downstream of EGFR inhibition was more effective than inhibiting bypass MET activation; however, this approach was ultimately circumvented by AKT–mTOR reactivation[120]. Similarly, both upstream EGFR signalling[165] and downstream MEK[124] signalling are potential therapeutic targets in combination with ALK TKI treatment in *ALK*-rearranged NSCLC.

Combining these approaches, computational simulations have been used to suggest switching strategies that alternate variable drug combinations to overcome the challenge of polytherapy toxicity and variable off-target pathway activation[242]. The optimal measurement and use of biomarkers to identify or predict resistance mechanisms are important areas of investigation.

Local therapy

A consequence of tumour heterogeneity is the potential for the existence of isolated sites of clinically progressive disease within an overall responsive tumour burden. Local ablation of these resistant lesions using either surgery or radiation can prolong the response to therapy, with an average time to next progression of 6–7 months[243,244]. The optimal management of oligoprogressive disease during treatment with earlier-generation therapies remains to be determined.

Conclusions

Understanding the multi-factorial biological basis of resistance to targeted therapy in NSCLC provides a rich insight into the molecular architecture of tumour development and progression, particularly how genetic alterations co-opt normal cellular processes to initiate and maintain a tumour and rewire cell signalling pathways to achieve plasticity and evolutionary robustness. Recognition of the complexity of the molecular alterations underlying the development of resistance to targeted therapeutics is necessary to understand the basis of tumour cell survival and clinical progression during therapy, as well as to design combinatorial and non-cross resistant therapeutic strategies. These strategies will require integration with ongoing advances in the field of immunotherapy for lung cancer. Successfully implementing personalized medicine in the treatment of NSCLC will require improved individualized assessments of pathways driving tumour growth, both at baseline and serially throughout the course of therapy, in order to design tailored treatments to forestall tumour evolution and drug resistance.

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Author contributions

J.R. researched data for the article. Both authors contributed equally to the discussion of the content, wrote the article and reviewed and/or edited the manuscript before submission.

Competing interests statement

The authors declare competing interests: see Web version for details.

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