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ALK-positive lung cancer: a moving target

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Abstract

Anaplastic lymphoma kinase (ALK) is a potent oncogenic driver in lung cancer. ALK tyrosine kinase inhibitors yield significant benefit in patients with *ALK* fusion-positive (ALK^+) lung cancers; yet the durability of response is limited by drug resistance. Elucidation of on-target resistance mechanisms has facilitated the development of next-generation ALK inhibitors, but overcoming ALK-independent resistance mechanisms remains a challenge. In this Review, we discuss the molecular underpinnings of acquired resistance to ALK-directed therapy and highlight new treatment approaches aimed at inducing long-term remission in ALK^+ disease.

Over the past few decades, advances in lung cancer diagnostics and treatments have transformed patient outcomes[1,2]. Translational research, clinical genotyping and drug discovery have enabled the molecular stratification of lung cancers, namely adenocarcinomas, based on the presence of oncogenic drivers and the development of targeted therapies matched to the respective oncogenes[2]. The *ALK* gene fusion defines one molecular subtype of non-small cell cancer (NSCLC), comprising 4–6% of lung adenocarcinomas[3]. A chromosomal rearrangement involving the *ALK* gene on chromosome 2 leads to ectopic expression of the tyrosine kinase-containing portion of ALK and its constitutive activation. ALK^+ lung cancers exhibit ALK dependency and are typically sensitive to ALK inhibition using tyrosine kinase inhibitors (TKIs). So far, five ALK TKIs have received approval by the US Food and Drug Administration (FDA) for treatment of advanced ALK^+ NSCLC, with more in clinical development.

Despite their remarkable responses to ALK TKIs[4–9], almost all patients with advanced ALK^+ lung cancers ultimately experience disease relapse through on-target and off-target resistance mechanisms[10]. Tumor cells with on-target resistance retain their dependence

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on ALK, whereas those with off-target mechanisms activate ALK-independent pathways to support proliferation and survival. Re-biopsies and genotyping of resistant clinical samples are key in elucidating the mechanisms of resistance and guiding sequential therapeutic approaches. Yet, challenges remain in addressing the heterogeneity of resistance mechanisms and preventing disease relapse.

In this Review, we provide an overview of the underlying biology of oncogenic ALK fusions, discuss the current understanding of acquired resistance to ALK-directed therapy and highlight the latest therapeutic strategies aimed at inducing long-term remission in advanced ALK^+ lung cancers, centered on the hypothetical, yet imperative, question of what it would take to cure metastatic ALK^+ lung cancer.

Physiological role of ALK

The *ALK* gene was first cloned in 1994 when the nucleophosmin (NPM1)–ALK fusion protein was identified in anaplastic large cell lymphoma (ALCL)[11]. *ALK* encodes a highly conserved receptor tyrosine kinase (RTK) in the insulin receptor superfamily[12]. The native ALK protein is thought to be essential for the development and functioning of the nervous system[13]. Structurally, ALK is composed of an N-terminal extracellular domain, a hydrophobic single-pass transmembrane region and an intracellular kinase domain (Fig. 1). ALK is activated when ALKAL proteins (endogenous ligands of ALK) bind to its extracellular domain, resulting in dimerization and autophosphorylation and activation of downstream signaling pathways critical for cell proliferation, survival and differentiation[14–16].

Oncogenic ALK fusions

In malignancies, *ALK* point mutations or chromosomal rearrangements result in aberrant activation of ALK and downstream signaling cascades[17]. In NSCLC and cancer types that include ALCL, diffuse large B cell lymphoma, inflammatory myofibroblastic tumors, glioma and colon cancer[14,18–20], the oncogenic driver is a structural *ALK* rearrangement whereby the kinase domain-encoding region of *ALK* at the 3′ end is fused to various partner genes at the 5′ end. Echinoderm microtubule-associated protein-like 4 (*EML4*)-*ALK* is the most common *ALK* fusion in NSCLC, identified in approximately 85% of cases[21,22]. Delineation of *ALK* fusion partners continues, with more than 90 distinct 5′ partner genes reported to date in NSCLC[22]. *ALK* fusions result in ligand-independent dimerization and hyperactivation of pro-mitogenic and anti-apoptotic signaling including the RAS–mitogen-activated protein kinase (MAPK)[23], PI3K–AKT[24] and JAK–STAT[25] cascades (Fig. 1). In the case of EML4–ALK, the MAPK pathway is a critical downstream effector, the activation of which is mediated by the HELP domain of EML4 (ref. [23]).

Within *EML4-ALK* alone, at least 12 distinct variants exist as determined by breakpoints in *EML4*. Variant 1 (E13;A20) and variant 3 (E6a/b;A20) are the most common and are associated with differences in protein stability, drug sensitivity[26] and distinct *ALK* resistance mutations[27]. The identity of the 5′ partner can influence the intrinsic properties of the fusion protein by altering kinase activity, protein stability, transformative potential and drug

sensitivities in vitro[28]. However, selection of ALK inhibitors currently remains agnostic of fusion type. Sequencing analysis showed that 5′ *ALK* is retained in the genome in some cases, in addition to 3′ *ALK*. Whether these reciprocal or nonreciprocal translocations are independent predictors of poorer outcomes in patients treated with next-generation ALK TKIs remains to be seen[29].

Most ALK fusions lack a transmembrane region and are not anchored to the plasma membrane, unlike native ALK. Early studies of ALK fusions in ALCL showed differential cytoplasmic, nuclear and granular subcellular localization depending on the ALK binding partner identity[30], likely representing an important feature in the modulation of protein–protein interactions, activation of distinct signaling cascades and stability and/or degradation of the fusion product. Recent studies have revealed that certain RTK fusion oncoproteins including ALK assemble de novo in their own subcellular compartment that can phase separate based on coalescence of cytoplasmic membraneless protein granules[31–33]. These discrete structures concentrate oncogenic ALK with components of MAPK, PLC-γ, PI3K and JAK–STAT cascades to coordinate RTK signaling, suggesting that disruption of protein granule assembly and function could be therapeutically targeted. However, more work is needed to identify factors that regulate these subcellular condensates.

ALK has served as a framework for investigating other fusion oncoproteins in lung cancer such as ROS1, RET, TRKA–TRKC and NRG1. Although ALK-driven NSCLC is an archetypal example of oncogene addiction, whether genomic heterogeneity in ALK$^+$ NSCLC, in terms of binding partners, reciprocal translocations and breakpoint variants, accounts for differences in treatment responses to TKIs remains unclear[34]. Furthermore, the etiology of *ALK* fusions in cancers is largely unknown, as is its propensity to affect young patients[35,36].

ALK as a therapeutic target

The field of ALK$^+$ NSCLC has been transformed by the development of successive generations of increasingly selective, potent and brain-penetrant ALK TKIs, serving as a paradigm of a successful bench-to-bedside approach and highlighting the clinical benefit of understanding molecular dependencies of cancer. The success in targeting ALK$^+$ lung cancer is also a model of reverse translation, as insights from molecular analysis of patient samples have directly guided basic science discoveries and shaped drug development.

Preclinical modeling of ALK$^+$ NSCLC has proven critical in recapitulating ALK pathophysiology in vitro and in vivo and facilitating the development of ALK inhibitors. For instance, overexpression of fusion ALK in untransformed cells[28,37,38] facilitated in vitro studies in isogenic backgrounds and enabled mutagenesis screening, drug testing and tracking of resistant clones[37]. Early germline transgenic mouse models and tissue-specific expression of EML4–ALK in alveolar epithelial cells established the transforming role of ALK fusion in vivo[39]. Tissue-specific Cre-inducible transgenic models and CRISPR–Cas9-based, viral-mediated delivery of *EML4-ALK* were developed to study drug resistance in vivo[40,41]. Although patient-derived cell lines (PDCs) and xenografts lack certain advantages of orthotopic ALK-driven NSCLC murine models, they have been invaluable in

elucidating clinically relevant mechanisms of resistance in *ALK*- and other oncogene-driven cancers[42,43].

Targeting ALK in the clinic

Preclinical modeling of ALK$^+$ lung cancers spurred the development of numerous ALK TKIs (summarized in Table 1). Crizotinib, the first-generation ALK inhibitor, is a multitargeted TKI that was originally developed as an inhibitor of mesenchymal–epithelial transition (MET)[44]. In 2011, crizotinib was granted accelerated FDA approval based on phase 1–2 studies showing clinical activity in advanced ALK$^+$ NSCLC[45], followed by two large phase 3 trials demonstrating its superiority to cytotoxic chemotherapy in this patient population[4,5]. However, the median progression-free survival (PFS) of patients treated with crizotinib was limited to 8–11 months, with relapse observed in the central nervous system (CNS) due to poor drug penetration of the blood–brain barrier[4]. Several second-generation ALK TKIs that were developed (of which ceritinib, alectinib and brigatinib have received FDA approval) showed clinical activity also in the CNS in the post-crizotinib setting and overcame some common crizotinib-refractory *ALK* resistance mutations[46]. Second-generation ALK TKIs were also effective in the absence of crizotinib-resistant *ALK* mutations, suggesting incomplete ALK inhibition by crizotinib.

Although they were initially evaluated in the post-crizotinib setting, second-generation ALK TKIs have since supplanted crizotinib as the preferred initial therapy in advanced ALK$^+$ lung cancer. Alectinib[7], brigatinib[8,47,48] and ensartinib[49] were compared directly to crizotinib in the first-line setting, demonstrating superior efficacy of second-generation TKIs across the board (Table 1). The global randomized phase 3 ALEX study[7,50–52] established alectinib as a preferred first-line therapy with mature data showing significantly prolonged PFS with alectinib compared to crizotinib (median PFS of 25.7 versus 10.4 months, respectively; hazard ratio (HR) for disease progression or death of 0.50).

Lorlatinib is a third-generation macrocyclic ALK TKI, designed to be highly potent, selective and CNS penetrant[53]. Preclinical studies demonstrated robust activity of lorlatinib against wild-type ALK and most known *ALK* mutations refractory to first- and second-generation TKIs, including the predominant ALK^{G1202R} solvent-front mutation[46]. Lorlatinib initially received accelerated FDA approval in the second- or third-line setting for advanced ALK$^+$ disease based on efficacy in patients with exposure to at least one prior ALK TKI in the phase 1–2 study, with an objective response rate (ORR) of 47% in this patient population[54]. The global randomized phase 3 CROWN trial[9] evaluated lorlatinib in the front-line setting in comparison to crizotinib and demonstrated significantly longer PFS (HR for disease progression or death of 0.28) and dramatic reduction in the risk for CNS progression (HR for CNS progression or death of 0.07), resulting in its FDA approval as a first-line agent in 2021 (refs. [9,55]).

Despite the success of iterative generations of ALK TKIs, the durability of response remains limited by drug resistance. Approximately half of patients treated with first-line alectinib for ALK$^+$ NSCLC will experience disease progression within approximately 2 years[50], an unfortunate but expected consequence of tumor evolution and emergence of resistance.

Mechanisms of ALK-dependent resistance

Resistance to ALK-targeted therapies can be broadly classified as ALK dependent and ALK independent. ALK-dependent, or 'on-target', resistance is largely defined by the emergence of single or compound mutations in the *ALK* gene, rendering tumor cells persistently dependent on ALK activity (Fig. 2). ALK-independent, or 'off-target', resistance is defined by lineage changes or activation of ALK-independent signaling pathways that obviate ALK dependency in ALK^+ tumor cells.

ALK mutations in acquired resistance

Sequencing of paired treatment-naive and ALK TKI-resistant tumor specimens has been key to discovery of *ALK* resistance mutations[46] that confer resistance by re-inducing kinase activation despite the presence of a TKI. Functional validation of putative resistance mechanisms in PDCs and xenograft models helped distinguish drivers of resistance from bystander mutations[56]. As tissue biopsies are not always feasible at the time of clinical relapse, analysis of circulating tumor DNA (ctDNA) from liquid biopsies has offered a complementary tool to track the evolution of resistance[57].

In 50–60% of patients treated with a second-generation ALK inhibitor, resistance arises through acquisition of a secondary *ALK* mutation[46]. These mutations occur universally in the kinase domain and confer resistance by direct steric hindrance of TKI binding, alteration in protein kinase conformation and/or changes in ATP binding[46,58,59]. In contrast to *EGFR*-mutant lung cancers in which T790M was the predominant *EGFR* alteration refractory to early-generation inhibitors[60], an impressively broad spectrum of *ALK* mutations confer resistance in ALK^+ NSCLC[46]. The first *ALK* resistance mutation identified was the L1196M gatekeeper mutation[61,62]. ALK^{G1269A} similarly affects the ATP-binding pocket, impairing crizotinib docking. Another class of alterations known as solvent-front mutations, including G1202R, G1202del, D1203N, S1206Y and S1206C, disrupt the solvent-facing surface of ALK and impair drug binding through steric hindrance[63]. ALK^{G1202R} confers resistance to both first- and second-generation TKIs at clinically achievable doses and accounts for approximately half of on-target resistance across all second-generation ALK TKIs[46]. Each ALK TKI, even those within the same generation, is associated with a distinct spectrum of secondary *ALK* resistance mutations. For example, although ALK^{G1202R} is the most common *ALK* mutation identified across specimens after treatment with ceritinib, alectinib and brigatinib, ALK^{I1171N}, ALK^{I1171T} and ALK^{I1171S} mutations are seen in 10–15% of alectinib-resistant samples but in no ceritinib- or brigatinib-resistant samples[46].

Lorlatinib was specifically designed to overcome *ALK* mutations refractory to first- and second-generation ALK TKIs[64,65] and has shown efficacy against most single *ALK* mutations including G1202R and I1171X[54]. However, resistance to lorlatinib also emerges, with a distinct profile of *ALK* mutations arising with sequential TKI use culminating with lorlatinib[37,66,67]. Sequencing of biopsies from patients relapsing on lorlatinib revealed that on-target resistance accounts for approximately a third of cases, composed of diverse compound (that is, two or more *ALK* mutations occurring on the same allele or in cis) and notably not single *ALK* mutations[37]. For instance, the $ALK^{G1202R + L1196M}$ compound mutation has been identified in patients at the time of relapse and was independently

identified in an untransformed cell line mutagenesis screen yielding lorlatinib-refractory clones[37]. Analyses of serial clinical biopsies demonstrated that sequential ALK TKI therapy culminating in lorlatinib induces compound *ALK* mutations, with ALK^{G1202R}- or ALK^{I1171N}-based compound mutations being the most common[37,68]. The multitude of single *ALK* mutations existing after treatment with prior ALK TKIs likely serves as the substrate for compound *ALK* mutations to develop on lorlatinib, supporting the notion of stepwise accumulation of resistance mutations.

A substantial fraction of lorlatinib-resistant compound *ALK* mutations such as G1202R + L1196M and G1202R + F1174C/L are refractory to all approved ALK TKIs[68,69], highlighting the need for fourth-generation ALK inhibitors. However, a limited range of double mutants are sensitive to currently available agents. An in vitro study showed that select ALK^{I1171N}-based compound mutations are sensitizing to brigatinib and ceritinib[69]. Furthermore, in an illustrative case report, a patient with ALK^{+} lung cancer who had received sequential TKIs (crizotinib, ceritinib, lorlatinib) and ultimately acquired a lorlatinib-resistant $ALK^{L1198F + C1156Y}$ mutation had a durable response to retreatment with crizotinib, as the acquired ALK^{L1198F} mutation re-sensitized tumor cells to crizotinib[59]. Although these examples are rare, they underscore the importance of serial biopsies in guiding next-line therapeutic strategies and the continued need to catalog and functionally test emerging *ALK* resistance mutations.

Mechanisms of ALK-independent resistance

In approximately half of the patients with ALK^{+} NSCLC who progress on a second-generation ALK TKI, *ALK* mutations are not identified at the time of clinical relapse, suggesting ALK-independent resistance and only modest benefit by subsequent-generation ALK inhibitors for this subset of patients[68,70]. Diverse off-target mechanisms that confer ALK TKI resistance can occur across patients, making ALK-independent resistance challenging to overcome.

Bypass pathway activation

One important category of ALK-independent resistance is activation of bypass signaling, which arises from genetic alterations, changes in protein expression and/or activation or dysregulation of autocrine feedback signaling. Multiple bypass tracks have been described in ALK TKI-resistant tumors including activation of RTKs MET[71], EGFR[72], SRC[56], IGF-1R[73], HER2 and HER3 (ref. [74]) and KIT[72] and alterations in downstream signaling factors MAP2K1 (refs. [23,56]), DUSP6 (ref. [23]), STAT3 (ref. [75]) and NF2 (ref. [66]). These co-occurring genetic alterations mediating resistance are not present at the time of diagnosis in treatment-naive patients[76].

The first bypass mechanisms were described in the context of crizotinib resistance. Comparison of crizotinib-sensitive and crizotinib-resistant cells and biopsies before and after treatment revealed increased EGFR tyrosine phosphorylation[72,77]. EGFR autophosphorylation can occur in the absence of acquired *EGFR* mutations or amplification, indicating non-genetic mechanisms such as altered EGFR dynamics and expression, enhanced EGFR ligand binding or dysregulation of feedback loops. Hyperactivation of

other RTKs has been reported, including HER2 and HER3 (ref. [74]) and activation of protein kinase C (PKC) signaling through P2Y purinergic receptor family G-protein-coupled receptors[78]. Characterization of the bypass resistance landscape after next-generation ALK inhibitors revealed similar findings, including increased activation of RTKs IGF-1R and HER3 and overexpression of the HER3 ligand neuregulin 1 (NRG1)[73,74,78,79].

MET alteration is a well-established driver of RTK-mediated resistance in ALK$^+$ and other NSCLC subsets[80], with amplification detected in ~15% of tumor biopsies from patients relapsing on next-generation ALK TKIs[71]. Patients receiving second-generation ALK TKIs in the first-line setting are more likely to develop *MET* amplification than those on next-generation ALK inhibitors following treatment with crizotinib, which inhibits MET[71]. Combined ALK–MET inhibition using crizotinib alone or lorlatinib plus a MET-selective TKI can effectively suppress proliferation of ALK$^+$, *MET*-amplified tumors[71], supporting the clinical testing of combined ALK and MET inhibitors (NCT04292119).

Intracellular signaling mediators have also been implicated in acquired resistance. Oncogenic ALK signaling requires activation of the MAPK pathway. ALK-independent MAPK pathway reactivation can occur through multiple mechanisms including *KRAS* copy number gain, mitogen-activated protein kinase kinase 1 (MAP2K1)-activating mutations[56] or loss of DUSP6, a negative regulator of MAPK[23]. Upfront co-inhibition of mitogen-activated protein kinase kinase (MEK) enhanced the therapeutic efficacy of ALK inhibition through diminished residual MAPK signaling[23], providing the rationale for clinical testing of dual ALK and MEK blockade (NCT03202940) to enable more durable responses by limiting tumor cell persistence and clonal expansion.

Functional inhibition of a protein with pleiotropic effects may offer a superior strategy for overcoming bypass pathways. For example, a short hairpin RNA dropout screen of multiple ALK TKI-resistant PDCs identified SH2-containing protein tyrosine phosphatase 2 (SHP2) as a potential target[42]. SHP2 mediates GTP loading of RAS downstream of multiple RTKs including EGFR, FGFR and MET for modulation of JAK–STAT, PI3K–AKT and MAPK pathways. Pharmacological inhibition of SHP2 attenuates ceritinib-induced ERK kinase reactivation, and combined inhibition of ALK and SHP2 restores sensitivity and overcomes resistance in drug-tolerant cell lines[42]. Dual ALK and SHP2 inhibition is being evaluated in early-phase trials (NCT04292119 and NCT04800822).

That bypass track engagement renders cells fully independent of ALK may be an oversimplified notion. ALK TKI-resistant PDCs often retain partial dependency on ALK, and maximal cytotoxicity is achieved with dual ALK and bypass inhibition[77]. In the clinic, disease flares can occur upon discontinuation of ALK TKIs, even in the context of known ALK-independent resistance mechanisms[81]. These observations may reflect intratumoral or intertumoral heterogeneity in which subsets of cells remain addicted to ALK. More studies are needed on the dynamics of sustained ALK dependency during acquired resistance. The field has been limited by tissue availability, as obtaining matched pretreatment and post-resistance biopsy specimens for functional analyses can be challenging. Comparative analysis of specimens before and after TKI treatment paired with functional validation of

putative pathways will be integral to fully elucidate genetic and non-genetic mechanisms of resistance and the breadth of off-target mechanisms.

Histologic transformation

Transformation of a tumor to a different histologic subtype is associated with loss of reliance on the oncogenic driver, leading to drug resistance[82]. Although virtually all cases of newly diagnosed ALK$^+$ NSCLC are adenocarcinoma, small cell lung cancer transformation has been identified in patients with ALK$^+$ lung cancer after treatment with all generations of ALK TKIs, albeit at low frequency (<3% according to retrospective analysis)[83–86]. In small cell-transformed *EGFR*-mutant and *ROS1* fusion-positive lung cancers, even though the original driver gene alteration was retained, its expression was lost upon transformation[87,88]. Transformation to squamous cell carcinoma has also been reported following treatment with alectinib[89] and lorlatinib[68].

Identifying histological transformation in the clinic is critical for the selection of subsequent histology-matched therapy. Given the rarity of transformed ALK$^+$ lung cancers, randomized prospective trials to inform treatment strategies following phenotypic changes are not feasible. Ongoing research centers on elucidating the molecular changes that rewire cellular phenotypes during ALK-targeted therapy and understanding their reversibility, with the goal of restoring adenocarcinoma histology and re-sensitizing cells to ALK inhibition.

Challenges of polyclonal resistance

Therapeutic targeting of ALK TKI-resistant tumors is complicated by the heterogeneity of resistance mechanisms (Fig. 3). Divergent pathways may evolve in distinct metastatic foci within one patient or in clusters of tumor cells within one disease site, resulting in polyclonal resistance. For instance, concomitant *ALK* mutations with *ALK* or *KIT* amplifications have been identified in biopsies after crizotinib treatment[72,90]. A report of a patient with ALK$^+$ NSCLC progressing on ceritinib followed by alectinib noted the concomitant detection of sequence encoding ALK^{G1202R} in ctDNA and small cell transformation in tumor[91]. In instances in which *ALK* resistance mutations and off-target resistance mechanisms co-occur, addressing ALK dependency alone is not sufficient.

Tissue biopsies are not always feasible at the time of disease progression. ctDNA analysis from liquid biopsies offers a complementary tool for monitoring the emergence and temporal evolution of acquired *ALK* mutations and capturing mechanisms of resistance across metastatic sites[57]. Comparative analysis of plasma and tumor specimens after alectinib treatment in a study with ~90% sensitivity of plasma genotyping to detect *ALK* resistance mutations in relapsing patients revealed that plasma was more likely to harbor at least two *ALK* mutations, indicating polyclonal resistance that was not captured with single-site tissue biopsies[57]. Various other gene alterations implicated in off-target resistance can be detected by plasma biopsies, including *BRAF*, *MAP2K1* and *PIK3CA* mutations[92], although certain copy number changes or structural gene alterations, such as *MET* amplification, may be harder to detect.

The increasing ability to identify disparate mechanisms of resistance in an individual patient may pose clinical dilemmas about which, if any, warrants therapeutic targeting, especially in cases in which multiple FDA-approved or investigational drugs are available.

Therapeutic strategies in ALK+ NSCLC

Below, we discuss new therapeutic strategies that focus on new ways to maximally inhibit ALK and to overcome or prevent ALK TKI resistance.

Sequencing of ALK TKIs

With several FDA-approved ALK TKIs as first-line treatment for advanced ALK+ NSCLC, the optimal front-line next-generation ALK TKI (in particular, alectinib, brigatinib or lorlatinib) remains controversial[93,94]. Second-generation ALK TKIs such as alectinib are more commonly used as the initial therapy (with lorlatinib reserved as salvage therapy[93]) on the basis of efficacy that can be achieved with upfront second-generation TKIs and a favorable toxicity profile with alectinib in particular. Whether this achieves the optimal clinical outcome is unclear. Starting with a less potent agent has the increased probability of selecting for refractory compound mutations, many of which are recalcitrant to all currently FDA-approved ALK inhibitors as discussed above[37,95].

Moving the most potent ALK inhibitor to first line may suppress or delay the emergence of on-target resistance and prolong the duration of response. In the phase 3 CROWN trial of first-line lorlatinib versus crizotinib in patients with advanced ALK+ NSCLC[9], lorlatinib resulted in significantly longer PFS with an impressive HR for disease progression or death of 0.27 based on an updated analysis[9,55], as compared to the HR for progression or death of close to 0.50 seen for the second-generation TKIs alectinib, brigatinib and ensartinib when compared to crizotinib[7,8,49]. Furthermore, lorlatinib has the highest level of CNS penetration and provides robust CNS protection[55,96], which is noteworthy given the CNS tropism of ALK+ disease[97]. Although there is no head-to-head comparison of lorlatinib versus a second-generation ALK TKI and the resistance landscape for first-line lorlatinib remains to be determined, these data support starting with a pan-inhibitory, highly potent and CNS-penetrant ALK TKI to ensure maximal cytoreduction and depth of response, limiting the tumor heterogeneity that can emerge with less potent second-generation ALK TKIs and delaying on-target resistance and CNS recurrence.

As efforts to reach consensus on ALK TKI sequencing continue, patients whose tumors harbor an *ALK* resistance mutation can be treated with an ALK TKI targeting that particular mutation, if available. Patients whose tumors lack *ALK* mutations can be considered for ALK-based combinatorial strategies or other investigational approaches to tackle ALK-independent resistance.

Overcoming on-target resistance with new ALK TKIs

A subset of ALK-driven tumors remains addicted to ALK even after lorlatinib treatment due to compound *ALK* mutations. Functional screening of a panel of lorlatinib analogs in vitro and in vivo indicated that distinct molecules have differential selectivity for ALK^{G1202R}- versus ALK^{I1171}-based compound mutations[68]. For example, two lorlatinib analogs, LA7

and LA9, demonstrated selectivity against ALK^{I1171N} and ALK^{G1202R} single and compound mutants, respectively. These data suggest that distinct ALK TKIs may be required against different classes of compound *ALK* mutations, with one new ALK TKI being unlikely to overcome all on-target lorlatinib resistance.

TPX-0131 (ref. [98]) and NVL-655 (ref. [99]) are fourth-generation ALK TKIs designed to target compound *ALK* mutations, with preclinical activity against single and some compound *ALK* mutations (for example, ALKG1202R + L1196M, ALKG1202R + G1269A, ALKG1202R + L1198F)[100]. Both agents are currently in phase 1 testing (NCT04849273 and NCT05384626, respectively). Consistent with lorlatinib analogs, TPX-0131 lacks activity against $ALKL^{I1171}$ mutations but has high potency against ALK^{G1202R} and ALK^{G1202R}-based double and triple mutations[98]. By contrast, gilteritinib, an agent used in *FLT3*-mutated acute myelogenous leukemia[101], acts against ALK^{I1171N}-based but not ALK^{G1202R}-based compound mutations[102].

The challenge of overcoming the diverse array of compound *ALK* mutations aside, should early-phase trials of fourth-generation ALK TKIs demonstrate favorable safety profiles, studies will be needed on how to integrate them into the already crowded landscape of ALK-targeted therapy. Each TKI will likely have a distinct potency spectrum against ALK mutants, and thus may be most useful after next-generation TKIs with known ALK-dependent resistance. Mutations conferring resistance to these new agents are anticipated, highlighting the challenge of perpetually chasing resistance in ALK^{+} NSCLC. One potential advantage of starting with a pan-single mutant-inhibitory ALK TKI such as lorlatinib is the prevention of refractory compound *ALK* mutations and obviation of the need for higher-generation TKIs.

Alternative ALK-centric approaches

Use of sequential ALK TKIs has exposed the problem of increasingly complex on-target resistance mutations. Thus, alternative approaches of targeting ALK outside of small-molecule TKIs merit attention. Targeting ALK through allosteric or covalent inhibition or protein degradation may serve as a complementary therapeutic approach to circumvent the increasingly complex on-target resistance mutations exposed by using sequential ALK TKIs. One strategy involves covalent ALK inhibitors targeting cysteine residues located outside of the active site[103]. Proteolysis targeting chimeric (PROTAC) technology is another approach, used to direct endogenous protein degradation by linking a protein of interest and an E3 ubiquitin ligase[104]. A flurry of PROTACs have been developed against oncoproteins including estrogen or androgen receptors, BTK and BCR–ABL1, several of which are in early-phase clinical trials[105]. Aside from those using an ALK TKI[106] as the PROTAC's ALK ligand, for example, using allosteric ALK inhibitors, ALK-directed PROTACs could be agnostic to *ALK* kinase domain mutations, thus overcoming TKI resistance. Preclinical testing of ALK-directed PROTACs reported potent decreases in total and phosphorylated ALK levels in a concentration- and time-dependent fashion[106,107]. In vivo evaluation is needed to clarify the biologically relevant intracellular ALK concentrations, as even residual amounts may be sufficient to activate proliferative pathways.

Another ALK-centric approach involves the disruption of protein–protein interactions. EML4–ALK homodimerizes through a coiled-coil domain within EML4 (ref. [21]) that requires a conserved pattern of hydrophobic residues and salt bridges[108]. Disruption of this interaction abrogates the transforming ability of the ALK fusion[21], and introduction of competitive coiled-coil-mimetic compounds abrogates tumor formation[109].

Whether these agents will be successfully developed clinically and whether they will be integrated into practice together with or in lieu of ALK TKIs remains unknown. However, despite maximal ALK inhibition, a substantial proportion of patients will develop ALK-independent resistance requiring alternative treatment strategies such as targeting bypass pathways or tumor microenvironmental factors.

Combinatorial strategies against ALK-independent resistance

The functional characterization of bypass pathways mediating ALK TKI resistance has led to development of rational combinatorial approaches for patients who relapse on ALK TKIs. *MET* amplification is a prototypical actionable bypass pathway in ALK$^+$ NSCLC[71] and has similarly been established as a bona fide bypass track in *EGFR*-mutant[110] and *RET* fusion-positive[111] NSCLC, in which co-targeting of the oncogenic driver and MET can overcome MET-driven resistance[112,113]. Dual ALK– MET inhibition re-sensitizes ALK$^+$ PDC models with MET-driven resistance[71]. Case reports have documented disease responses to crizotinib monotherapy or lorlatinib-based combinations with a MET inhibitor in patients who are ALK$^+$ with acquired *MET* amplification[71,114]. A phase 1–2 study is evaluating the combination of lorlatinib plus crizotinib to address MET-driven resistance (NCT04292119). Several additional ALK TKI-based combination trials are underway (Table 2). Most trials (outside of ALK–MET inhibitor combinations) do not require biomarkers to guide stratification. Further investigation is needed to identify biomarkers associated with responses to combinations.

These rational combination strategies have thus far yielded underwhelming results without notable efficacy. One challenge is the augmentation in toxicities that can limit dosing of each drug. Another potential pitfall is the timing of treatment, as combinations may exert a greater impact in forestalling the emergence of resistance, rather than overcoming resistance once established[23]. In light of these challenges, whether the evolutionary trajectory of an ALK$^+$ lung cancer could be discerned at initial diagnosis and tumors preemptively pressured toward a particular path to exploit targetable vulnerabilities remains in question.

Targeting persister cell populations

Despite marked responses typically seen upon ALK TKI initiation, residual disease often remains and can lead to relapse, even after several years of stability on therapy. Growing evidence indicates that drug-tolerant persister cells are responsible for residual disease (Fig. 3). The persister state is thought to be reversible, with most cells remaining in cell cycle arrest in the presence of drug but with a small subset having the capacity to re-enter the cell cycle. Persisters may subvert TKI inhibition through adaptive mechanisms, including epigenetic modifications, bypass activation, metabolic reprogramming and altered interactions with the tumor microenvironment[115].

Non-genetic mechanisms have emerged as important drivers of persister cell survival. For instance, cycling and non-cycling drug-resistant persister cells from *EGFR*-mutant lung cancer were shown to arise from distinct cell lineages, with discrete transcriptional and metabolic programs[116]. A shift to fatty acid oxidation was associated with persister proliferative capacity in *EGFR*-mutant NSCLC cells and across multiple cancer subtypes, underscoring a proliferative response that may enable transition to oncogene independence following treatment. As an example, the transcriptional regulator YAP1 was shown to be activated following treatment of ALK^+ cells with alectinib in vitro and in vivo, an effect attenuated by combinatorial inhibition of ALK and YAP1 (ref. [117]). Genetic and pharmacologic blockade of YAP1 suppressed tumor growth in drug-resistant cells, xenograft models and *EML4-ALK*-transgenic mice[118]. High expression of YAP1 in treatment-naive samples was found to be a negative prognostic sign for response to ALK TKIs[118]. Selective inhibitors of YAP, its isoform TAZ and its binding partner TEAD are in early-phase clinical trials[119] (NCT04665206, NCT04857372, NCT05228015 and NCT04659096). Illuminating the full spectrum of non-mutational mechanisms that bolster persister fitness will be critical in targeting pro-survival escape pathways promoting clinical relapse.

In the clinic, eradication of persister cells is one rationale for using local consolidative approaches (for example, radiation) to ablate sites of residual disease after TKI initiation. More data are needed to ascertain clinical benefit of ablating persister populations after targeted therapies before relapse[120]. One prospective study is evaluating the impact of integrating stereotactic body radiation therapy after induction with TKIs in stage IV oncogene-driven NSCLC (NCT02314364). Adding chemotherapy or epigenetic modulators to an ALK TKI after initial cytoreduction could serve as alternative strategies to eliminate persisters, although not yet clinically evaluated. One caveat with existing strategies is the reliance on radiographic findings to identify residual disease at the macroscopic rather than microscopic level. Ultrasensitive, blood-based techniques may prove beneficial for monitoring occult persistent and/or recurrent disease.

Immune-based therapies for ALK^+ lung cancer

Although programmed cell death protein 1 (PD-1)–programmed cell death ligand 1 (PD-L1) checkpoint inhibitors have revolutionized the management of NSCLC, their efficacy has been minimal in patients with ALK^+ lung cancer[121], even among patients with high PD-L1 expression[121,122]. Retrospective and real-world studies show lower response rates and shorter PFS for immune checkpoint monotherapy among patients with ALK^+ or *EGFR*-mutant NSCLC than those with ALK–EGFR-wild-type disease[121,123,124]. Although most prospective or randomized phase 3 studies have excluded patients with *EGFR* mutations or who are ALK^+ (ref. [125]), those that allowed patients with ALK^+ NSCLC have been largely underpowered to draw conclusions[126,127]. ALK^+ tumors have low tumor mutational burden, effectively limiting the neoantigen landscape, and low colocalization of PD-L1 expression with $CD8^+$ tumor-infiltrating lymphocytes, which likely underlies the limited anti-tumor immune responses regardless of oncogene-mediated upregulation of PD-L1 (refs. [121,122,128]). Despite data supporting refractoriness of ALK^+ NSCLC to immune checkpoint inhibitor monotherapy, the impact of combining them with other treatments (such as chemotherapy) remains to be clarified[129,130]. Combinations with ALK TKIs have been

explored, largely demonstrating lack of synergistic efficacy and, in some cases, heightened toxicities[131–133]. Alternative immune-based strategies may be a more promising avenue in the context of ALK-driven disease.

For instance, ALK represents an attractive target for vaccines as it is recognized as a tumor antigen and autoantibodies against it are detected in NSCLC and ALCL, suggesting that some patients may be able to generate a spontaneous anti-ALK immune response[134,135]. Mapping of specific epitope sequences of anti-ALK autoantibodies in a small cohort of patients with NSCLC demonstrated clustering outside the kinase domain[134]. High levels of these spontaneous anti-ALK antibodies were detected in 17% of patients with ALK^+ NSCLC, underscoring the fact that ALK can be spontaneously immunogenic in a small subset of patients. Hypothetically, an ALK vaccine could potentiate an anti-tumor response in patients who already have autoantibodies and may induce an immune response for those without autoantibodies. The first ALK vaccine developed in 2015 consisted of a DNA plasmid coding for the intracytoplasmic domain of ALK and produced remodeling of the immune microenvironment and a $CD8^+$-mediated cytotoxic response in mouse models of orthotopic ALK^+ lung tumors[136]. The first-in-human trial of an ALK peptide vaccine is anticipated to open in 2023. The optimal place for an ALK vaccine in the therapeutic landscape remains to be determined.

Because the ALK fusion protein is localized intracellularly in NSCLC, certain therapeutic modalities such as chimeric antigen receptor T cells and antibody–drug conjugates that require the presence of a tumor antigen on the cell surface are not feasible. However, ALK-targeted antibody–drug conjugates[137] and chimeric antigen receptor T cells[138] are being developed in *ALK*-mutated neuroblastoma and other pediatric tumors where ALK is expressed on the plasma membrane. Identification of intracellular ALK antigen fragments presented by major histocompatibility complex (MHC) molecules may serve as a foundation for engineering modified T cell receptor (TCR) T cells. In advanced uveal melanoma, tebentafusp, a bispecific protein consisting of an affinity-enhanced TCR specific for an HLA-A*02:01-presented glycoprotein 100 (gp100) epitope fused to an anti-CD3 effector, had a survival benefit compared to the control group of pembrolizumab, ipilimumab or dacarbazine[139]. This result serves as a proof of concept that tumors with low tumor mutational burden that are refractory to immune checkpoint inhibitors can be responsive to immune strategies.

A deeper understanding of the immunobiology of ALK^+ NSCLC will be critical for devising immune-based therapies. ENIGMA+ (‘Elucidating novel immune and genomic markers for ALK^+’; NCT04881916) is a research platform aiming to enable remote consent and participation of patients with ALK^+ lung cancer nationwide to illuminate vulnerabilities for immune-based therapeutics in ALK^+ NSCLC.

Addressing lineage plasticity

The molecular mediators that govern lineage plasticity in ALK^+ lung cancer and how this leads to TKI resistance remain to be determined. More exploratory studies are needed to elucidate the role of repressor RB1 and tumor protein p53 (TP53) in ALK^+ SCLC transformation and associated dependencies in tumors that have undergone

lineage changes[140,141]. In patients with *EGFR*-mutant lung cancers, in which small cell transformation accounts for 10–15% of acquired resistance to EGFR TKIs[142,143], patients with concurrent TP53 and RB1 loss at diagnosis are at significantly higher (43×) risk of small cell transformation than those without[144,145]. One study implicated Aurora kinases as a unique dependency in RB1-deficient SCLC[146], raising the possibility that Aurora kinase inhibitors may be used to target subpopulations of *EGFR*-mutant and ALK$^+$ cancers that acquire RB1 loss in the context of lineage change.

Modulation of the epigenetic landscape is also being studied as an avenue for reversing lineage plasticity and re-sensitizing cells to ALK inhibitors. EZH2 is an epigenetic modulator that is upregulated in SCLC[147] and inhibition of which suppresses lineage plasticity in prostate cancer[148]. EZH2 inhibitors are being evaluated as single agents or in combination in relapsed small cell lung cancer (NCT03460977). Whether transformed ALK$^+$ SCLC exhibits upregulation of EZH2 or other epigenetic modulators is unknown. Moreover, questions remain about appropriate timing of epigenetic therapy. If applied early in ‘at risk’ patients, it may block lineage changes and prevent transformation. If phenotypic changes are reversible, epigenetic therapies may be used at the time of transformation aimed at restoring TKI sensitivity.

Future directions

The treatment of ALK$^+$ lung cancer represents a paradigm of precision oncology, offering lessons on targeted therapies applicable across cancer types. Over a short timeframe since the discovery of the *EML4-ALK* fusion in NSCLC, tremendous strides have been made in the development of ALK-directed therapies, resulting in dramatic improvements in patient survival[50]. The field has exemplified how integration of bench and bedside investigations can uncover important molecular insights that translate into real-time clinical benefit for patients.

Yet metastatic ALK$^+$ lung cancer remains incurable, and many challenges remain in the treatment of this disease. Although genotyping of clinical samples at the time of relapse has informed resistance biology and led to development of later-generation ALK TKIs, it has also revealed increasingly TKI-refractory *ALK* resistance mutations and heterogeneous off-target ALK escape mechanisms. Elucidating the molecular drivers of resistance and continuing to develop innovative therapies to overcome resistance is critical. For instance, tumor microenvironment factors that modulate ALK TKI sensitivity remain to be elucidated. More work is needed to illuminate how manipulation of the microenvironment, including local immune cells and fibroblasts, could be exploited for therapeutic purposes.

Fundamental questions remain regarding how to stem the emergence of polyclonal resistance, eradicate persister cells and ultimately take the transformative step toward curing patients with metastatic disease. A singular ALK TKI or even a combination approach is unlikely to achieve this goal. An optimal treatment approach will require encompassing the following aspects: (1) the use of a pan-inhibitory ALK TKI upfront to block on-target resistance; (2) routine incorporation of orthogonal treatment modalities such as radiation, chemotherapy, epigenetic or immune-based strategies to eliminate residual disease; (3)

development of highly sensitive diagnostics to track tumor response and detect microscopic residual or recurrent disease; and (4) early implementation of rational combinations before the clinical evidence of relapse. Plasma monitoring to define the disease status remains investigational, and further studies are needed to clarify whether adaptive therapeutic escalation or de-escalation based on serial plasma monitoring may improve outcomes.

Furthermore, questions remain regarding the potential use of ALK TKIs in the adjuvant and neoadjuvant settings for early-stage ALK$^+$ lung cancer. The seminal phase 3 trial ADAURA demonstrated significantly longer disease-free survival in patients with surgically resected *EGFR*-mutant NSCLC who received the EGFR TKI osimertinib[149]. A phase 3 study of adjuvant alectinib versus chemotherapy in patients with resected ALK$^+$ NSCLC is ongoing (NCT03456076). In addition, the NAUTIKA1 phase 2 trial is evaluating various targeted therapies in the neoadjuvant setting for patients with early-stage resectable NSCLC harboring appropriate biomarkers, including alectinib for ALK$^+$ disease (NCT04302025). Results from these adjuvant and neoadjuvant studies will inform the optimal management of early-stage ALK$^+$ lung cancer.

A deeper understanding of the unique biology of ALK$^+$ lung cancer and continued therapeutic advances will ultimately catalyze waves of translational research efforts dedicated to the overarching goal of prolonging lives and inducing cures in patients with ALK$^+$ lung cancer.

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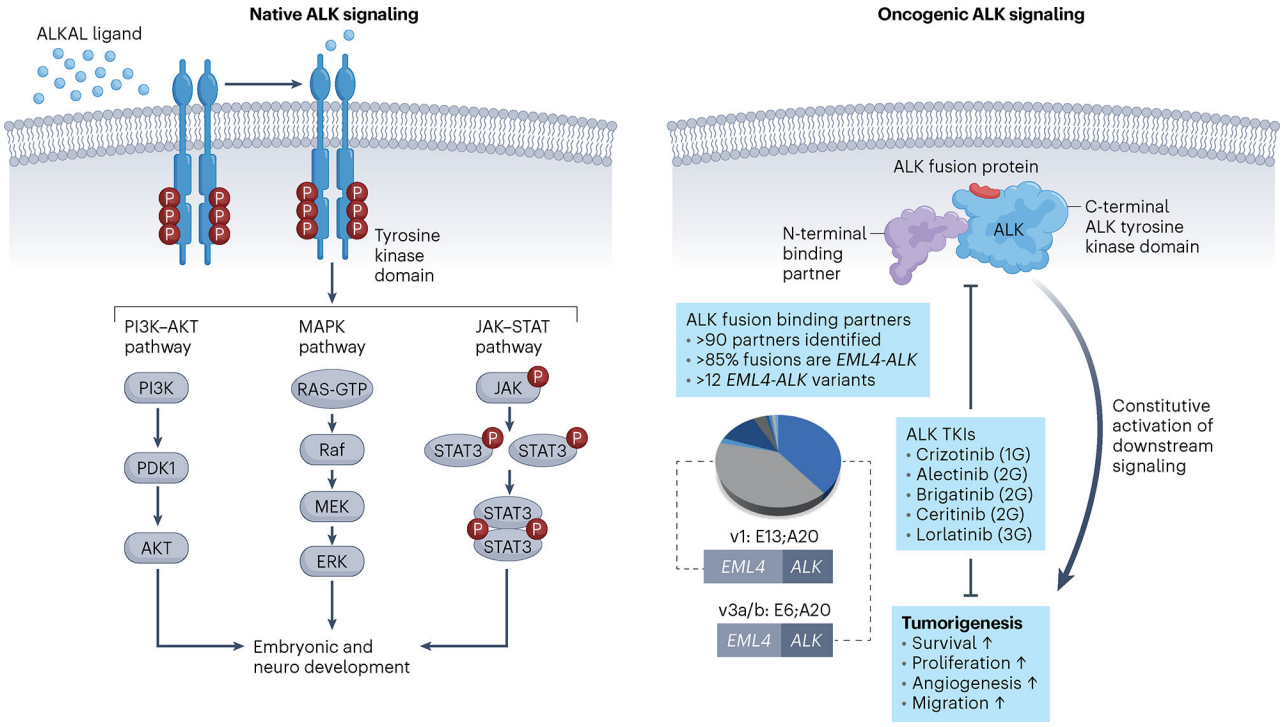

Fig. 1 |. Oncogenic ALK signaling.

Left, wild-type ALK is a plasma membrane-bound RTK that undergoes autophosphorylation upon ligand binding and receptor oligomerization. ALK activates downstream signaling pathways that contribute to organ development and homeostasis. Right, a chromosomal translocation leads to formation of an *ALK* fusion gene and translation of an ALK chimeric oncoprotein that is composed of the C-terminal kinase domain of ALK joined with various N-terminal, non-kinase fusion partners. Constitutive activation of ALK promotes cell survival pathways and tumorigenesis. Although many ALK binding partners have been elucidated across all tumor types, the EML4–ALK fusion is the most common, of which *EML4-ALK* variant 1 (E13;A20) and variant 3 (E6;A20) are the most prevalent in lung cancer. FDA-approved ALK TKIs and their generation are depicted. PDK1, pyruvate dehydrogenase kinase 1; v, variant; 1G, first generation; 2G, second generation; 3G, third generation.

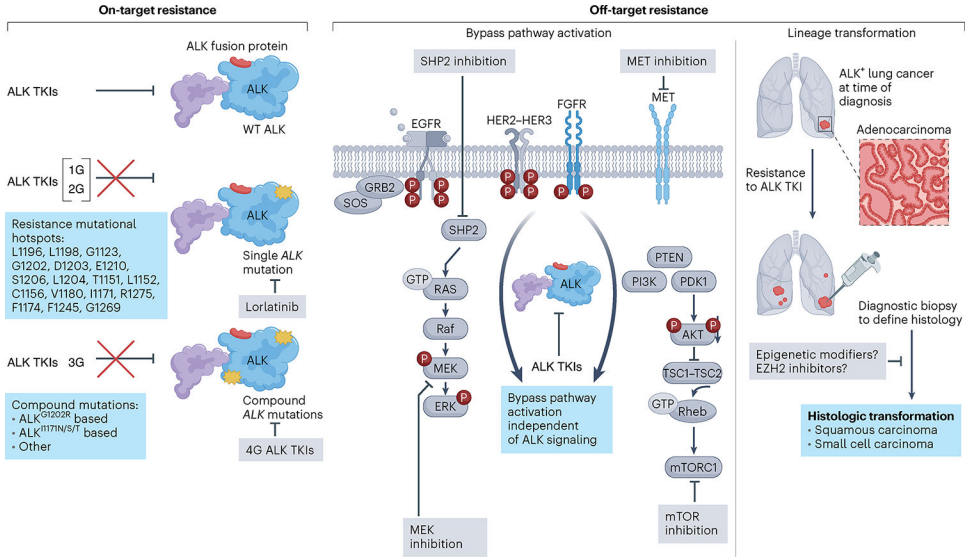

Fig. 2 |. Resistance in ALK$^+$ lung cancer and therapeutic interventions.

Resistance to ALK TKIs occurs through three main mechanisms. Left, on-target resistance is mediated by mutations in the ALK tyrosine kinase domain, which disrupt TKI binding to ALK, rendering tumor cells insensitive to ALK inhibition. ALK residues involved in ALK TKI resistance are listed. Single *ALK* mutations are most common after first- or second-generation ALK TKIs, while compound mutations are most common after sequential use of early-generation inhibitors culminating with a third-generation inhibitor, lorlatinib. This stepwise accumulation of *ALK* mutations confers resistance to ALK TKIs, with fourth-generation (4G) ALK TKIs designed to target compound mutations that are refractory to current FDA-approved ALK inhibitors. Middle, off-target resistance is mediated by bypass signaling activation or lineage transformation. Bypass pathway activation can occur through genetic mechanisms (amplifications, activation mutations, structural alterations) and non-genetic mechanisms (receptor hyperactivation), resulting in activation of signaling pathways that bypass ALK dependency. Rational combinations of ALK plus bypass pathway inhibition are being evaluated and are depicted in gray boxes. Right, lineage transformation is another off-target resistance mechanism that can lead to ALK TKI insensitivity. Diagnostic biopsies to define histology are necessary to select histology-specific chemotherapy regimens in squamous cell- or small cell-transformed tumors. Studies are underway to determine whether histologic changes are reversible and whether epigenetic modifiers may resensitize tumor cells to ALK inhibition. GRB2, growth factor receptor-bound protein 2; PTEN, phosphatase and tensin homolog; Rheb, Ras homolog enriched in brain; SOS, son of sevenless; SHP2, SH2 containing protein tyrosine phosphatase-2; TSC, tuberous sclerosis proteins 1 and 2; WT, wild type.

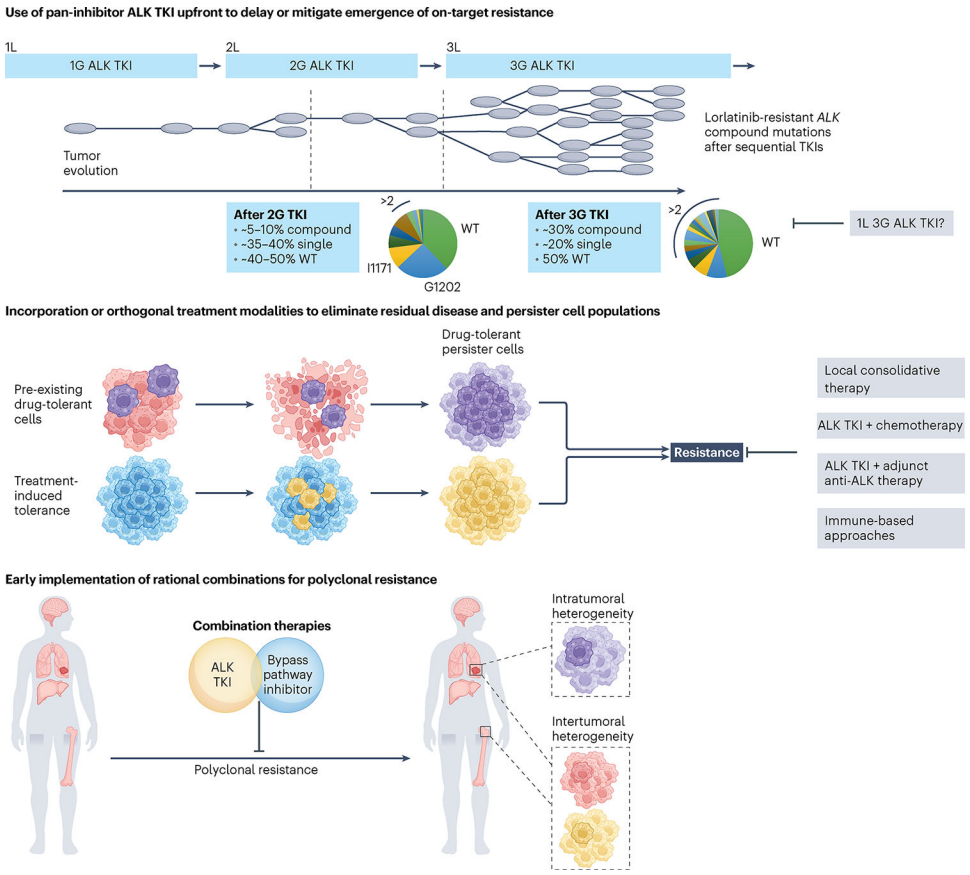

Fig. 3 |. Forward-looking treatment paradigms in advanced ALK^{+} lung cancer.

Top, sequential ALK TKI therapy culminating in lorlatinib induces compound *ALK* mutations, with ALK^{G1202R}- or ALK^{I1171N}-based compound mutations being the most common. The schematic depicts tumor clonal evolution with the multitude of single *ALK* mutations serving as a substrate for compound *ALK* mutations, highlighting the notion of stepwise accumulation of resistance mutations. Treatment with a highly potent pan-inhibitory third-generation ALK TKI in the first-line (1L) setting may allow for maximal cytoreduction and depth of response, limiting tumor heterogeneity that can emerge with less potent ALK TKIs. Middle, drug-tolerant cells that are present at the time of treatment may undergo expansion under therapeutic selective pressure, leading to treatment failure and clinical relapse. In parallel, persister cells that survive initial treatment may acquire de novo resistance alterations, serving as a nidus for the development of polyclonal resistance. Depicted in gray are potential adjunctive therapeutic strategies aimed at eliminating persister cells. Bottom, intratumoral heterogeneity can occur across different regions of the primary tumor and/or metastatic sites, with spatial heterogeneity represented by the presence of subclones with different genetic features. Intertumoral heterogeneity can also occur across different metastatic sites, which can be missed using single-site tissue biopsies. Studies are underway to evaluate the utility of early rational combinations and to stem polyclonal resistance. In parallel, efforts are ongoing to develop ultrasensitive diagnostic tools to track tumor response and detect microscopic disease. 2L, second line; 3L, third line.

Table 1 |

Summary of ALK inhibitors approved by the FDA or in clinical testing

ALK TKI	TKI generation	Status	Trial name[a] (phase)	Comparator	*n*	Median PFS[b], months (HR, 95% CI)	ORR, % (95% CI)	IC-ORR[c], % (95% CI)	*ALK* mutations refractory to TKI	References
Crizotinib	1G	Approved for 1L and beyond	PROFILE 1014 (phase 3)	Chemotherapy (platinum-based doublet)	343	10.9 versus 7.0 (0.45, 0.35–0.60)	74% (67–81%) versus 45% (37–53%)	N/A	L1196M G1202R I1171T/N/S G1269A/S S1206Y I1151Tins L1152P/R C1156Y/T F1174C/L/V V1180L S1206C/Y E1210K	4,5,43,60
Ceritinib	2G	Approved for 1L and beyond	ASCEND-4 (phase 3)	Chemotherapy (platinum-based doublet)	376	16.6 versus 8.1 (0.55, 0.42–0.73)	73% (66–79%) versus 27% (21–34%)	73% (50–89%) versus 27% (11–50%)	G1202R/del I1151Tins L1152P/R C1156Y/T F1174C/L/V D1203K G1269A	6,45
Alectinib	2G	Approved for 1L and beyond	ALEX (phase 3)	Crizotinib	303	25.7 versus 10.4 (0.50, 0.3–0.70)	83% (76–89%) versus 76% (68–82%) (INV)	81% (58–95%) versus 50% (28–72%) (INV)	G1202R/del V1180L I1171T/N/S L1196M	7,45,49-51
Brigatinib	2G	Approved for 1L and beyond	ALTA-1L (phase 3)	Crizotinib	275	24.0 versus 11.1 (0.48, 0.35–0.66)	74% (66–82%) versus 62% (54–70%)	78% (52–94%) versus 26% (10–48%)	G1202R/del E1210K	8,45-47,90
Ensartinib	2G	Phase 3	eXalt3 (phase 3)	Crizotinib	290	25.8 versus 12.7 (0.51, 0.35–0.72)	74% (66–81%) versus 67% (58–74%)	64% versus 21%	G1269A G1202R/del E1210K	48,90
Lorlatinib	3G	Approved for 1L and beyond	CROWN (phase 3)	Crizotinib	296	NR versus 9.3 (0.27, 0.18–0.39)	76% (68–83%) versus 58% (49–66%)	82% (57–96%) versus 23% (5–54%)	C1156Y + L1198F I1171N + L1198F G1202R + F1174L G1202R + L1196M D1203N + L1196M G1202R + S1206Y G1202R + C1156Y G1202R + G1269A C1156Y + G1269A I1171N/T + D1203N G1202R + G1269A G1202R + L1204V +	9,36,52-54,65,68

ALK TKI	TKI generation	Status	Trial name[a] (phase)	Comparator	*n*	Median PFS[b], months (HR, 95% CI)	ORR, % (95% CI)	IC-ORR[c], % (95% CI)	*ALK* mutations refractory to TKI	References
									G1269A G1202R + S1206F + G1269A D1203N + E1210K + G1269A	
TPX-0131	4G	Phases 1–2	FORGE-1 (NCT04849273)	None	N/A	N/A	N/A	N/A	N/A	97
NVL-655	4G	Phases 1–2	ALKOVE-1 (NCT05384626)	None	N/A	N/A	N/A	N/A	N/A	98

[a]Seminal global randomized phase 3 trials are listed for the FDA-approved agents and for ensartinib, which is approved as first-line treatment in China.

[b]Median PFS according to blinded independent review committee assessment is shown.

[c]Intracranial response rates in patients with baseline measurable brain metastases are shown, according to blinded independent review committee assessment unless indicated otherwise. CI, confidence interval; HR, hazard ratio; IC-ORR, intracranial ORR; INV, per-investigator assessment; NR, not reached; N/A, not available.

Table 2 |

Summary of ongoing trials with combination therapy in ALK+ NSCLC

Clinical trial identifier	Drugs		Bypass pathway targeted	Phase	Biomarker required outside of ALK
	ALK TKI	Other agent			
NCT02321501	Ceritinib	Everolimus	mTOR	1	No
NCT03202940	Alectinib	Cobimetinib	MAPK (MEK)	1–2	No
NCT04005144	Brigatinib	Binimetinib	MAPK (MEK)	1	No
NCT04227028	Brigatinib	Bevacizumab	VEGF	1	No
NCT04292119	Lorlatinib	Binimetinib	MAPK (MEK)	1–2	No
		Crizotinib	MET		Yes (*MET* amplification)
		TNO155	SHP2		No
NCT04800822	Lorlatinib	PF-07284892	SHP2	1	No

mTOR, mammalian target of rapamycin; VEGF, vascular endothelial growth factor.