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Targeting Infrequent Driver Alterations in Non-Small Cell Lung Cancer

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Abstract

The discovery of oncogenic driver mutations led to the development of targeted therapies with non-small cell lung cancer (NSCLC) being a paradigm for precision medicine in this setting. Nowadays, the number of clinical trials focusing on targeted therapies for uncommon drivers is growing exponentially, emphasizing the medical need for these patients. Unfortunately, similar to what is observed with most targeted therapies directed against a driver oncogene, the clinical response is almost always temporary and acquired resistance to these drugs invariably emerges. Here, we review the biology of infrequent genomic actionable alterations in NSCLC as well as the current and emerging therapeutic options for these patients. Mechanisms leading to acquired drug resistance and future challenges in the field are also discussed.

Glossary

Epithelial to mesenchymal transition (EMT): process by which epithelial cells lose their epithelial phenotype and gain mesenchymal properties.

MAPK signaling pathway: signaling cascade (RAS-RAF-MEK-ERK) that mediates signals from cell surface receptors regulating cell growth, differentiation and survival.

Median progression-free survival (mPFS): length of time during and after the treatment that a patient lives with the disease and does not get worse.

Non-small cell lung cancer (NSCLC): major subtype of lung cancer (accounts for 80-90% of all lung cancers) comprising both squamous neoplasms and adenocarcinoma (LUAD).

Objective/overall response rate (ORR): proportion of patients in a trial whose tumor is significantly reduced by a drug.

The challenge of targeted therapy in NSCLC driven by uncommon genetic alterations

The expanding spectrum of oncogenic driver mutations and clinically available signaling pathway inhibitors had a major impact on **non-small cell lung cancer (NSCLC)** (see Glossary) patients management in the last decade [1]. Cancer driver mutations not only initiate the disease but also sustain tumor progression and therefore their inhibition results in a therapeutic benefit. Driver oncogenes are often identified due to their recurrence in patients, a complicated feature when they appear with low prevalence as those covered in

this review. Yet the oncogenic nature and the addiction of cancer cells to this set of infrequent drivers have been clearly established as well as their mutually exclusive pattern with other oncogenes. With the exception of BRAF, all belong to the receptor tyrosine kinase (RTK) family and when in the oncogenic form constantly activate essential downstream signaling pathways, including the **MAPK** (see Glossary), PI3K and JNK, resulting in sustained growth, increased survival and enhanced dissemination. Recently, Food and Drug Administration (FDA) has approved specific inhibitors targeting infrequent NSCLC oncogenic drivers (*ROS1*, *MET*, *RET*, *BRAF*, and *NTRK*). Beyond those, promising preliminary data with compounds targeting other emerging driver alterations (e.g. *HER2*) have been reported [2]. Used as first or latter lines of treatment (after standard chemotherapy and/or immunotherapy), these targeted therapies have greatly increased patients' outcome [3-9]. Unfortunately, as anticipated by the lessons learned from the clinical management of *EGFR*-mutated and *ALK*-rearranged LUAD (**Box 1**), therapeutic resistance eventually appears in most patients and, therefore, advanced NSCLC remains largely incurable (for review see [10]). In the present review, we describe the biological relevance and the therapeutic options of a series of infrequent drivers for which clinical treatments have been approved or are under evaluation. Likewise, the clinical management of on-target resistance is discussed per driver while that due to bypass signaling is collectively addressed given its pan-driver conservation.

ROS1

Biological and clinical features

ROS1 is a conserved orphan RTK initially identified in glioblastoma and was the first chromosomal rearrangement described in NSCLC [11]. Since then, several *ROS1* breakpoints have been described involving the entire *ROS1* kinase domain and accounting for 1-2% of NSCLC. Twenty fusion partners (e.g. *SLC34A2*, *EZR* and *SDC4*) have been reported, among which *CD74-ROS1* is the most common [12-14]. Whether distinct partners are functionally equivalent is unknown, although *in vitro* data indicates that they impose changes in subcellular localization affecting signaling and oncogenic properties [15]. Unlike *ALK* rearrangements, most of the known *ROS1* fusion partners lack constitutive dimerization domains and the mechanism of *ROS1* oncogenic activation remains unknown. Along with *RET* and *NTRK*, *ROS1* rearrangements are associated with younger age at diagnosis, none or

low tobacco exposure and generally have adenocarcinoma histology [16, 17]. Diagnosis of ROS1 fusions by immunohistochemistry (IHC) is the most cost-effective method requiring confirmation by break-apart probe fluorescent *in situ* hybridization (FISH) or hybrid RNA-based next-generation sequencing (NGS) if the RNA quality is optimal [18, 19].

Frontline targeted therapy

ROS1-positive lung cancer seems particularly sensitive to pemetrexed-based chemotherapy [20]. The ALK inhibitor crizotinib was supposed to efficiently target ROS1-rearranged tumors given that ROS1 and ALK belong to the insulin receptor superfamily [21] sharing >80% of amino acid sequence within their ATP-binding sites. Furthermore, crizotinib binds with high affinity to both kinases and inhibiting cell line proliferation. Indeed, this hypothesis was corroborated in the clinic with crizotinib showing marked therapeutic effect in patients with ROS1-rearranged NSCLC [22]. Accordingly, crizotinib, first approved for the treatment of ALK-rearranged NSCLC, received FDA and European Medicines Agency (EMA) expanded approval in 2016 for ROS1-rearranged NSCLC [22-26] (**Figure 1 and Table 1**). Similarly, in 2020, entrectinib has obtained FDA and EMA approval in this tumor type [8, 27]. In addition, other kinase inhibitors including ceritinib and lorlatinib have been evaluated in ROS1-positive crizotinib naïve NSCLC [28, 29] (**Figure 1 and Table 1**).

Addressing on-target mechanisms of resistance

The accumulated knowledge after a decade of using RTKi for the treatment of ALK- and EGFR-mutant LUAD (**Box 1**) anticipated that the selection of on-target mutations within the kinase domain would be the main mechanism of acquired resistance in ROS1 and the rest of infrequent drivers covered in this review. Indeed, several mutations have been reported affecting the solvent-front, the gatekeeper residue, the activation loop or the DFG motifs and all are predicted to prevent drug binding due to steric hindrance. **Figure 2A** summarizes the different on-target mutations identified and their functional analogy among the different drivers together with ALK and EGFR for comparative purposes.

Fortunately, also guided by the successful clinical management of resistant disease in EGFR- and ALK-mutant patients (**Box 1**), new generation inhibitors have been identified or purposely designed to combat drug resistance. **Figure 2B** summarizes pre-clinical and clinical data regarding the sensitivities of the most common secondary mutations to a panel of inhibitors for each driver. In the case of ROS1, resistance profiling studies using both *in vitro*

and *in vivo* models demonstrated that cabozantinib (and its structural analog foretinib) as well as DS-6051b (a novel ATP-competitive ROS1/NTRK inhibitor) markedly inhibited the growth of *ROS1* G2032R mutants [30-35]. Indeed, several case reports of *ROS1*-rearranged LUAD patients resistant to crizotinib and ceritinib reported responses to cabozantinib [36, 37]. With similar efficacy, DS-6051b had better toxicity profile [38] and two ongoing clinical trials are evaluating its efficacy in *ROS1*-rearranged NSCLC^{i,ii}. In addition, repotrectinib (TPX-0005), a selective and highly potent TKI against ROS1, NTRK, and ALK, demonstrated potent anti-proliferative activity against the G2032R and D2033N *ROS1* mutations in cellular inhibitory assays and xenografts [39]. This has been tentatively confirmed in a clinical setting (TRIDENT-1, NCT03093116) reporting that repotrectinib achieved tumor regression in 45% of patients with *ROS1*-positive NSCLC resistant to prior TKI(s) [40, 41]. Interestingly, not all on-target mutations are equally sensitive to new generation inhibitors and patient stratification will be required. Indeed, each TKI is structurally distinct with varying degrees of activity against the different resistance mutations, giving them unique activity profiles. This is illustrated by lorlatinib that demonstrated promising activity in 40 patients with *ROS1*-positive NSCLC who progressed to crizotinib with an **overall response rate (ORR**, see Glossary) of 35% and a **median progression-free survival (mPFS**, see Glossary) of 8.5 months. While patients harboring K1991Q and S1986F *ROS1* mutations achieved durable responses to lorlatinib, none of the patients with the more frequent G2032R mutation did benefit [29].

NTRK

Biological and clinical features

The Neurotrophic Tropomyosin Receptor Kinase (NTRK) genes (*NTRK1*, 2 and 3) encode TRKA, TRKB and TRKC, respectively. They are activated by a family of four ligands (NGF, BDNF, NT-3 and NT-4) known as neurotrophins that display different affinities for each RTK and play essential roles in the development and function of the nervous system. Activating mutations in *NTRK* have been identified in NSCLC, yet their oncogenic role remains controversial [42]. Similarly, activating splice variants and overexpression have been reported in pan-cancer studies but, undoubtedly, the most frequent aberrations involve intra- and inter-chromosomal gene-fusions implicating *NTRK* that occur at a frequency below 0.4% in NSCLC. The biology of *NTRK* oncogenes has been comprehensively reviewed

elsewhere [43, 44]. Of note, a *NTRK* fusion was the first non-*RAS* oncogene identified almost 40 years ago (indicated as *OncD* in [45]; for review see [46]). Of the three *NTRK*, pan-cancer-related fusions more commonly affect *NTRK1* and *NTRK3*, and 48 different partners have been reported [47]. In lung, the fusions involve *NTRK1* and *NTRK3* with five and two distinct partners respectively, being *ETV6-NTRK3* the most prevalent [47, 48]. The NGS-based FoundationOneCDx test has just received FDA-approval for the identification of *NTRK* fusions.

Frontline targeted therapy

Early phase basket trials of solid tumors harboring *NTRK* gene rearrangements were performed with first-generation TKIs such as larotrectinib, which is selective for TRKA/B/C, and entrectinib, which targets TRKA/B/C as well as ALK and ROS1. Both compounds have demonstrated marked tumor-agnostic efficacy in pan cancer trials [6, 7] (**Figure 1 and Table 1**). Based on these results larotrectinib received accelerated FDA approval for the treatment of TRK fusion-positive cancers in 2018 followed by entrectinib one year later. Both drugs also obtained histology-independent approval in solid tumors harboring *NTRK* rearrangements by EMA. Ongoing trials may help to better estimate efficacy and duration of response in NSCLC.

Addressing on-target mechanisms of resistance

Next-generation TRK inhibitors, such as selitrectinib (LOXO-195) and repotrectinib (TPX-0005), have been designed to combat on-target acquired resistance mutations and their activity is being evaluated in phase I/II trials or compassionate use protocols for *NTRK*-altered cancers. Indeed, selitrectinib was developed as a second-generation TRK inhibitor using modeling studies even before acquired resistance appeared in the clinic. An initial evaluation performed on a first-in-human basis proved its activity against solvent-front and xDFG mutants inducing rapid tumor responses [49]. This has been extended in an ongoing phase I/II trial upon enrolling patients with prior treatment with *NTRK* inhibitorsⁱⁱⁱ. Preliminary results indicate an ORR of 45% in patients with mutations affecting the kinase domain [50]. Similarly, repotrectinib evaluation in a phase I/II first-in-human dose-escalation study (NCT03093116) demonstrated efficacy in patients harboring *NTRK3* solvent front resistant mutations [39]. Whether these next-generation TRK inhibitors will exert a similar benefit in resistant LUAD patients with kinase-domain acquired mutations is still unknown.

RET

Biological and clinical features

Unlike other RTKs, RET (REarranged during Transfection) does not directly bind to its ligands but requires an additional co-receptor. Indeed, ligands of the glial cell line-derived neurotrophic factor (GDNF) family bind to one of the four receptors of the GDNF family receptor- α (GFR α), which subsequently allow RET dimerization, autophosphorylation and activation [51]. Despite a few studies reporting concomitant genomic alterations (for review see [52]), *RET* rearrangements are considered mutually exclusive with other alterations in NSCLC indicating an independent carcinogenic role and have been identified in 1-2% of cases. Chromosomal inversions or translocations involving *RET* results in a juxtaposition of its kinase domain and the coiled coil domain of the partner. Similar to the oncogenic activation of ALK fusions, this coiled coil domain is responsible of the ligand-independent homodimerization and constitutive RET activation [53]. In NSCLC, up to 12 fusion partners (e.g. *CCDC6*, *NCOA4*, *TRIM33*) have been described with the most frequent being *KIF5B* [54]. Unlike *ALK* and *ROS1* rearrangements, *RET* fusion genes cannot be adequately detected by IHC due to low sensitivity and highly variable specificity [52]. Therefore, NGS is the most accurate option as FISH and RT-PCR may lead to false negatives due to technical limitations.

Frontline targeted therapy

Similar to *ALK* and *ROS1*-positive tumors, durable benefits have been obtained with pemetrexed-based chemotherapy in *RET*-rearranged NSCLC [55]. Considering the structural similarities of both the kinase domain and the ATP-binding site with other RTKs [56], several multi-kinase inhibitors (MKIs; e.g. vandetanib, cabozantinib, lenvatinib as well as alectinib, a VEGFR-sparing MKI) were initially used for the treatment of patients bearing *RET* fusions (for review see [52, 57, 58]) (**Figure 1 and Table 1**). Recently, more specific and potent RET inhibitors are under investigation. These include pralsetinib (BLU-667) and selpercatinib (LOXO-292), which have received FDA approval for *RET*-altered NSCLC [4, 5, 59-61] (**Figure 1 and Table 1**).

Treatment evolution of *RET*-driven disease is an illustrative example of the continuous need to design specific and potent inhibitors. New putative oncogenic fusions are being discovered [62, 63] that could also require initial treatment with less-efficient MKIs in parallel to the development of specific compounds.

Addressing on-target mechanisms of resistance

The differential response of various on target mutants has also been reported in *RET*-altered tumors. Whereas most MKIs are ineffective against resistant mutations, both ponatinib [64] and AD80 [65] showed pre-clinical efficacy against specific mutations (V804 and G810). Whether this may provide clinical benefit is under evaluation for ponatinib^{iv}. In addition, while pralsetinib displayed inhibitor activity against V804M/L and S904F mutations but failed to inhibit G810 alterations [60], TPX-0046, a structurally different RET/SRC inhibitor, showed potent *in vitro* and *in vivo* activity against the *RET* solvent-front mutation G810R and is currently tested in a phase I/II trial^v.

MET

Biological and clinical features

In NSCLC, c-Mesenchymal-Epithelial Transition factor (c-MET) gene rearrangements, fusions and somatic mutations have been identified but are rare [66]. MET overexpression, however, is frequent in unselected NSCLC patients although its clinical value is unclear as this criterion is largely dependent on the antibody assay and the determination of a positive threshold. *MET* amplification is also a common mechanism of resistance to EGFR-TKIs in NSCLC. Anyhow, increased expression is considered a causative driver only if co-occurring with clinically relevant *MET* genomic alterations like *MET* gene amplification or mutations resulting in exon 14 skipping (*METΔ14*). This alteration results in a MET protein lacking the juxtamembrane domain with extended half-life after ligand stimulation and is considered an oncogenic driver. *METΔ14* is predominantly associated to sarcomatoid carcinoma histology [67, 68] and detected in 2-4% of NSCLC patients while *MET* amplification affects approximately 2% of LUAD.

Several MET inhibitors, including TKIs and monoclonal antibodies against MET or its ligand HGF have been assessed in NSCLC (for review see [69, 70]). Various agents have reached phase III trials and failed in patients with *MET* alterations, illustrating the need for better stratification upfront. Indeed, a significant fraction of patients display co-existing oncogenes and only a small fraction of highly amplified tumors may actually be *bona-fide* MET-addicted cases, which are associated to more effective responses to targeted therapies [71].

Frontline targeted therapy

Crizotinib received FDA approval for *ALK* and *ROS1* NSCLC patients but was initially developed as a MET inhibitor [72]. Significant clinical activity was reported in patients harboring *METΔ14* alterations including complete responses. Yet, the ORR of crizotinib in this cohort was lower compared to those achieved with targeted therapies for other NSCLC drivers [73] (**Figure 1 and Table 1**). Neither variations in the mutation type or splice-site region nor the *MET* copy number appear to be major differentiating response factors, so the underlying causes of the heterogeneous clinical outcome are unknown. Novel and highly selective type Ib MET inhibitors such as tepotinib and capmatinib have been evaluated in phase II trials [9, 74] (**Figure 1 and Table 1**). In general, *METΔ14* tumors display lower initial response rates to TKIs compared to other RTK drivers in NSCLC. This could be due to high rate of co-existing genomic alterations inducing both MAPK and PI3K pathway activation resulting in innate/primary resistance [75, 76].

Addressing on-target mechanisms of resistance

In the case of MET-resistant disease, mutations like those affecting the Y1230 and D1228 residues may predict response to type II MET inhibitors such as cabozantinib and glesatinib [77]. Others, including substitutions implicating L1195 or F1200, may not be sensitive to treatment with these compounds as they preclude binding to the DFG-out pocket [75]. In a similar study, 7 out of 20 (35%) *METΔ14* patients who progressed to MET TKI had on-target kinase domain mutations (H1094, G1163, L1195, D1228, Y1230) or elevated *MET* amplification. Cabozantinib has also been shown to be active against the D1228V mutation with concomitant MET amplification [78].

HER2/ERBB2

Biological and clinical features

Human epidermal growth factor receptor 2 (HER2), also called ERBB2, is one of the four members of the EGFR family. It remains an orphan receptor activated by heterodimerization with other ERBB family members (for review see[79]). In NSCLC, HER2 dysregulations can be caused by gene amplification, protein overexpression and mutations. Despite the efficacy of anti-HER2 agents in breast and gastric cancer, clinical interest on HER2-positive NSCLC was dampened when the first clinical trials evaluating trastuzumab, an anti-HER2 humanized antibody reported negative results [80, 81]. In parallel, HER2 kinase domain mutations were

identified in NSCLC patients not associated with HER2 amplification/overexpression [82, 83]. These results were further confirmed [84] indicating that *HER2* amplification and mutations represent two distinct oncogenic drivers and potential therapeutic targets. These findings, coupled with the encouraging results of TKIs in EGFR mutants, launched the clinical evaluation of anti-HER2 agents specifically in NSCLC patients bearing *HER2* mutations (90 % of them are located in exon 20). *HER2* exon 20 alterations comprise point mutations (e.g. L755S, V777L and G776C) or, more frequently, insertions [85]. The most prevalent (over 80%) is a 12 base pair in-frame insertion causing duplication of amino acids YVMA at codon 775 (A775_G776insYVMA) [86]. Mutations in exon 20 are analogous to *EGFR* exon 20 mutations and lead to HER2 constitutive activation [87-89]. Overall, *HER2* mutations account for 2-4% of the NSCLC cases and tend to be women and light/never smokers with LUAD histology [85].

Frontline targeted therapy

HER2 targeted therapy is not yet standard of care in NSCLC and, currently, chemotherapies remain the main strategy. Patients with *HER2*-mutant LUAD, especially YVMA insertion, demonstrated a markedly inferior response to first line pemetrexed-based chemotherapy compared to those harboring *ALK/ROS1* rearrangements, which strengthen the need for effective anti-HER2 agents [90]. Retrospectives studies displayed encouraging disease control rates in NSCLC patients bearing *HER2* exon 20 insertions treated with HER2-targeted drugs [91, 92]. Several clinical studies have been carried out or are ongoing using both anti-HER2 antibodies and small molecules (for review see [2]) (**Figure 1 and Table 1**). Among these compounds, poziotinib, a pan-HER TKI, has structural features that can circumvent the steric changes induced by exon 20 insertions and increase its affinity compared to larger TKIs [93]. Next-generation TKIs with increased selectivity towards the EGFR and HER2 mutants (such as TAK-788) are being developed to limit the toxicity associated to wild-type EGFR inhibition [94, 95]. In addition, trastuzumab and its antibody-drug conjugate, trastuzumab-emtansine (T-DM1), showed mixed results in the clinic due in part to the molecular heterogeneity of *HER2*-altered NSCLC (for review see [2]). Trastuzumab-deruxtecan (DS-8201a) showed promising efficacy among NSCLC patients with HER2 exon 20 mutations [96] (**Figure 1 and Table 1**). In this context, patients with *HER2*-mutated NSCLC often develop brain metastases with disease progression and trastuzumab would be inefficient due to its

inability to cross the blood-brain barrier. Therefore, monitoring and treatment of CNS metastases may require specific approaches.

BRAF

Biological and clinical features

BRAF, together with ARAF and CRAF, constitute the RAF family of serine/threonine kinases, a core component of the MAPK cascade. Since the first evidence in 2002 [97], almost 200 distinct *BRAF* mutations have been reported in around 8% of cancers. In NSCLC, they represent 1.5-3.5% of the cases and affect the kinase domain, either in the glycine-rich loop in exon 11 or in the activation segment in exon 15, resulting in increased ERK signaling (for review see [98]). Clinical characteristics associated with *BRAF* mutations in lung cancer vary without obvious segregation in specific parameters, except the significant association with LUAD histology. Most patients with *BRAF* mutations are smokers, although patients with V600 mutations are more likely to be light/never-smokers compared with patients with non-V600 mutations [99-102]. *BRAF* mutations as well as *HER2* and *MET* alterations are generally well covered by NGS gene panels. *BRAF* mutants are classified in three categories [103]. Class 1 (V600) and class 2 (G464, G469, L597 and K601) mutants are mutually exclusive, RAS-independent and signal constitutively as active monomers or active dimers, respectively. Class 3 (e.g. G466 and D594) have impaired/null kinase activity and activation of downstream signaling is RAS- and CRAF-dependent and sensitive to ERK-mediated feedback. In this case, RAS is generally activated by RTK signaling [103]. Notably, expression of an endogenous class 3 BRAF mutant triggers LUAD in mice, indicating that kinase-inactive BRAF isoforms are oncogenic drivers [104].

Frontline targeted therapy

Experience with *BRAF*-mutant melanoma has heavily influenced the management of NSCLC. Targeted therapy has evolved from BRAF monotherapy to combined BRAF/MEK inhibition showing superior efficacy and favorable safety profile in *BRAF* V600E-mutant NSCLC [3, 105-109] (**Figure 1 and Table 1**). The combination of dabrafenib plus trametinib has received both FDA and EMA approval for the treatment of metastatic NSCLC harboring the *BRAF* V600E mutation regardless of previous therapies. Another pair of BRAF and MEK inhibitors,

encorafenib and binimetinib, is under investigation and has shown encouraging CNS activity in a patient with *BRAF* V600E-mutated LUAD [110].

Next-generation RAF inhibitors have been reported to be active in *BRAF*-mutant NSCLC in the clinic (for review see [111]). Notably, the pan-RAF inhibitor PLX8394 selectively disrupts both BRAF homo- and BRAF-CRAF heterodimers [112, 113]. Furthermore, a phase I study evaluating ulixertinib, a first-in-class ERK inhibitor, reported partial responses in NSCLC patients bearing both V600 and non-V600 mutations as well as CNS activity [114]. In addition, *BRAF*-mutant NSCLC is likely to have high level of PD-L1 expression [115]. When treated with immune checkpoint inhibitors, no significant differences in ORR and mPFS were reported among V600E and non-V600E mutants. The optimal therapeutic sequence has yet to be established in both V600 and non-V600 (**Box 2**) *BRAF*-mutated LUAD patients.

Signaling bypass activation and other resistance mechanisms

In accordance with *EGFR*- and *ALK*-driven NSCLC (**Box 1**), the development of resistance to targeted therapies in lung cancer bearing infrequent alterations also implicates signaling bypass tracks, although the underlying molecular mechanisms are less understood. The activation of bypass pathways described so far in both preclinical and clinical studies are depicted for each uncommon driver in **Figure 3**.

Alterations affecting the RAS-MAPK pathway or upstream receptors are the most frequent. Notably, *EGFR* [116-119] and *HER2* [120, 121] activating mutations or amplification have been reported in *ROS1*- and *MET*-altered patients after progressing to crizotinib. Interestingly, EGFR activation may not only contribute to the resistant phenotype by supporting bypass signaling but also by protein-protein interactions that reactivate drug-inhibited ROS1 [118]. EGFR signaling has also been identified as a critical adaptive survival mechanism to RET [118, 122, 123] and NTRK [118] blockade in NSCLC preclinical models. Beside EGFR and HER2, other RTKs have been associated to drug resistance in lung cancer. Particularly, *MET* amplification has been identified in afatinib-resistance cell lines harboring *HER2* alterations [124], in pralsetinib resistant *RET*-rearranged tumors [125] and in entrectinib resistant *NTRK*-rearranged biopsies [126]. Furthermore, FGFR autocrine pathway activation through FGF1 secretion was shown to be associated with increased sensitivity to FGFR inhibitor in resistant *BRAF* V600E LUAD [127].

In addition, *KRAS* and *NRAS* mutations on codons 12 and 61 have been identified both in preclinical models of resistance to MKIs in *RET*-altered cells [123] and in *MET*, *NTRK* and *BRAF*-altered patients after progressing to frontline targeted therapies [126, 128-132]. Similarly, *MEK1* activating mutations were described in case reports of *BRAF* V600E NSCLC after progression to dabrafenib and trametinib [133] as well as in *NTRK*-rearranged patients after TKIs treatment [126]. Furthermore, case-reports highlighted *BRAF* V600E mutation or amplification in post-treatment biopsies of *ROS1*, *NTRK* and *METΔ14*-altered NSCLC patients [126, 129, 134, 135]. Intriguingly, RTK-RAS-MAPK activating mutations tend to be mutually exclusive in primary tumors, a finding justified due to functional redundancy and lack of selective advantage. However, several studies have demonstrated that synthetic toxicity due to excessive signaling could equally underlie oncogene incompatibility [136-138]. In this context, intermittent therapy resulting in excessive signaling proves to be advantageous in drug resistant melanoma harboring co-existing oncogenes [139]. Yet, in NSCLC, this approach could only be applied to certain oncogenic combinations [140]. Additional clinical support from both prospective and retrospective studies is required to validate this therapeutic strategy.

Another frequently altered pathway upon TKI treatment is PI3K-AKT-mTOR. Indeed, activating mutations of *PIK3CA* have been reported in both *ROS1*-altered [135, 141] and *HER2* exon 20-mutated patients [142] after progression to targeted therapies.

Finally, other alterations potentially contributing to drug resistance have been reported such as *KIT* [143] and *c-myc* [144] in preclinical models of *ROS1* and *MET*-altered NSCLC, respectively. Additionally, acquired amplification of *MDM2*, which encodes the E3 ubiquitin-protein ligase MDM2, has been identified in *RET*-rearranged cabozantinib-resistant patients [145]. Likewise, the Hippo pathway with its effector YAP has been recognized as a mechanism of resistance to *BRAF* and *MEK* targeted therapy in both preclinical and clinical samples bearing *BRAF* V600 mutations [146]. Yet, given their low prevalence, their clinical significance requires further confirmation.

Importantly, the activation of bypass signaling pathways often results in the acquisition of vulnerabilities that could be therapeutically exploited. In a recent study, novel activating MAPK alterations (deletions of several nucleotides in *MAP3K1* and *MAP2K1* as well as *NF1* loss-of-function mutations) were acquired in patients harboring *ROS1*-rearranged tumors being treated with *ROS1* inhibitors. Cells bearing these alterations are resistant to *ROS1*-TKIs

but sensitive to a combination of ROS1 and MEK inhibitors *in vitro* and *in vivo* [147]. Importantly, a *MET* Δ 14-altered patient who acquired a *KRAS* gain after crizotinib treatment achieved clinical response upon addition of trametinib [75].

The identification of *PIK3CA* or *PTEN* mutations suggests that activation of the PI3K-AKT-mTOR pathway could represent an acquired vulnerability and supports the potential use of mTOR inhibitors for the treatment of resistant NSCLC. In a phase I clinical trial, a *BRAF* V600E-mutated NSCLC patient who progressed to single-agent dabrafenib and multiple lines of chemotherapy achieved a partial response with the combination vemurafenib plus everolimus, a mTOR inhibitor [148]. Preliminary results from a clinical trial (NCT01582191) combining vandetanib and the mTOR inhibitor everolimus showed significant activity in *RET*-positive NSCLC [149] as well as CNS responses [150]. Overall, combined inhibition of uncommon drivers and bypass signaling pathway (either with mTOR inhibitors or MEK inhibitors) could be a promising therapeutic strategy that should be assessed in resistant patients lacking on-target resistance mutations.

Finally, phenotypic changes such as **epithelial to mesenchymal transition (EMT)** (see Glossary) has been reported in one ROS1 crizotinib-resistant patient lacking resistant *ROS1* mutations or alternative pathway mutations [117]. EMT along with acquisition of cancer stem cell-like properties have been identified in both TKI resistant *MET*- and *HER2*-altered cell lines [124, 151]. No cases of histological transformation to a small-cell lung cancer (SCLC), a phenomenon observed in EGFR and ALK TKI-resistant tumors, has been reported so far in the context of uncommon drivers.

Concluding remarks and future perspectives

Alongside *EGFR*- and *ALK*-driven NSCLC, patients bearing infrequent drivers display limited benefit from targeted therapies due to the onset of resistance. Despite the growing body of evidence about their clinical management many key knowledge gaps remain (see Outstanding Questions). First, lung cancers genomes display substantial heterogeneity and complex branching evolution that can be influenced by treatment-imposed selective pressure [152-154]. The understanding of the subclonal origin of resistance in the context of rare drivers will require deep sequencing studies of either multiregional biopsies or single tumor cells that are currently missing. This information could help develop tailored treatments to tackle resistance by anticipating the emergence of on-target or bypass

mechanisms. Interestingly, recent preclinical evidence has demonstrated that enhanced therapeutic benefit and delayed onset of resistance upon multiple low dosing of different nodes within the same pathway [155]. Beside targeted therapies, these patients may benefit from combining chemotherapy and immunotherapy, but further information is needed. Recent retrospective studies highlighted that immune checkpoint inhibitors may provide an effective option for NSCLC patients with certain infrequent alterations, especially in those associated with smoking such as *BRAF* mutations [115, 156, 157]. Future clinical trials should determine the ideal regimen of combination therapies including dosing and nature of targeted agents, anti-angiogenic drugs and/or chemo plus immunotherapy. When defining the ideal clinical regimen caution should be applied if transposing information from other cancer types harboring the same infrequent driver. For example, the extended survival upon combinatorial treatments in *BRAF* V600E mutant melanoma has also been achieved in LUAD patients. However, the therapeutic response of colorectal carcinoma patients bearing the same alteration was disappointing due to EGFR-mediated intrinsic resistance [158]. In any case, an ideal clinical regimen would require radiological and molecular monitoring of disease progression. Resistance mechanisms could be captured by non-invasive methods like liquid biopsy [119, 125, 159] (see Outstanding Questions). A recurrent and important problem is the occurrence of brain metastases, thereby requiring the use of compounds with good intracranial activity. In conclusion, a better understanding of the alterations underlying the development of resistance and the design of tailored treatments will allow delaying tumor progression and achieving improved clinical outcomes in NSCLC patients bearing infrequent alterations.

Box 1. Targeting *EGFR* and *ALK* alterations in NSCLC

EGFR mutations occur in 15% of NSCLC, being in-frame deletion in exon 19 (19Del) or L858R mutation the most frequent. Both first-generation (erlotinib and gefitinib) and second-generation (afatinib) inhibitors demonstrated more durable responses than chemotherapy [160-164], receiving FDA-approval as first-line treatments in 2013 and 2014. In almost half of patients treated with these compounds, resistance is mediated by the T790M gatekeeper mutation (equivalent to those described in *ROS1*, *RET* and *NTRK*, **Figure 2A**). Third-generation *EGFR* inhibitors such as osimertinib demonstrated impressive response in

patients with EGFR T790M-positive NSCLC after progression on early-generation TKIs, receiving FDA-approval in 2017 [165]. Due to its ability to spare wild-type EGFR, inhibit EGFR 19Del/L858R/T790M and CNS penetration, osimertinib is approved as first-line treatment since 2018 [166]. Unfortunately, new resistance mechanisms have emerged like the tertiary C797S mutation affecting the covalent binding of the drug [167, 168] or the development of T790 wild-type clones [169].

ALK rearrangements are observed in 5% of NSCLC, being *EML4-ALK* the most prevalent. At least 15 *EML4-ALK* variants have been identified so far, with some expressed as multiple isoforms [170]. All contain the *ALK* entire intracellular kinase domain (exons 20-29) but differ in the breakpoint with the *EML4* gene. First-generation crizotinib received FDA approval in 2011, becoming the first approved *ALK* inhibitor for NSCLC [171]. Almost one third of crizotinib-treated patients acquire resistance by kinase domain mutations, being L1196M gatekeeper and G1269A mutations the most frequent. In 2014, second-generation *ALK* inhibitor ceritinib was approved after confirmation of its efficacy in both crizotinib-naïve and crizotinib-resistant patients with L1196M and G1269A/S mutations [172, 173]. In approximately half of cases, resistance is caused by emergence of additional *ALK* mutations, the most common being the solvent-front substitution G1202R. Therefore, two other second-generation *ALK* inhibitors — alectinib and brigatinib — were granted accelerated FDA approval for *ALK*-positive patients who had failed on crizotinib treatment, demonstrating superior inhibitory profile against all known 17 secondary *ALK* mutations [174, 175]. Both alectinib and brigatinib were later approved also as first line therapy in treatment-naïve patients, although alectinib has become the preferred agent. Recently, the third-generation *ALK/ROS1* inhibitor lorlatinib, specifically designed to target resistance mutations and to penetrate the blood-brain barrier, received accelerated FDA approval for the treatment of patients with *ALK*-rearranged advanced NSCLC after progression on crizotinib and at least one other *ALK* inhibitor [176]. The phase III trial CROWN comparing lorlatinib with crizotinib as first-line therapy is currently ongoing^{vi}.

Box 2. BRAF non-V600 mutations in NSCLC

While more than 90% of *BRAF*-altered melanoma bear a V600 mutation, *BRAF*-positive NSCLC are equally divided in V600 and non-V600 mutations and, therefore, represent one of the cancers with the higher proportion of non-V600 mutations. The tumor-initiating

potential of many of the rare BRAF mutations has yet to be demonstrated *in vivo*. In addition, data demonstrating clinical activity of MAPK-directed therapies in non-V600 *BRAF*-mutant lung cancers are lacking. Therefore, there is currently no consensus on how to optimally manage patients with class 2 and 3 *BRAF* mutations. In the EURAF retrospective series that included 6 NSCLC patients with *BRAF* mutations other than V600E, only one patient (G596V) achieved a partial response to Vemurafenib [105]. These findings are consistent with the statement that class 2 and 3 mutants signal as RAF dimers rather than monomers [103], making them resistant to the BRAF monomer inhibitors. However, *in vitro*, both class 2 and 3 mutated cell lines are sensitive to MEK inhibition [177]. Additionally, the combination of BRAF and MEK inhibitors enhanced MAPK inhibition and cell proliferation reduction in both class 2 and 3 *BRAF*-mutant NSCLC preclinical models compared to MEK inhibitor alone [178-181]. Interestingly, oncogenic signaling triggered by class 3 BRAF mutants depends on a variety of RTKs that should be identified per patient [103]. Alternatively, the inhibition of SHP2, a scaffold protein downstream of RTKs promoting RAS activation, or the use of pan-RTK inhibitors (such as afatinib) could be considered [182]. Incidentally, concomitant SHP2 and BRAF/MEK inhibition showed synergistic effects in a class 3 *BRAF*-mutant cell line [183]. Additionally, allosteric inhibitors targeting the heterodimer interface between BRAF class 3 mutants with CRAF may represent an alternative approach [184]. Further studies are required to (1) precise the molecular mechanisms of each *BRAF* mutants (i.e. homo *versus* heterodimer), (2) define the importance of the additional molecular determinants that may play a role in modulating therapeutic response (i.e. expression level of CRAF and concomitant genetic alterations) and (3) identify the most promising strategies with potential drug combination to achieve successful clinical response in patients with non-V600 *BRAF* mutations.

Competing interests

E. N. participated in advisory boards from Bristol Myers Squibb, Merck Sharpe & Dohme, Lilly, Roche, Pfizer, Takeda, Boehringer Ingelheim, Amgen and AstraZeneca. E. N. has received research funding from Roche and Pfizer. All other authors have nothing to disclose.

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Resources

ⁱ <https://clinicaltrials.gov/ct2/show/NCT02279433>

ⁱⁱ <https://clinicaltrials.gov/ct2/show/NCT02675491>

ⁱⁱⁱ <https://clinicaltrials.gov/ct2/show/NCT03215511>

^{iv} <https://clinicaltrials.gov/ct2/show/NCT01813734>

^v <https://clinicaltrials.gov/ct2/show/NCT04161391>

^{vi} <https://clinicaltrials.gov/ct2/show/NCT03052608>

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1062

1063 **Table 1: Comparative clinical efficacy of targeted therapies in *ROS1*, *MET*, *RET*, *NTRK*, *HER2***
1064 **and *BRAF*-altered lung cancers.**

Driver	Drug	Clinical trial identifier ^a /name (phase)	NSCLC, n	ORR, %	mPFS, months	Intracranial disease control, n (%)	FDA approval	Refs
ROS1	Crizotinib	EUROS1 (retrospective)	32	80	9.1		2016	[24]
		NCT00585195/PROFILE 1001 (I)	50	72	19.2			[22]
		NCT01945021 (II)	127	71.7	15.9			[25]
		NCT02183870/EUCROSS (II)	34	70	20			[26]
		NCT02034981/AcSé (II)	78	47.2				[23]
	Ceritinib	NCT01964157 (II)	32 (30 crizotinib naïve)	62 (overall) and 67 (naïve)	9.3 (overall) and 19.3 (naïve)	5/8 (63%)		[28]
	Lorlatinib	NCT01970865 (I/II)	40 (previously treated) and 21 (TKI naïve)	35 (previously treated) and 62 (naïve)	8.5 (previously treated) and 21 (naïve)	Previously treated: 12/24 (50%) Naïve: 7/11 (64%)		[29]
	Entrectinib	NCT02097810/STARTRK-1 (I), NCT02568267/STARTRK-2 (II) and EudraCT 2012–000148–88/ALKA-372-001 (I)	53	77	19	11/20 (55%)	2019	[8, 27]
MET	Crizotinib	NCT00585195/PROFILE 1001 (I)	65	32	7.3			[73]
	Tepotinib	NCT02864992/VISION (II)	99	46	8.5	6/11 (55%)		[74]
	Capmatinib	NCT02414139/GEOMETRY mono-1 study (II)	69 (previously treated) and 28 (naïve)	41 (previously treated) and 64 (naïve)	5.4 (previously treated) and 12.4 (naïve)	12/13 (92%)	2020	[9]
RET	Vandetanib	UMIN000010095/LURET (II)	19	47	4.7			[185]
		NCT01823068 (II)	17	18	4.5			[186]
	Cabozantinib	NCT01639508 (II)	25	28	5.5			[187]
	Lenvatinib	NCT01877083 (II)	25	16	7.3			[188]
	Cabozantinib, vandetanib, sunitinib, sorafenib, alectinib, lenvatinib, nintedanib, ponatinib or regorafenib	GLORY (retrospective)	53	26	2.3			[54]
	RXDX-105	NCT01877811 (I/Ib)	31 (RET inhibitor naïve)	19 (0% with KIF5B-RET and 67% with non-KIF5B partners)				[189]
	Alectinib	UMIN000020628 (II) and NCT03131206 (II)	ongoing					
	Pralsetinib	NCT03037385/ARROW (I/II)	116 (26 naïve)	65 (overall) and 73 (naïve)		5/9 (56%)	2020	[5]
		NCT04222972/AccelerRET (III)	ongoing					
	Selpercatinib	NCT03157128/LIBRETTO-001 (I/II)	105 (previously treated) and 39 (naïve)	64 (previously treated) and 85 (naïve)	16.5 (previously treated)	10/11 (91%)	2020	[4]
		NCT04194944/LIBRETTO-431 (III)	ongoing					
NTRK	Larotrectinib	NCT02122913 (I)	12	88 (pan cancer)			2018	[6]
		NCT02576431 (II)	ongoing					
	Entrectinib	NCT02097810/STARTRK-1 (I), NCT02568267/STARTRK-2 (II) and EudraCT 2012–	10	57 (pan cancer)	11.2 (pan cancer)		2019	[7]

		000148–88/ALKA-372-001 (I)						
HER2	Afatinib	Retrospective	10	33				[190]
		Retrospective	23	13				[191]
		NCT02369484/NICHE (II)	13	53.8	15.9			[192]
	Dacomitinib	NCT00818441 (II)	26	12	3			[193]
	Neratinib	NCT01953926/SUMMIT (II)	26	4	5.5			[194]
	Pyrotinib	NCT02535507 (II)	15	53.3	6.4			[195]
		NCT02834936 (II)	ongoing					
	Mobocicetinib (TAK-788)	NCT02716116 (I/II)	21					[95]
	Pozotinib	NCT03066206 (II)	12	42	5.6			[93, 196]
BRAF	Trastuzumab-deruxtecan	NCT03505710/DESTINY-Lung 01 (II)	42	61.9	14			[96]
	Vemurafenib, dabrafenib or sorafenib	European EURAF (retrospective)	35	53	5			[105]
	Vemurafenib	NCT01524978/VE-BASKET (II)	62 (8 naïve)	37.1 (overall), 37 (previously treated) and 37.5 (naïve)	6.5 (overall), 6.1 (previously treated) and 12.9 (naïve)			[107]
		NCT02304809/AcSé (II)	101	44.8	5.2			[106]
	Dabrafenib	NCT01336634 (II)	78 (previously treated) and 6 (naïve)	33 (previously treated) and 67 (naïve)	5.5 (previously treated)			[108]
	Dabrafenib and trametinib	NCT01336634 (II)	57 (previously treated) and 36 (naïve)	63.2 (previously treated) and 64 (naïve)	10.2 (previously treated) and 10.9 (naïve)		2017	[3, 109]
	Encorafenib and binimetinib	NCT03915951/PHAROS (II)	ongoing					
	Ulixertinib	NCT01781429 (I)	12 (BRAFi/MEKi naïve)	25		1/12 (8%)		[114]
	LXH254 and LLT462	NCT02974725 (I)	ongoing					

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Figure legends

Figure 1: Comparative clinical efficacy of targeted therapies in *ROS1*, *MET*, *RET*, *HER2* and *BRAF*-altered lung cancers. Each circle on the plot represents the efficacy of the indicated inhibitor in patients for each specific driver (indicated with the different colors). Inspired by Figure 5 in Drilon A. and colleagues [57]. X and Y axis represent the objective response rate and the median progression-free survival duration, respectively, as reported from phase I and II clinical trials (see main text and Table 1 for details and references).

Figure 2: Schematic representation of the on-target resistance mutations and activity profile of kinase inhibitors targeting these specific resistance mutations among the different oncogenic drivers in NSCLC. **A.** The different types of mutations affecting the solvent-front, the gatekeeper residue, the activation loop or the DFG motifs are represented. Most resistance mutations seen in uncommon driver oncogenes have analogous resistance mutations identified in EGFR and ALK. **B.** Blue boxes indicate kinase inhibitor for which anti-tumoral activity against the indicated mutants has been reported in preclinical models (preclinical validation). Orange boxes designate kinase inhibitor that failed to show any inhibitory activity *in vitro* and/or *in vivo* (preclinical invalidation). Green boxes highlight reports of clinical activity (tumor shrinkage) while red dots indicate agents that failed to achieve an objective response in patients. Asterisks (*) indicate FDA approved drugs. Abbreviations: GK, gatekeeper; SF, solvent-front; AL, activation loop. References [36, 60, 75, 117, 120, 129, 197-207].

Figure 3: Signaling pathways driving resistance to targeted therapies in *ROS1* (A), *RET* (B) and *NTRK* (C) fusions-positive NSCLC and *MET* (D), *HER2* (E) and *BRAF* (F)-mutated NSCLC. Oncogenic drivers and their downstream signaling pathways are represented in blue and green, respectively. On-target resistance mutations as well as histological transformation are indicated (boxes). Protein alterations that have been implicated in the resistance to targeted agents are shown in orange. Potential therapeutic strategies are also highlighted in red.

Outstanding questions

- Is it ethical to have an approved targeted treatment against an uncommon driver and not implement the diagnostic tests as routine to identify the patients?
- Are large randomized clinical trials comparing targeted agents with platinum-based chemotherapy needed in patients with rare oncogenic drivers?
- How could we design and validate combination treatments in the clinic that would outperform single agent therapies? Could we develop in parallel predictive biomarkers for stratifying patients?
- How can we improve the early identification of emergent on-target mutations? Could multiple samplings along the treatment through liquid biopsies and circulating DNA represent an alternative option to monitor resistance onset?
- Could concomitant low dosing therapies targeting different effectors within the RTK-RAS-MAPK pathway be implemented in the clinic?

Highlights

- Lung cancer is responsible of around 1.8 million deaths per year worldwide and is widely the leading cause of cancer-related mortality.
- The development of specific inhibitors is currently extending to less common oncogenic drivers (i.e. *ROS1*, *MET*, *RET*, *NTRK*, *HER2* and *BRAF*) with an exponential growth of dedicated clinical trials.
- While, individually, each of these drivers appears with low prevalence, altogether, they account for 15% of all lung cancer cases, thereby affecting a large population of patients worldwide.
- Similar to what is observed with most targeted therapies directed against an oncogenic driver, the initial clinical response to targeted kinase inhibitors is almost always temporary and acquired resistance to these drugs invariably emerges, restricting their clinical utility.
- The characterization of intrinsic and acquired resistance relies on tissue or liquid biopsy upon disease progression. However genomic testing is not always capturing the underlying mechanism of resistance.
- Both on-target and off-target resistance mechanisms to targeted therapies could be tackled in the clinic but the design of the most effective treatment still requires further evaluation.

Figure 1

A

Figure 3