New horizons for uncommon mutations in non-small cell lung cancer: BRAF, KRAS, RET, MET, NTRK, HER2

Olmedo ME et al. New horizons for NSCLC uncommon mutations

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Abstract
The 2004 discovery of EGFR mutations, followed by ALK rearrangements, ushered in a targeted therapy era for advanced non-small cell lung cancer (NSCLC). Tyrosine kinase inhibitors targeting gene alterations have substantially improved survival and quality of life for patients with NSCLC. In the last decade, rearrangements of the ROS1 oncogene have been incorporated into healthcare practice that are applicable to another small subgroup of patients who benefit from similar targeted strategies. Recent genome studies of lung adenocarcinoma have identified other possible therapeutic targets, including RET, NTRK fusions, c-MET alterations, and activating mutations in KRAS, BRAF, and HER2, all with frequencies greater than 1%. Lung cancers harbouring these genome changes can potentially be treated with agents approved for other indications or under clinical development. This review updates the therapeutic arsenal that especially targets those genes.

Key Words: BRAF; NTRK; KRAS; MET; RET; HER2; Non-small cell lung cancer; Targeted therapy; Uncommon mutations

**Core Tip:** Compared to other types of cancer, non-small cell lung cancer (NSCLC) is highly genetically altered. Outside of *EGFR, ALK,* and *ROSI,* reflecting 15%-20% of clinical practice, other molecular alterations with important recent advances in their therapeutic arsenal and already in phase II/III trials are *BRAF, KRAS, RET, MET, NTRK,* and *HER2.* The goal is to achieve, compared to conventional treatments such as chemotherapy, better symptom control, better response rates, and improved progression-free survival and overall survival in patients with NSCLC.

**INTRODUCTION**

Approximately 60% of lung adenocarcinomas harbour molecular alterations in driver oncogenes, with incidence, which varies according to ethnic origin and alteration, as follows: epidermal growth factor receptor (*EGFR*) mutation, 15%-20%[1]; anaplastic lymphoma kinase (*ALK*) rearrangement, 5%-7%[2]; and c-ros 1 (*ROSI*) rearrangement, approximately 1%[3]. There has been an impressive improvement in survival in response to tyrosine kinase inhibitors (TKIs), which also have a better toxicity profile compared to standard chemotherapy.

The consequent improvement in molecular understanding of non-small cell lung carcinoma (NSCLC) has allowed increasingly exhaustive molecular classification as well as identification of a subset of patients susceptible to specifically targeted therapy. The outcome of massive gene-sequencing platforms with higher throughput than gene-to-gene determinations is that patients can be offered more treatments that more specifically impact on their quality of life and survival. The current recommendation is to carry out a comprehensive molecular analysis using multiplex platforms - next-
generation sequencing (NGS) – if available, considering advantages in terms of coverage, time, and a favourable economic profile[4]. NGS is capable of detecting less common or difficult-to-identify oncogenes, such as Kirsten rat sarcoma viral oncogene homolog (KRAS) mutations (30%-35%), V-raf murine sarcoma viral oncogene homolog B (BRAF) mutations (4%-5%), mesenchymal-epithelial transition factor (c-MET) alterations, exon 14 insertions and/or amplifications (5%-9%), rearrangements during transfection (RET) (1%-2%), human epidermal growth factor receptor 2 (HER2) mutations (2%), and neurotrophic receptor tyrosine kinase (NTRK) fusions (< 1%)[5]. Identifying these alterations is increasingly important, as new specific drugs in clinical development show promise in terms of modifying the natural history of NSCLC. We focus on direct inhibitors of pathways and their practice-changing results.

**BRAF**

Present in 2%-3% of NSCLC cases, the BRAF mutation is mostly encountered in patients diagnosed with adenocarcinoma[6]. The most common variant is V600E, found in 50%-60% of patients with BRAF-mutated (BRAFm) NSCLC. Not clear is the prognostic value of BRAF-V600E compared with non-V600E or with the rest of patients with NSCLC[7]. The drugs used to date for this molecular alteration are the same TKIs that have proven to be effective in treating melanoma, a tumour with high BRAFm frequency.

Table 1 summarizes the efficacy of the main drugs used to date. The best results have been reported for dabrafenib combined with trametinib, which attempt to block the MAPK pathway at two different sites (BRAF and MEK), thus overcoming possible tumour resistance to TKIs. The BRF113928 study in patients who received 2-4 Lines of therapy reported an objective response rate (ORR) of 63.2%, and a first-line ORR of 64%[8-12].

However, the absence of comparative data for first and subsequent lines of therapy as currently used for this group of patients means that it is not possible to confirm significant clinical benefit and efficacy over alternative therapies. Dabrafenib and
trametinib may therefore be of use for patients for whom standard therapies are not possible or have failed.

Phase II studies are also currently recruiting for the encorafenib + binimetinib (NCT04526782) and cobimetinib + vemurafenib (NCT03178552) combinations.

**KRAS**

KRAS is the most common mutation in NSCLC, present in up to 30% of adenocarcinomas[13]. In 80% of cases it is located at codon 12, and the most frequent mutation is KRAS-G12C, reflected in 13% of all lung adenocarcinomas. It is considered practically exclusive in relation to any other clinical practice drivers, although co-occurrences have been found with alterations in TP53, cyclin dependent kinase inhibitor 2A/B (CDKN2A/B), STK11, and KEAPI (Kelch Like ECH Associated Protein 1)[14].

While KRAS has been a therapeutic target for decades, no direct therapeutic option has been established. In recent years, new direct inhibitors of KRAS-G12C have emerged. Phase II trial results for sotorasib, an irreversible and highly selective KRAS-G12C inhibitor, have positioned it as a major lung cancer milestone for the KRAS mutation[15,16]; for 126 included patients, the ORR was 37.1%, there were three complete responses (CRs) and 43 partial responses (PRs), and the disease control rate was 80.6%, for a median progression-free survival (PFS) of 6.8 mo and a good tolerability profile. Based on those data, an application for marketing authorization has been submitted to the FDA and EMA.

In two presentations at the 32nd Symposium on Cancer Therapeutics and Molecular Targets EORTC-NCI-AACR[17,18], investigators from the KRYS TAL-1 phase I and II clinical trial reported that adagrasib clinical activity has been demonstrated in previously treated patients with NSCLC and the KRAS-G12C mutation. Promising preliminary data for this drug are to be further evaluated in trials, along with combinations, including with pembrolizumab in the KRYS TAL-7 phase 2 trial (NCT04613596) of untreated patients[19].
RET

RET gene fusions and activating point mutations are primary oncogenic drivers that are usually mutually exclusive with other oncogenic driver alterations. Among the various oncogene drivers in NSCLC, the RET gene is involved in various chromosomal rearrangements, found in 1%-2% of all NSCLC patients.

Most of the drugs active against RET are TKIs. Multikinase inhibitors initially studied in phase II clinical trials include cabozantinib, nintedanib, lenvatinib, vandetanib, and sorafenib, each with a different ORR (Table 2).

Selpercatinib (LOXO-292) is a highly selective, potent, central nervous system (CNS)-active, small-molecule RET kinase inhibitor. Selpercatinib has nanomolar potency against wild-type RET and other RET alterations, including the KIF5B-RET fusion and V804M gatekeeper mutation, in both enzyme and cellular assays, with minimal activity against other kinase and non-kinase targets.

In the LIBRETTO-001 phase I/II trial, selpercatinib treatment demonstrated clinically meaningful responses and sustained antitumour activity, for a manageable toxicity profile, in both heavily pre-treated and treatment-naïve patients, and including patients with brain metastases and RET fusion-positive NSCLC (intracranial CNS (n = 10/11): ORR 91%). In May 2020, selpercatinib was approved by the FDA under the Accelerated Approval programme for the treatment of RET-altered cancers (NSCLC and thyroid cancer).

Pralsetinib (BLU-667) is a novel small-molecule RET inhibitor, designed for high potency and selectivity against oncogenic RET alterations, including the most frequent RET rearrangements (e.g., KIF5B-RET and CCDC6-RET). The global phase I/II ARROW study has demonstrated broad and durable antitumour activity for pralsetinib in a variety of advanced RET-altered solid tumours, including RET fusion+ NSCLC. For 354 patients with advanced solid tumours who received pralsetinib as first-line treatment, the ORR was 73%, for a 12% CR rate (n = 26). Treatment-related adverse events were most frequently grade 1-2. Table 2 summarizes the activity of the different TKIs against RET.
RXDX-105 differs from the other multi-targeted TKIs because it has RET activity but limited activity against the vascular endothelial growth factor (VEGF) receptors. In RET TKI-naïve patients, the drug showed modest activity. Subset analysis revealed that the ORR varied by fusion partner. ORRs were 0% (0/20) in the RET-KIF5B rearrangement subset (the most common rearrangement) and 67% (6/9) in the RET-non-KIF5B rearrangement subset\(^{39}\).

**MET**

c-MET is an oncogene that encodes a tyrosine kinase receptor whose ligand is hepatocyte growth factor (HGF). Alterations in c-MET (mutation, amplification, or overexpression) cause abnormal receptor activity that is associated with rapid tumour growth, greater tumour aggressiveness, and resistance to cancer treatments\(^{30}\).

c-MET amplification is present in 1%-6% of patients with NSCLC. Skipping mutation of exon 14 occurs in 3%-4% of cases, most frequently for non-squamous and sarcomatoid histologies (20%-30%). This alteration occurs most frequently in older patients and in smokers.

Selective and non-selective c-MET inhibitors (Tables 3 and 4) are currently available that can impact on survival in patients with NSCLC. The first drug to demonstrate efficacy with this tumour subtype was crizotinib: in the PROFILE 1001 study, the ORR was 32% and PFS was 7.3 mo\(^{31}\).

Capmatinib is another drug that has been shown to be active: in the GEOMETRY MONO-1 study, the ORR was 41% and PFS was 5.4 mo in previously treated patients; in first-line patients, the ORR was 68% and PFS was 12.4 mo, while ORR was 54% for intracranial activity\(^{32}\). In the VISION study, tepotinib achieved an ORR greater than 40%, irrespective of the therapy line, PFS of 8.5 mo, and an ORR of 55% for intracranial activity\(^{33}\). Regarding MET amplification, TKIs have only significantly benefited tumours with a high level of amplification (MET/CEP7 > 5), for an ORR of 40% with crizotinib and of 47% with capmatinib.
Amplification, which may appear de novo or as a mechanism of resistance to the targeted treatment of EGFR tumours, is present in 4% of cases of progression to first/second generation inhibitors, and in 15% of cases of progression to osimertinib. Being explored, therefore, is the combination of EGFR inhibitors and MET inhibitors.

The TATTON study explored osimertinib combined with savolitinib in patients with NSCLC and mutated EGFR. In the group that received initial treatment with a first/second generation inhibitor, the ORR was 52%, while in the group that received osimertinib, the ORR was 25%, for an acceptable toxicity profile\textsuperscript{34}.

As for immunotherapy, despite the fact that the tumours may present with elevated PD-L1 expression, the benefit reported for retrospective studies by a French group was limited, at an ORR of 16% and PFS of 3.4 mo\textsuperscript{35}.

\textbf{NTRK}

The tropomyosin receptor kinase (TRK) family consists of three tyrosine kinase receptors - TRKA, TRKB, and TRKC isoforms, encoded by the NTRK1, NTRK2, and NTRK3 genes, respectively - that are mainly expressed in the nervous system. Their fusions involve some 80 associated genes and they are known oncogenic drivers\textsuperscript{33-38}. The incidence of NTRK fusions in NSCLC is estimated to be 0.1%-0.2%, affecting a population that is unselected in terms of sex, age, or smoking\textsuperscript{37}.

Currently, two first-generation TKIs targeting NTRK fusions have been approved by the FDA and the EMA: entrectinib (multikinase ALK, ROS1, and pan-TRK inhibitor) and larotrectinib (selective pan-TRK inhibitor). Both have demonstrated great efficacy (irrespective of histology or fusion gene) and intracranial activity, as well as good toxicity profiles\textsuperscript{38-41}.

Larotrectinib efficacy and safety in patients with solid tumours and NTRK fusions have been evaluated in three phase I/II studies (NCT02122913, NCT02637687, NCT02576431). Joint analysis of those studies, with a total of 159 patients, yielded an
ORR of 79%, a CR rate of 16%, and median PFS of 28.3 mo. For the 12 included patients with NSCLC, the ORR was 75%. Reported adverse events were mostly grade 1-2\[38\].

Entrectinib was evaluated in the phase I ALKA-372-001 trial, phase I STARTRK-1 trial and phase II STARTRK-2 basket trial. For the 10 patients with NSCLC, the ORR was 70%, the CR rate was 10%, and PFS was 14.9 mo. Entrectinib showed a good toxicity profile; most adverse events were grade 1 or 2 and reversible, e.g., dysgeusia, constipation, fatigue, diarrhoea, oedema, and dizziness\[39\].

Selitrectinib (LOXO 195), repotrectinib (TPX-0005), and taletrectinib (DS-6051b/AB-106) are second-generation drugs capable of inhibiting on-target resistance of NTRK\[37,40\]. They are currently being evaluated in phase I/II clinical trials in patients with NTRK-positive tumours who have progressed to first-generation inhibitors (NCT03215511, EudraCT 2017-004246-20, NCT04094610, TRIDENT-1: NCT03093116, NCT02279433).

**HER2**

HER2 is a cell growth promoting protein, a member of the ERBB family of tyrosine kinase receptors expressed on the surface of many types of tumours. Overexpression, which occurs in 2%-20% of cases depending on the immunohistochemistry (IHC) level (IHC2+/3+), is associated with a poor prognosis. HER2 amplification occurs, especially in adenocarcinomas, in around 3% of cases without prior treatment and in approximately 10% of cases of EGFR resistance to TKIs\[42\].

HER2 mutations (HER2m) - usually consisting of insertions in exon 20, especially in codon 776 - appear mainly in women, in adenocarcinoma cases, and in the Asian population, and never in smokers. The insertions cause constitutive activation of the receptor, making it sensitive to dual TKI action against EGFR and HER2, but not exclusively to EGFR inhibition\[43\].

The therapies commonly used to target HER2 in breast cancer have not had the same results for NSCLC. The emergence of new TKIs and conjugated antibodies have given a
new boost to therapies for this molecular alteration in NSCLC (Table 5). Reported for the largest retrospective EUHER2 study, which included patients with HER2 exon 20 insertions, was an ORR of 7.4% for treatment with the TKIs afatinib, lapatinib, and neratinib; for the trastuzumab antibody and the trastuzumab emtansine (T-DM1) antibody-drug conjugate, the ORR was a more effective 50.9%, but that treatment was in most cases combined with chemotherapy\textsuperscript{[44,45]}. Two phase II studies, of neratinib combined with trastuzumab in HER2m patients in first or successive therapy lines (NCT01953926) and of neratinib with temsirolimus (NCT01827267), have reported ORRs of 17% and 19%, respectively\textsuperscript{[46]}. Zhou et al\textsuperscript{[47]} explored the efficacy of pyrotinib in monotherapy, reporting an ORR of 30%, median PFS of 6.9 mo, and overall survival (OS) of 14.4 mo; the main toxicity, as with other HER2-targeting TKIs such as neratinib and lapatinib, was diarrhea. In the phase II ZENITH20 trial of poziotinib, another pan-HER TKI, for the HER2m treatment the ORR was 28%, PFS was 5.5 mo, and the toxicity profile was similar to that for pyrotinib\textsuperscript{[48]}. In addition to the HER2 TKIs, also being evaluated in this setting are antibody-drug conjugates such as T-DM1 and trastuzumab deruxtecan (DS-8201, T-Dxd). Peters et al\textsuperscript{[49]} explored responses to TDM-1 in 49 patients with IHC2+/3+ overexpression, reporting no response for the IHC2+ cohort and 4 PRs for the IHC3+ cohort (20%). Better data is available for trastuzumab deruxtecan. For 42 patients with HER2m in the DESTINY-Lung01 cohort, the ORR was 62%, PFS was 14 mo; median OS was not achieved, while OS was 24.5% in the IHC2+/3+ overexpression cohorts\textsuperscript{[50]}. To confirm the PFS benefit, a phase III trial of pyrotinib vs docetaxel called PYRAMID-1 (NCT04447118) is ongoing.

**CONCLUSION**

Compared to traditional chemotherapy, the improved TKI targeting of EGFR mutations and ALK/ROS1 translocations has led to significant efficacy and quality of life improvements in the management of patients with NSCLC. While this subgroup of
patients inevitably develops resistance to TKIs, this can be overcome by developing new next-generation TKIs or drugs aimed at overcoming resistance from the outset or from the time of discovery\[^{51,52}\].

These developments may also be transferable to the treatment of patients with other molecular alterations of \textit{BRAF, KRAS, RET, MET, NTRK} and \textit{HER2}. As can be seen above, a growing number of drugs and combinations are becoming available that target these alterations, often producing a significant improvement in response and survival rates.

Given the many common and rare molecular alterations in NSCLC, full-panel multigene NGS is recommended rather than gene-by-gene sequencing, as not only is it more cost-effective, it allows patients with a target to be easily identified and treated, whether with an approved drug or in a clinical trial of a promising drug\[^{53-55}\].

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