Biology and clinical management of non-V600 *BRAF* alterations in non-small cell lung cancer

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ABSTRACT

BRAF mutations are detected in approximately 3-8% of non-small cell lung cancer (NSCLC) patients. In contrast to melanoma, in which the majority of *BRAF* mutations occur at the V600 codon, only about 35% of *BRAF*-mutant NSCLC tumors harbor V600 mutations. Among the remaining cases, 60-70% present non-V600 mutations, primarily in exons 11 and 15. *BRAF* mutations are classified into three classes according to their kinase activity and their dependence on RAS activation. Compared to class I (V600), patients with class II and class III mutations are associated with poorer clinical outcomes partly due to the lack of effective targeted therapeutic strategies. Indeed, while dual BRAF and MEK inhibition has demonstrated clinical benefit in *BRAF* V600-mutant NSCLC, there is currently no consensus on treatment strategies for patients with class II/III mutations. Beyond point mutations, other *BRAF* alterations (*e.g.,* gene fusions, deletions and amplifications) have been identified in treatment-naïve tumors and in the context of acquired resistance to targeted therapies in other oncogene-driven NSCLC subtypes. However, the biology and clinical implications of these alterations remain poorly characterized. In this review, we provide a comprehensive overview on the biology, epidemiology and therapeutic strategies of class II/III *BRAF* mutations as well as *BRAF* fusions, deletions and amplifications in NSCLC. We highlight current challenges in the clinical management of *BRAF*-mutant NSCLC as well as emerging inhibitors and combinatorial therapeutic strategies developed to treat non-V600E *BRAF*-driven cancers. Finally, we briefly discuss *BRAF* alterations in the context of resistance to targeted therapies in other oncogene-driven NSCLC.

Epidemiology of *BRAF* mutations

Lung cancer remains the leading cause of cancer related mortality, with non-small cell lung cancer (NSCLC) accounting for roughly 85% of cases [1]. Although *BRAF* mutations are found in a relatively small percentage (3-8%) of lung adenocarcinoma cases (the most common NSCLC subtype), this translates to an estimated 85,000 new cases annually [2]. Beyond lung cancer, *BRAF* mutations are also commonly found in melanoma, thyroid cancer and colorectal cancer, ranking among the most frequently mutated kinases in human cancers [3]. *BRAF* mutations are divided into three functional classes based on their kinase activity and their dependence on RAS activation [3]. Class I (V600) *BRAF* mutations represent 80-90% of *BRAF*-mutant melanoma and colorectal cancer cases whereas, in NSCLC, these mutations account for only one third (32-39%) of *BRAF* mutant cases. Indeed, over half of the cases involve class II (38-33%) and class III (28-30%) mutations, also considered together as non-V600 mutations [4] (**Figure 1A**). In addition, a very small proportion of NSCLC harbor *BRAF* alterations such as amplification, fusion, deletion and insertion from which the functional consequences and the kinase activity remain unclear [5–8].

Among class I mutations, the *BRAF* V600E variant is the most common, as it is found in 90% of cases. In contrast, class II and III mutations exhibit greater heterogeneity, with a wider variety of mutation sites observed (**Figure 1B**). Specifically, class II mutations are frequently represented by G469A and K601E, while D594G/N and G466V are the most prevalent class III mutations [5,9–12]. These mutations can co-occur with other genetic alterations that may influence tumor biology and treatment response. For instance, co-mutations in *KRAS* and *NF1* are predominantly associated with class II and III *BRAF*-mutant tumors compared to class I [11,13–15]. In a cohort of 133 patients with *BRAF*-mutated NSCLC, genetic alterations in *STK11* were significantly overrepresented in class II and III, while mutations *SETD2* were more frequent in class I [16].

Emerging evidence suggests that the prevalence of *BRAF* mutations in NSCLC may vary significantly across geographic regions. This variability is likely driven by complex interactions among smoking behavior, environmental exposures, ethnicity, genetic predisposition and

clinical factors [17]. Smoking behavior appears particularly relevant in *BRAF*-mutant NSCLC. Indeed, despite one report showing no clear correlation between smoking history and *BRAF* mutation classes [6], several studies indicate that patients with class II/III mutations present more frequently with a history of smoking, whereas class I mutations are more prevalent among non-smokers [13,18–21].

Air pollution and radon gas are the leading contributors to environmental-related lung cancer. Although no specific link has been established between air pollution and *BRAF*-driven tumors, a small prospective study reported radon concentrations exceeding 100 Bq/m³ - the threshold recommended by the World Health Organization - in homes of patients with *BRAF* V600E-mutant NSCLC [22]. Large-scale initiatives, such as the EORTC 1920 BIORADON trial, are currently underway to assess the contribution of radon and other environmental carcinogens in molecularly defined NSCLC populations, including *BRAF*-mutant tumors [23].

Beyond smoking behavior and environmental exposures, germline predisposition also appears to influence the biology of *BRAF*-driven NSCLC. A comprehensive genomic profiling (CGP) study involving over 87,000 patients identified pathogenic germline variants (PGVs) in 7.5% of *BRAF*-mutant tumors, a frequency among the highest across all oncogenic drivers [24]. Notably, PGVs in the tumor suppressor gene *CHEK2* were significantly enriched in *BRAF*-mutated tumors (odds ratio 1.6; $p = 0.04$), suggesting a potential interaction between germline susceptibility and *BRAF*-driven oncogenesis.

The contrast with melanoma, where V600E predominates, likely reflects lineage-specific mutational processes and evolutionary pressures. For instance, the high mutational burden linked to tobacco exposure in NSCLC favors a broader spectrum of point mutations [25], whereas UV-induced DNA damage in melanoma promotes a more restricted signature enriched for V600E substitutions [26]. In addition, cell-of-origin differences may play a role as melanocytes and bronchial epithelial cells may have distinct requirements for MAPK activation. Class III mutations, which rely on upstream receptor tyrosine kinase (RTK) activity (as further described below), may not sufficiently activate the MAPK pathway in melanoma, where constitutive BRAF activity (as with V600E) provides a stronger selective advantage. Conversely, in NSCLC,

where RTK signaling (e.g., via EGFR, MET) is often increased, class III mutations may function effectively in concert with these signals [27].

Age at diagnosis adds yet another dimension to the biological complexity of *BRAF*-mutant NSCLC. Young adults diagnosed with NSCLC often present with more advanced disease, increased rates of brain metastases, and a higher prevalence of oncogenic drivers. These alterations are especially common among never-smokers, women, and those with adenocarcinoma histology. While *EGFR* mutations and gene fusions (*ALK, ROS1, RET, NTRK*) are the most commonly reported drivers in this demographic, *BRAF* mutations are also reported, although less frequently. Indeed, *BRAF* V600E occur in 0-2.3% of young patients with NSCLC, whereas class II/III *BRAF* alterations are reported in approximately 0.5-3% of this particular subgroup [28].

Importantly, *BRAF* non-V600 mutations are also associated with different clinical parameters and worse clinical outcomes. First, the metastatic sites of *BRAF*-mutant tumors vary according to mutation class, with class II and III tumors more often linked to brain involvement, while intrathoracic spread is reported more frequently in class I and III tumors [11,29]; however, results remain inconsistent across studies [10]. Second, some evidence support more aggressive clinical features and poorer outcomes for patients with *BRAF* class II/III mutations compared to class I [11,30]. In particular, the study by Murciano-Goroff *et al.* showed that patients with class I *BRAF*-mutant lung cancer had a median overall survival (OS) of 26 months, compared to 9.1 months for those with class II/III mutations, from the date of sequencing and under standard treatment [30]. However, it should be noted that in the study of Dagogo-Jack *et al.*, when patients with class I mutations who had received targeted therapies were excluded, survival differences between class I and III were no longer observed [11]. This suggests that the apparent advantage of class I mutations may in part reflect differences in exposure to targeted therapies, as also discussed in other studies [6,20]. All these discrepancies may also be due to underpowered and retrospective study designs, as well as insufficient sample sizes.

Together, these observations reinforce the notion that *BRAF*-mutant NSCLC represents a biologically complex disease, shaped by the interaction of multiple layers of influence. These

include external risk factors (e.g., smoking, environmental carcinogens) and patient-specific characteristics (e.g., germline predisposition, age, sex, and histologic subtype); which may influence not only tumor behavior and prognosis, but also response to treatment. A deeper understanding of how these variables converge in *BRAF*-mutant disease - particularly in class II and III subtypes - is essential to improve risk stratification, inform therapeutic decision-making, and support the design of more precise, biology-driven clinical trials.

Biochemistry and biology behind *BRAF* mutations

BRAF domains and activation cycle in physiological conditions

Together with ARAF and CRAF, BRAF is a serine/threonine kinase belonging to the Rapidly Accelerated Fibrosarcoma (RAF) family. RAF proteins consist of three conserved regions (CR) – CR1, CR2, and CR3. CR1, located towards the N-terminal of the protein, contains a RAS-binding domain, as well as a cysteine-rich domain (CRD) with a 14-3-3 recognition site that plays a role in autoinhibition. CR2 includes a region rich in serine and threonine residues, while CR3 comprises the kinase domain and a second 14-3-3 recognition site, followed by a carboxy-terminal tail (CTT) [31]. The kinase domain features all the key elements required for BRAF's catalytic activity, such as the P-loop for ATP binding, the αC helix, the activation segment, the catalytic loop as well as the DFG motif, which is involved in the regulation of the active and inactive conformations of BRAF (**Figure 1B**).

In its inactive state, BRAF exists as a monomer in the cytosol and is autoinhibited through intramolecular interactions between its N-terminal region, catalytic domain, and a 14-3-3 dimer. The binding of 14-3-3 to BRAF prevents dimerization and activation until RAS signaling occurs. In addition, this autoinhibited conformation exposes basic residues essential for both BRAF autoinhibition and RBD-RAS binding [32]. Upon RAS activation, BRAF is recruited to the plasma membrane, where interaction with GTP-bound RAS and dephosphorylation of both the 14-3-3 binding site and the N-terminal region relieve autoinhibition, promote dimerization and trigger kinase activation via autophosphorylation. The BRAF dimer then recruits and phosphorylates MEK, activating the Mitogen-Activated Protein Kinase (MAPK) pathway, which subsequently

induces a negative feedback loop that disrupts RAF dimers. Oncogenic mutations, occurring in specific regions of the proteins, function by relieving the inhibition imposed by the N-terminal region, promoting dimerization of the kinase domain, and stimulating phosphorylation of the activation segment. Persistent activation of the MAPK pathway contributes to uncontrolled cell proliferation and cancer development [31].

Non-V600 BRAF mutations

While the oncogenic role of V600 mutations was highlighted in the early 2000s, the impact of non-V600 mutations on carcinogenesis was only demonstrated 10 to 15 years ago. In addition, more than 200 *BRAF* mutant alleles have been identified in human tumors, but only a small percentage of them have been functionally characterized with respect to their impact on signaling pathways and biological processes. Non-V600 mutations represent a very large and diverse class of alterations, which makes their functional characterization significantly more complex compared to the more homogeneous V600 mutations. Functional studies in preclinical models demonstrate that both kinase-active (class II) and kinase-inactive (class III) non-V600 isoforms can promote tumor growth and initiate lung oncogenesis, highlighting the capacity of non-V600 mutations to act as oncogenic drivers in NSCLC [33,34].

The majority of class II and III *BRAF* mutations are located within the kinase domain. Among the most common, G464, G469, and G466 are located in the P-loop; K601 and L597 are in the activation segment; and, D594 and G596 are in the DFG motif (**Figure 1B**). Using cryo-electron microscopy, a recent study demonstrated that class II and III variants exhibit the same global reorganization of the kinase domain as observed in V600E mutants; specifically, the adoption of an active-like kinase conformation driven by inward movement of the αC helix, including disruption of autoinhibitory interactions [35].

Neal Rosen and collaborators were the first to classify *BRAF* mutants based on their kinase activity and dependence on upstream RAS activation [36,37]. They reported that some *BRAF* mutations, now defined as class II, increase ERK signaling, but to a lesser extent than class I, and retro-inhibit RAS activity in an inducible *BRAF* mutant NIH3T3 embryonic fibroblastic cell

model. In contrast, class III mutants neither activate ERK signaling nor cause RAS inhibition. Additionally, in RAS-less mouse embryonic fibroblasts, class I and II mutations rescue MEK/ERK phosphorylation, unlike class III or wild-type BRAF, suggesting that class II mutations are RAS-independent while class III mutations remain RAS-dependent [37,38] (**Figure 2**).

Furthermore, disruption of BRAF dimerization (using the R509H mutation in SKBR3 or H293 cells) abolishes ERK signaling in cells with class II/III *BRAF* mutations, indicating that these variants function as homo- or heterodimers with A/CRAF, whereas class I mutations act as a monomer with constitutive ERK signaling activity [37,39]. Concordantly, class II mutations enhance ERK signaling through RAS-independent dimerization, while class III mutations, which have low or null kinase activity, require both dimerization and upstream RAS for activation. The classification of class II mutations is further refined into IIa (activation segment, e.g., L597, K601) and IIb (P-loop, e.g., G464, G469), reflecting differences in dimer stability and therapeutic response (**Figure 1B**). Noteworthy, class IIa variants show greater MAPK pathway inhibition with MEK inhibitors, whereas class IIb mutations form stronger dimers that may contribute to resistance against monomeric BRAF inhibitors [40].

BRAF fusions, insertions, deletions and amplifications

Besides point mutations, other *BRAF* alterations (insertions, deletions, amplifications and fusions) are also found in 2-5% of *BRAF*-altered lung adenocarcinoma (**Figure 1A**). Due to the wide variety and low prevalence of these genetic alterations, there is currently limited information regarding their functional significance and impact on treatment response. A recent study in a Chinese cohort of 371 *BRAF* fusion-positive samples identified multiple fusion partners, including *DLG1, STAT3, CNOT9, NRF1, TRIM24* among others [41]. Mechanistically, *BRAF* fusions were shown to induce a high kinase activity and function as RAS-independent dimers [37]. In addition, other types of *BRAF* rearrangements, such as T599-V600insT, V600-K601delinsE, and G469del, were introduced into NIH3T3 cells using a lentiviral vector to assess their functional impact. Both T599-V600insT and V600-K601delinsE mutations led to

increased ERK signaling activation compared to wild-type *BRAF*, although the activation was less pronounced than that induced by the V600E mutation. In contrast, the G469del mutation resulted in reduced kinase activity compared to wild-type *BRAF* [6]. In addition, shRNA-mediated silencing of BRAF expression in the H2405 lung cancer cell line, which harbors the *BRAF* L485_P490delins mutation, significantly impaired cell growth, supporting its role as an oncogenic driver in NSCLC [6].

BRAF fusions are rare acquired alterations in *EGFR*-mutant NSCLC, often contributing to resistance mechanisms against EGFR inhibitors [42–44]. MEK inhibitors have shown efficacy as monotherapy in patients with *BRAF* fusion-driven NSCLC [45]; additionally, some case reports suggest that combining EGFR inhibitors and MEK inhibitors, such as trametinib, may help overcome EGFR-inhibitor resistance driven by *BRAF* fusions [43,44,46].

Of note, the development of preclinical models that reliably capture the diversity of non-V600 *BRAF* mutations and structural alterations remains a major challenge but is essential to decipher the context-dependent oncogenic signaling and therapeutic vulnerabilities of these cancers. Beyond the few commercially available cell lines, efforts are underway to generate patient-derived xenografts (PDXs), organoids, and CRISPR-engineered cell lines. Ideally, these models should incorporate co-occurring genomic alterations (e.g., *KRAS*, *NF1*, *STK11*, or *EGFR* mutations) that may modulate pathway activity and influence treatment response.

Therapeutic strategies for class II and class III *BRAF* alterations

Access to targeted therapies in *BRAF* V600E patients has significantly improved outcomes for patients with *BRAF* V600E-mutant NSCLC [47–50]. However, patients bearing class II/III *BRAF* mutants generally show overall poor response rates to BRAF-inhibitor monotherapy or to combined treatment of BRAF and MEK inhibitors [51,52]. In preclinical models, the response to targeted therapies is highly variable, with half maximal inhibitory concentration (IC_{50}) values to some inhibitors differing by up to 1000-fold across various *BRAF* mutations. This highlights the marked heterogeneity both between and within *BRAF* mutation classes (**Figure 2**).

Here, we report the available data regarding the preclinical and clinical therapeutic strategies for the treatment of class II/III *BRAF* mutations in NSCLC (**Figure 3**).

First-generation BRAF inhibitors

BRAF non-V600 mutations often exhibit resistance to first-generation BRAF inhibitors (e.g., vemurafenib, dabrafenib, encorafenib) [15,36,37,51–54], as these inhibitors preferentially stabilize the "αC-OUT" configuration [55], thus targeting monomeric BRAF, while many non-V600 mutations signal as constitutive dimers. Indeed, vemurafenib monotherapy was not associated with any confirmed tumor responses in patients from the *BRAF* non-V600 cohort (G469A/V, G466V/A, N581S, K601E/N) in the AcSé vemurafenib study; while it resulted in a partial tumor response in one *BRAF* G596V-mutant patient from the EURAF cohort [56].

ATP-competitive RAF inhibitors are designed to inhibit RAF kinase activity and consequently suppress MAPK signaling in *BRAF*-mutant cells. However, in contexts where MAPK pathway activation arises from upstream alterations (e.g., *RAS* mutations or RTK signaling), these inhibitors can promote RAF dimerization, leading to paradoxical activation of downstream MEK and ERK signaling [57–60]. Paradox breakers not only inhibit monomeric BRAF V600, but also BRAF-containing dimers (e.g., BRAF:BRAF, BRAF:CRAF) [61,62]. In this context, plixorafenib (PLX8394) treatment was effective in decreasing cell growth of *BRAF* G469A (class II) and G466V (class III)-mutant NSCLC cells (H1395 and H1666, respectively) [34]; however, this result was not confirmed in a subsequent study [61]. In class II *BRAF* G469A-mutant cells, plixorafenib treatment resulted in cell line dependent cell growth inhibition while it had no effect on H2405 (*BRAF* L485_P490delinsY) or in H2087 (class II, L597V) cells (**Figure 2**). *In vivo*, plixorafenib treatment suppressed tumor growth, RAF-MEK-ERK signaling and tumor cell proliferation in H1755 xenograft NSCLC tumors (class II, G469A) [34].

In a phase 1/2a study in advanced solid tumors (NCT02428712), including patients with activating *BRAF* non-V600 alterations (e.g., point mutations, gene amplification, fusions, insertions or deletions), plixorafenib treatment resulted in complete tumor response, during 51.8 months, in a patient with *AGK-BRAF* fusion positive melanoma [63,64] (**Table 1**). A study to

assess the efficacy and safety of plixorafenib in cancer patients harboring *BRAF* V600E mutations or *BRAF* fusions (NCT05503797) is currently ongoing [65]. In 2022, the Food and Drug Administration (FDA) granted plixorafenib an orphan drug designation for the treatment of primary brain tumors and a fast-track designation for the treatment of patients with tumors harboring class I/II *BRAF* alterations, for whom all previous therapies have been exhausted (**Table 1, Figure 3**).

Second-generation BRAF inhibitors

Building on the limitations of first-generation RAF inhibitors in non-V600E mutations, next-generation αC-helix-IN inhibitors, and within this category RAF dimer inhibitors, have demonstrated activity against class II and III BRAF mutations in NSCLC, which are typically characterized by an α-C-helix-IN shift toward the active site [15,66].

Among these, exarafenib (KIN-2787), a selective inhibitor that targets the RAF protein dimer in the αC-helix-IN conformation, has shown anti-tumor efficacy in preclinical NSCLC models of *BRAF* class I, II (*BRAF* deletion delta NVTAP) and III (D594G), and in *in vitro* models of *NRAS*-driven melanoma [66]. A phase 1/1b study (NCT04913285, KN-8701) is currently ongoing to assess the safety and efficacy of exarafenib in cancer patients with *BRAF* class I, II or III mutations, including NSCLC, melanoma and other selected solid tumors (**Table 1**). Preliminary results showed therapeutically meaningful exarafenib exposures and promising tolerability in *BRAF* or *NRAS* alteration-driven solid tumors (**Table 1, Figure 3**). Among 48 evaluable patients, treatment with exarafenib monotherapy resulted in stable disease in NSCLC patients with class II (i.e., G469S, K601N) and class III (i.e., D594N) *BRAF* mutations; and in a partial tumor response in a *TEM105B:BRAF* fusion-positive NSCLC patient [67] (**Table 1**). Of note, the combination of exarafenib with binimetinib resulted in partial response in two cases of class II *BRAF*-altered solid tumors [68]. More recently, a preclinical study showed that the combination of belvarafenib (a pan-RAF dimer inhibitor) and binimetinib is significantly more effective than encorafenib plus binimetinib at inhibiting growth and MAPK signaling in several non-V600 *BRAF*-mutated cancer models [69].

BDTX-4933, a brain-penetrant orthosteric inhibitor that targets RAF hetero- and homodimers, showed activity in tumor cell lines with genetic alterations in *BRAF* (e.g., *BRAF* fusions, *BRAF* indels and L597V and L245F mutations), and anti-tumor activity in xenograft models of class I, II and III *BRAF* mutations [70]. BDTX-4933 is currently being evaluated in a phase 1 clinical study in patients with solid tumors harboring oncogenic *BRAF* and *RAS* alterations (NCT05786924) (**Table 1, Figure 3**).

Naporafenib (LXH254), a type II RAF inhibitor with a strong selectivity against BRAF and CRAF, mainly binds the inactive, DFG-out kinase configuration, and fosters the formation of BRAF/CRAF heterodimers [71]. Naporafenib showed more potent inhibition of NSCLC cell lines that harbor either G469A or L597V *BRAF* mutations, compared to dabrafenib or encorafenib [15] (**Figure 2**). In a single-agent naporafenib escalation study (NCT02607813), a partial response was observed in a patient affected by head and neck cancer with *HRAS* G13R and *BRAF* D594A mutations [72]. A recent study (NCT02974725) evaluated the safety, tolerability and antitumor activity of naporafenib in combination with either rineterkib (ERK1/2 kinase inhibitor), trametinib (MEK inhibitor) or ribociclib (CDK4/6 inhibitor), in patients with advanced *KRAS*- or *BRAF*-mutant NSCLC or *NRAS*-mutant melanoma (**Table 1, Figure 3**). Among 22 evaluable patients treated with naporafenib plus rineterkib, partial tumor responses were observed in two patients with NSCLC (*BRAF* K601E and *BRAF-MIPOL1* fusion), and stable disease was reported in three additional patients with *BRAF* non-V600E-mutant NSCLC [73] (**Table 1**). Moreover, combined naporafenib and trametinib treatment led to stable disease in 6 out of 11 NSCLC patients with *BRAF* non-V600E alterations (**Table 1**) [73]. In this study, patients with *KRAS*- or *BRAF*-mutant NSCLC harboring a *TP53* or *KEAP1* mutation in baseline ctDNA, or mutations in effectors of the PI3K or NRF2 pathways, had significantly shorter median OS than those without these co-mutations [73].

LY3009120 is a pan-RAF inhibitor that targets both monomeric and dimeric RAF. Although it promotes BRAF-CRAF dimerization, LY3009120 blocks the phosphorylation of downstream MEK and ERK, indicating its ability to effectively suppress the kinase activity of BRAF-CRAF heterodimers [74]. Treatment with LY3009120 resulted in cell growth inhibition and decreased

ERK phosphorylation in NSCLC cell lines of class II (L597V, G469A) or class III (G466V) *BRAF* mutations (**Figure 2**), and led to tumor growth arrest in a *BRAF* L597V-mutant NSCLC xenograft mouse model [75]. Furthermore, in a NSCLC xenograft mouse model of the *BRAF* deletion L485_P490delinsY, treatment with LY3009120 resulted in almost complete tumor growth regression, while vemurafenib treatment did not show antitumor activity [76]. In a phase 1 clinical trial (NCT02014116), 8 patients had stable disease, with no complete or partial tumor responses observed (**Figure 3**). Although plasma exposure levels were sufficient, anticipated pharmacodynamic effects were not observed [77].

Recent reports presented preclinical evidence on the activity of DCC-3084, a brain penetrant switch-control inhibitor of BRAF and CRAF. DCC-3084 inhibited cell proliferation in NSCLC cells that harbor the *BRAF* L485_P490delinsY deletion (H2405). Treatment with DCC-3084 resulted in tumor regression in melanoma xenografts harboring the *SKAP2-BRAF* fusion or the class III *BRAF* D594G mutation, as well as in *BRAF* ΔV487-P492A pancreatic adenocarcinoma [78,79]. A phase 1/2 study (NCT06287463) opened in 2024 to evaluated DCC-3084 alone or in combination with other antitumor agents in cancer patients with MAPK pathway alterations, including *BRAF* (**Table 1**).

Other RAF monomer-dimer inhibitors are currently in preclinical development. Ponatinib, an FDA-approved drug (**Figure 3**), effectively targets both BRAF monomers and dimers [80]. Based on the structural characteristics of the ponatib-BRAF V600E dimer, PHI1 (Ponatinib Hybrid Inhibitor 1) was developed to uncover the allosteric site and enhance inhibition of the second protomer when the first protomer is occupied. In H1666 (class III, G466V) and H2087 (class II, L597V) NSCLC cell lines, PHI1 achieves sub-micromolar p-ERK inhibition after pre-treatment with encorafenib [80]. Two more examples are the type II kinase BRAF inhibitors GNF-7 and SIJ1227, which induce apoptosis and decrease cellular migration and invasion of lung cancer cells harboring the class III *BRAF* G466V mutation [81] (**Figure 2**).

Non-kinase RAF inhibitors: Proteolysis-targeting chimeras (PROTACs) and peptide inhibitors

Long-term effectiveness of BRAF inhibitors is hindered by dimerization-dependent resistance mechanisms that result in RAF activation. Proteolysis-targeting chimeras (PROTACs) are dual-function molecules that promote the breakdown of specific proteins inside cells by simultaneously attaching to both the target protein and an E3 ubiquitin ligase, triggering the ubiquitination process and ultimately leading to the degradation of the target protein by the proteasome [82]. A vemurafenib-based PROTAC, SJF-0628, enables the degradation of all classes of BRAF mutants proteins, sparing WT RAF family members [83]. Treatment of NSCLC H1666 and CAL-12-T cell lines (class III, G466V) with SJF-0628 results in a dose-dependent loss in BRAF protein levels and a significant inhibition of ERK phosphorylation. Moreover, SJF-0628 demonstrates *in vivo* efficacy in a class II *BRAF*-mutant melanoma xenograft model [83].

In parallel, the allosteric BRAF peptide inhibitor (i.e., 10-mer Braftide) inhibits the kinase activity of BRAF homo- and heterodimers, including the *BRAF* G469A mutation, and avoids negative cooperativity and paradoxical activation. By targeting the dimer interface of BRAF, this scaffold promotes the degradation of both RAF and MEK proteins [84]. This mechanism is particularly relevant in the context of class III mutations. For example, Cope *et al.* recently demonstrated that heterodimers formed by BRAF D594G (one of the most prevalent class III mutations) and CRAF are more active than CRAF alone, with an activity comparable to that of the BRAF:CRAF heterodimer, one of the hallmarks of paradoxical MAPK activation [85]. In this setting, while pan-RAF ATP-competitive inhibitors led to short-term inhibition of HEK293 cells transiently transfected with BRAF D594G and CRAF, treatment with the 10-mer peptide resulted in sustained MAPK signaling inhibition and the degradation of the BRAF D594G:CRAF heterodimer [84,85].

Combined BRAF and MEK inhibition: kinase inhibitors and molecular glues

Although BRAF inhibitors lead to modest inhibition of signaling and growth of *BRAF* non-V600E mutations, they may still offer benefit when combined with MEK inhibitors.

In preclinical models, MEK inhibitors (e.g., trametinib, binimetinib, cobimetinib and mirdametinib) showed a stronger anti-proliferative effect compared to BRAF inhibitor

monotherapy in H2087 (class II, L597V), H1755 (class II, G469A) and H1666 (class III, G466V) cells [15,86,87] (**Figure 2**). Conflicting results have been observed in H1395 cells (class II, G469A) upon MEK inhibitor treatment [15,86]. In contrast, combined BRAF and MEK inhibition such as dabrafenib-trametinib, encorafenib-binimetinib or vemurafenib-cobimetinib exhibited higher effectiveness than single-agent, in these models. However, all these cell lines were found to be resistant to single-agent ERK inhibitors (e.g. ulixertinib and ravoxertinib) [15,86] (**Figure 2**).

In patients treated with MAPK-targeted therapies, progression-free survival (PFS) is higher in patients with class II compared to class III *BRAF* mutations, with response rates (RR) of 42% and 12%, respectively [40,52]. Of note, trametinib monotherapy led to stable disease in a lung adenocarcinoma patient harboring a *BRAF* D594G mutation [15] (**Table 2**). Moreover, a small number of case reports supports the clinical efficacy of combined dabrafenib plus trametinib treatment in NSCLC patients with class IIa (i.e., L597R [15], K601E [88], ex15 p.T599dup [89,90]), IIb (i.e., G469A /V [91,92]) and class III (i.e., G466V [93], N581S [94], D594G [95]) *BRAF* mutations, with time on treatment ranging from 2 to 30 months and few reported toxicities (**Table 2**). Besides kinase inhibitors, the RAF/MEK clamp avutometinib (VS-6766) inhibits MEK phosphorylation and induces a dominant negative RAF/MEK complex, blocking the phosphorylation of MEK by ARAF, BRAF and CRAF. In preclinical studies, this compound exhibited strong anti-tumor activity across diverse MAPK-altered tumor cell lines and showed synergistic effects on tumor cell viability when combined with other agents [96].

A phase 2 study evaluated the combination of avutometinib with defactinib (FAK inhibitor) in *KRAS*-mutant and *BRAF*-mutant NSCLC, including non-V600E *BRAF* mutations (RAMP202 trial, NCT04620330). The study is currently completed and the results on the non-V600E NSCLC cohort have not yet been presented (**Table 1**).

RAS-related inhibitors and receptor tyrosine kinase inhibitors

Owing to the limited efficacy of current MAPK pathway inhibitors in targeting non-V600 *BRAF* mutations, there is growing interest in therapeutic strategies aimed at targeting upstream modulators of the pathway. Indeed, in class III *BRAF* mutants, RAS is activated by RTK

signaling. Consequently, these mutants have proven to be sensitive to the inhibition of RAS signaling [37] as well as EGFR tyrosine kinase inhibitors (TKIs) [27,97].

In a large preclinical screening, cell lines with *BRAF* class II or III mutations were often sensitive to RMC-7977, a RAS(ON) multi-selective inhibitor [98]. A recent report demonstrated that patient-derived models of class II *BRAF* G469V-positive lung adenocarcinoma display sensitivity to multiple EGFR TKIs [99] (**Table 2, Figure 3**). This study showed that *BRAF* knockdown, but not *EGFR*, resulted in cell death in *BRAF* G469V-mutant cells, confirming G469V as the oncogenic driver in this context. Moreover, structural analyses revealed that the G469V substitution promotes binding of EGFR TKIs to BRAF [99]. In good agreement with both preclinical data and data from the Cancer Cell Line Encyclopedia, a recent case reports show that two patients harboring class III (D594N/G) mutated NSCLC had partial or complete responses to erlotinib after progression on standard chemotherapy [27].

Another strategy consists of targeting non-kinase modulators of the MAPK pathway, such as the nonreceptor protein tyrosine phosphatase SHP2 (which is encoded by *PTPN11*), recruited by RTK and acting as a positive regulator of the RAS-RAF-MEK-ERK pathway [100]. In this context, treatment with RMC-4550, an allosteric SHP2 inhibitor, showed efficacy against class III *BRAF* mutations *in vitro*, leading to suppression of both RAS–GTP and pERK levels in H1666 and CAL-12T (class III, G466V) NSCLC cells [101] . This study also revealed a dose-dependent tumor growth inhibition on repeated daily oral dosing of RMC-4550 *in vivo*, using NSCLC PDX models of class III D594N and N581 *BRAF* mutations [101]. Furthermore, the SHP2 inhibitor SHP099, combined with either dabrafenib or trametinib, led to a synergistic decrease in cell viability in H1666 cells (class III, G466V), compared to monotherapy [102].

Chemotherapy

Chemotherapy remains a standard treatment option for patients with class II/III *BRAF*-mutant NSCLC in the absence of approved targeted therapies. While some studies suggest that class II/III *BRAF* mutations may be associated with poorer responses to chemotherapy compared to class I mutations, the available evidence is inconsistent and primarily derived from

retrospective analyses. For example, Sakai et *al.* reported a shorter PFS in patients with class III mutations receiving platinum-based chemotherapy compared to those with class I mutations [21]. Similar trends were observed in two additional retrospective studies, which found significantly shorter PFS and OS in patients with class II/III mutations treated with platinum/pemetrexed chemotherapy [10,11]. However, these differences may be confounded by treatment selection bias, particularly due to the greater use of targeted therapies as first-line treatment for patients with class I *BRAF*-mutant NSCLC. Indeed, as mentioned above, when patients receiving targeted therapies were excluded from the analysis, no significant differences in outcomes were observed between *BRAF* mutation classes [11], in line with the results reported by two other retrospective studies [103,104]. Given the retrospective nature of these studies, potential biases, and lack of randomized data, these findings should be interpreted with caution. Prospective studies are needed to more definitively assess the impact of *BRAF* mutation class on chemotherapy efficacy.

Immunotherapy

Characterization of how *BRAF* mutation affects the tumor immune microenvironment (TIME), using gene expression analyses, revealed an enrichment of gene transcripts related to effective immune cells (i.e., CD8+ T cells, NK cells, M1 macrophages, cytotoxic cells and Th1 cells) and immune suppressors (i.e., Tregs, mast cells, and neutrophils) in *BRAF*-mutated NSCLC compared to *BRAF* wild-type tumors specimens [105]. In this study, a comparable TIME profile was observed between the *BRAF* V600E (n=14) and non-V600E (n=8) NSCLC tumors, in terms of cell type scores or CD8 T-cell /Treg ratios, indicating that these patients may benefit from treatment with immune checkpoint inhibitors (ICI) [105].

Furthermore, median PD-L1 expression in *BRAF*-mutant NSCLC is relatively higher compared to *EGFR/ALK*-driven [106] or unselected NSCLC tumors [107]. Although few studies have stratified PD-L1 levels by *BRAF* mutation class, available data suggest either similar [105,108] or lower [109] PD-L1 expression in non-V600 tumors compared to those with V600E. Tumor mutational burden (TMB) constitutes another biomarker for populations who would benefit from

immunotherapy. In a small cohort of 11 patients with *BRAF*-mutant NSCLC, a median TMB of 5 and 11 mutations per Mb was found in V600E and non-V600E tumors, respectively. [108]. More recently, a study showed a higher TMB in V600 tumors (3.5-4.9 mutations/Mb) compared to non-V600 tumors (8.8-9.6 mutations/Mb) [109], probably reflecting the past exposure to tobacco in patients from the latter group.

Analyses of the IMMUNOTARGET registry, which evaluated the activity of ICI across NSCLC harboring oncogenic alterations including *BRAF* V600E (n=17) and non-V600E (n=18) cases, showed that long-term responders were more frequent in *KRAS*, *MET* and *BRAF* subgroups, compared to *EGFR*, *ALK*, *HER2* and *RET* subgroups [106]. In line with these observations, *BRAF* mutations were associated with slightly better outcomes compared with *EGFR* mutations [106].

Multiple retrospective analyses, including those from the IMMUNOTARGET registry and other institutional cohorts, have evaluated the efficacy of ICI in *BRAF*-mutant NSCLC. Response rates to immunotherapy-based systemic treatment regimens are comparable between patients with V600E and non-V600E-positive NSCLC [105,108,110] . For instance, in a multi-institutional retrospective study where 56% (22/39) of patients with *BRAF*-mutant NSCLC received ICI-based treatment (i.e., nivolumab, n=11; pembrolizumab, n=10; and atezolizumab, n=1), the Objective Response Rate (ORR) was 33% in non-V600E and 25% in V600E cases [108]. These results mirror those by Guisier *et al.* (n=44) where response rates for V600 and non-V600 *BRAF* altered NSCLC were 26% and 35%, respectively [111]. In this study, the majority of non-V600E patients (11/18; 61%) received ICI as second-line treatment [111]. Recently, a real-world study (V600E, n=35; non-V600E, n=18) reported the efficacy of ICI-based regimens in the first-line setting, with ORR of 38% for V600E and 43% for non-V600E patients, with comparable rates of adverse events between these subgroups [110]. Overall, nearly half of patients present progressive disease (PD) as best response to ICI either in patients with *BRAF*-mutant NSCLC [106] or specifically in patients with *BRAF* non-V600E [111].

Median PFS of ICI-based treatment varies between studies but is generally short (around 3-4 months), with slight numeric differences favoring non-V600E patients in some cohorts. In the study of Dudnik *et al.*, of the 18 patients who progressed on ICI by the time of analysis, median

PFS was 4.1 months in non-V600E, compared to 3.7 months in V600E [108]. Similar observations were reported by two independent studies [110,111]. Furthermore, in cases from the IMMUNOTARGET registry, PFS was numerically longer, but not statistically significant, in non-V600E (4.1 months) compared to V600E (1.8 months) [106]. Importantly, in this study, PFS was positively associated with smoking status in *BRAF*-mutant NSCLC, as smokers had significantly better PFS (4.1 months) than never-smokers (1.9 months) [106].

Although treatment with ICI-based regiments results in similar OS patients with wild-type (n=358) and *BRAF*-mutant (n=59) NSCLC [105], *BRAF*-mutant NSCLC patients treated with ICI present higher median OS compared to those with driver alterations in *EGFR* and *ALK* [106]. Interestingly, survival analyses according to *BRAF* mutation type show either lower [111] or equivalent [112] OS in patients with non-V600E mutations compared to V600E patients.

Overall, these reports show that response rates to ICI are comparable between V600E and non-V600E, and similar to that observed in non-oncogene driven NSCLC [111,113], suggesting that checkpoint inhibitors can be considered for both mutation classes. However, these observations are based on generally small, retrospective studies and are not consistent [109]. Larger, prospective studies are needed to better characterize the tumor microenvironment of non-V600 mutant tumors, determining whether ICI outcomes depend on *BRAF* mutation class and to assess whether chemo-immunotherapy regimens are to be preferred over ICI monotherapy in non-V600 cases, given the generally poor performance of the latter in oncogene-driven NSCLC [113].

Emerging therapeutic strategies

Antibody–drug conjugates in BRAF-mutant NSCLC

Antibody–drug conjugates (ADCs) represent an innovative therapeutic approach in solid tumors, including NSCLC, with growing interest in certain oncogene-driven subgroups. Datopotamab deruxtecan (Dato-DXd), a TROP2-directed ADC, has demonstrated notable clinical activity in advanced non-squamous NSCLC, regardless of the presence of actionable

genomic alterations [114,115]. Results from the TROPION-Lung01 and TROPION-Lung05 trials showed that Dato-DXd improved overall survival and response rates in heavily pretreated patients, including those harboring *EGFR* and *ALK* alterations. Although specific efficacy data for patients with *BRAF*-mutant NSCLC have not been reported, the inclusion of oncogene-driven subtypes in these trials along with the widespread expression of TROP2 in NSCLC, supports investigating Dato-DXd in this patient population. Further prospective studies are needed to define the role of ADCs, such as Dato-DXd, in *BRAF*-mutant NSCLC, particularly in class II/III alterations, which currently lack effective targeted treatment options.

Adoptive cell therapy

Considering the increasing impact and development of adoptive cell therapies, tumor-infiltrating lymphocyte (TIL) therapy has emerged as a promising strategy in *BRAF*-driven malignancies, notably in melanoma and, more recently, in NSCLC. In melanoma, TIL-based therapy has demonstrated durable responses even in heavily pretreated patients with *BRAF*-mutant tumors, including those resistant to immune checkpoint blockade and targeted therapy. In NSCLC, a recent phase 1 clinical trial explored the feasibility and activity of TILs in patient's refractory to anti-PD-1 therapy. Although *BRAF* mutations were not the primary selection criterion, the trial confirmed that TILs can be expanded from NSCLC tumors and may elicit clinical responses, including durable complete responses in some patients, and also in patients with *KRAS*- or *EGFR*-mutant NSCLC [116]. Despite the limited number of patients treated and the absence of dedicated cohorts for *BRAF*-mutant lung cancers, these initial signs of efficacy support the inclusion of adoptive cell therapies among the investigational strategies in *BRAF*-driven tumors.

Acquired *BRAF* mutations as a resistance mechanism to targeted therapies in other oncogene-driven NSCLC

Genetic alterations in *BRAF* can arise under selective pressure from TKIs targeting EGFR, ALK, ROS1, or RET, and serve as bypass mechanisms to reactivate downstream MAPK

signaling. Multiple studies, based on case reports or small cohorts, have reported class II/III mutations as well as other *BRAF* genetic alterations in post-treatment biopsies from patients with oncogene-addicted NSCLC [43,94,117–122] (**Table 3**). Acquired *BRAF* alterations may be subclonal and coexist with the primary driver or other resistance alterations (e.g., *EGFR* mutation, *MET* amplification) [123,124]. The presence of class II/III *BRAF* mutations in this context hinders the identification of treatment strategies beyond disease progression, as approved BRAF inhibitors (designed for V600E) are often ineffective against these mutations. In this setting, longitudinal genetic profiling using liquid biopsies might help capture the dynamic evolution of BRAF-mediated resistance and support the rational design of combination therapies to prevent or overcome such adaptations.

Conclusions and future perspectives

Patients with NSCLC harboring class II/III *BRAF* mutations represent a clinically relevant yet underexplored subgroup. Unlike the more homogeneous *BRAF* V600E mutation, these alterations are biologically diverse, often co-occurring with other genomic events (e.g., in *KRAS*, *NF1*, *STK11* tumors) that may shape oncogenic signaling and therapeutic response. Epidemiologically, non-V600 mutations predominate in NSCLC, particularly among smokers, and their distribution differs markedly from other tumor types, reflecting potential distinct mutational processes likely dependent on the cell-of-origin. However, the biology of these alterations remains poorly understood, and preclinical models that faithfully recapitulate class II/III or structural *BRAF* alterations are still limited. This lack of biological insight, combined with their relatively low prevalence, contributes to their underrepresentation in clinical studies and hampers the development of evidence-based therapeutic strategies.

Current management of patients with non-V600 *BRAF*-mutant NSCLC relies mainly on chemotherapy and immunotherapy, with only anecdotal benefit from MAPK pathway inhibitors in case reports and early-phase clinical trials. While outcomes remain modest, several promising strategies are being explored, including next-generation RAF inhibitors (dimer inhibitors, pan-RAF inhibitors, PROTACs), rational combinations with MEK, EGFR or SHP2

inhibitors, and immunotherapy-based regimens. In addition, innovative approaches such as ADCs (e.g., datopotamab deruxtecan) and adoptive cell therapies (e.g., TILs) are emerging as potential options that warrant further clinical evaluation.

Altogether, these advances highlight the pressing need of developing collaborative research networks as well as of designing large, prospective, multicenter studies integrated with real word data and biologically informed preclinical models, to better guide treatment decisions and ultimately improve outcomes for patients with NSCLC harboring non-V600 *BRAF* alterations.

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FIGURE LEGENDS

Figure 1 : **Prevalence and structural distribution of *BRAF* mutations in NSCLC. A**. Frequency of oncogenic *BRAF* mutations and alterations in *BRAF*-mutant lung tumors and distribution among the 3 *BRAF* mutation classes. Other *BRAF* alterations include fusions, insertions, deletions and amplifications. Data (extracted from cBioPortal) are derived from the AACR GENIE and MSK-IMPACT projects (n = 19,666 sequenced tumors). Duplicate entries with the same sample ID and mutation were removed (assuming each sample ID represents a unique patient). Mutation class and oncogenic/non-oncogenic proportions were calculated relative to the total number of oncogenic *BRAF* mutations and total *BRAF*-mutated samples, respectively. **B**. Distribution of single-nucleotide mutations throughout the BRAF protein sequence and within the different functional domains. CR : conserved region; DIF : the dimerization interface; CL : catalytic loop; AS : activation segment.

Figure 2 : Classification of *BRAF* mutations and sensitivity to different inhibitors in *BRAF*-mutated NSCLC. In the upper part of the figure, MAP kinase signaling and kinase activity of the 3 *BRAF* mutation classes. Class I, with high kinase activity as monomers and RAS-independent; Class II, active as dimers with moderate kinase activity, also RAS-independent; and Class III, with low or absent kinase activity, requiring RAS activation and dimerization with wild-type RAF. In the lower part of figure, half-maximal inhibitory concentration (IC_{50}) values of different BRAF, MEK, ERK, EGFR and SHP2 inhibitors in the commercially available *BRAF*-mutated lung cancer cell lines. Green indicates compounds with high efficacy (IC_{50} in the nanomolar range), while orange to red represents compounds with lower efficacy (IC_{50} in the micromolar range). Figure created with BioRender.com.

Figure 3: MAPK signaling pathway and the various classes of targeted therapies developed to interfere with its components in *BRAF*-mutated NSCLC. Compounds are color-coded according to their clinical development stage: green for clinically approved compounds for *BRAF* V600E cases, orange for those approved for other indications, blue for

phase II, purple for phase I, and red for terminated clinical trials. EGFR inhibitors listed according to Huo *et al.,* 2022 [99]. Figure created with BioRender.com.

References

1. Lung cancer statistics | World Cancer Research Fund International. WCRF International. Accessed October 29, 2024. https://www.wcrf.org/cancer-trends/lung-cancer-statistics/

2. Rosell R, Karachaliou N. Large-scale screening for somatic mutations in lung cancer. *Lancet*. 2016;387(10026):1354-1356. doi:10.1016/S0140-6736(15)01125-3

3. Dankner M, Rose AAN, Rajkumar S, Siegel PM, Watson IR. Classifying BRAF alterations in cancer: new rational therapeutic strategies for actionable mutations. *Oncogene*. 2018;37(24):3183-3199. doi:10.1038/s41388-018-0171-x

4. AACR Project GENIE Consortium. AACR Project GENIE: Powering Precision Medicine through an International Consortium. *Cancer Discov*. 2017;7(8):818-831. doi:10.1158/2159-8290.CD-17-0151

5. Owsley J, Stein MK, Porter J, et al. Prevalence of class I–III BRAF mutations among 114,662 cancer patients in a large genomic database. *Exp Biol Med (Maywood)*. 2021;246(1):31-39. doi:10.1177/1535370220959657

6. Cardarella S, Ogino A, Nishino M, et al. Clinical, pathologic, and biologic features associated with BRAF mutations in non-small cell lung cancer. *Clin Cancer Res*. 2013;19(16):4532-4540. doi:10.1158/1078-0432.CCR-13-0657

7. Sheikine Y, Pavlick D, Klempner SJ, et al. BRAF in Lung Cancers: Analysis of Patient Cases Reveals Recurrent BRAF Mutations, Fusions, Kinase Duplications, and Concurrent Alterations. *JCO Precis Oncol*. 2018;(2):1-15. doi:10.1200/PO.17.00172

8. Seker-Cin H, Tay TKY, Kazdal D, et al. Analysis of rare fusions in NSCLC: Genomic architecture and clinical implications. *Lung Cancer*. 2023;184:107317. doi:10.1016/j.lungcan.2023.107317

9. Lim GHT, Balbi KJ, Poskitt B, Bennett P, Moore DA. Prevalence and breakdown of non-small cell lung cancer BRAF driver mutations in a large UK cohort. *Lung Cancer*. 2022;173:71-74. doi:10.1016/j.lungcan.2022.09.008

10. Perrone F, Mazzaschi G, Minari R, et al. Multicenter Observational Study on Metastatic Non-Small Cell Lung Cancer Harboring BRAF Mutations: Focus on Clinical Characteristics and Treatment Outcome of V600E and Non-V600E Subgroups. *Cancers (Basel)*. 2022;14(8):2019. doi:10.3390/cancers14082019

11. Dagogo-Jack I, Martinez P, Yeap BY, et al. Impact of BRAF Mutation Class on Disease Characteristics and Clinical Outcomes in BRAF-mutant Lung Cancer. *Clin Cancer Res*. 2019;25(1):158-165. doi:10.1158/1078-0432.CCR-18-2062

12. Paik PK, Arcila ME, Fara M, et al. Clinical Characteristics of Patients With Lung Adenocarcinomas Harboring BRAF Mutations. *Journal of Clinical Oncology*. 2011;29(15):2046. doi:10.1200/JCO.2010.33.1280

13. Tissot C, Couraud S, Tanguy R, Bringuier PP, Girard N, Souquet PJ. Clinical characteristics and outcome of patients with lung cancer harboring BRAF mutations. *Lung Cancer*. 2016;91:23-28. doi:10.1016/j.lungcan.2015.11.006

14. Özgü E, Kaplan BG, Sivakumar S, et al. Therapeutic vulnerabilities and pan-cancer landscape of BRAF class III mutations in epithelial solid tumors. *BJC Rep*. 2024;2(1):1-11. doi:10.1038/s44276-024-00086-2

15. Negrao MV, Raymond VM, Lanman RB, et al. Molecular Landscape of BRAF-Mutant NSCLC Reveals an Association Between Clonality and Driver Mutations and Identifies Targetable Non-V600 Driver Mutations. *J Thorac Oncol*. 2020;15(10):1611-1623. doi:10.1016/j.jtho.2020.05.021

16. Niu J, Flannery CA, Hall DW, et al. BRAF mutation classes and co-occurring mutations in NSCLC. *JCO*. 2022;40(16_suppl):9083-9083. doi:10.1200/JCO.2022.40.16_suppl.9083

17. Laguna JC, García-Pardo M, Alessi J, et al. Geographic differences in lung cancer: focus on carcinogens, genetic predisposition, and molecular epidemiology. *Ther Adv Med Oncol*. 2024;16:17588359241231260. doi:10.1177/17588359241231260

18. Marchetti A, Felicioni L, Malatesta S, et al. Clinical features and outcome of patients with non-small-cell lung cancer harboring BRAF mutations. *J Clin Oncol*. 2011;29(26):3574-3579. doi:10.1200/JCO.2011.35.9638

19. Auliac JB, Bayle S, Vergnenegre A, et al. Patients with non-small-cell lung cancer harbouring a BRAF mutation: a multicentre study exploring clinical characteristics, management, and outcomes in a real-life setting: EXPLORE GFPC 02-14. *Curr Oncol*. 2018;25(5):e398-e402. doi:10.3747/co.25.3945

20. Litvak AM, Paik PK, Woo KM, et al. Clinical Characteristics and Course of 63 Patients with BRAF Mutant Lung Cancers. *Journal of Thoracic Oncology*. 2014;9(11):1669-1674. doi:10.1097/JTO.0000000000000344

21. Sakai T, Matsumoto S, Ueda Y, et al. Clinicogenomic Features and Targetable Mutations in NSCLCs Harboring *BRAF* Non-V600E Mutations: A Multi-Institutional Genomic Screening Study (LC-SCRUM-Asia). *Journal of Thoracic Oncology*. 2023;18(11):1538-1549. doi:10.1016/j.jtho.2023.07.024

22. Mezquita L, Benito A, Ruano-Raviña A, et al. Indoor Radon in EGFR- and BRAF-Mutated and ALK-Rearranged Non-Small-Cell Lung Cancer Patients. *Clin Lung Cancer*. 2019;20(4):305-312.e3. doi:10.1016/j.cllc.2019.04.009

23. Mezquita L, Turrisi F, Mauer ME, et al. 1508TiP The 1920-EORTC-BIORADON study: Molecular characterization of non-small cell lung cancer (NSCLC) and indoor radon exposure in Europe. *Annals of Oncology*. 2023;34:S849. doi:10.1016/j.annonc.2023.09.2539

24. Mezquita L, Kuang Z, Sivakumar S, et al. MA14.08 Pathogenic Germline Variants in Patients with Non-Small Cell Lung Cancer (NSCLC) Detected by Tissue Comprehensive Genomic Profiling. *Journal of Thoracic Oncology*. 2023;18(11):S151. doi:10.1016/j.jtho.2023.09.217

25. Wang X, Ricciuti B, Nguyen T, et al. Association between Smoking History and Tumor Mutation Burden in Advanced Non–Small Cell Lung Cancer. *Cancer Research*. 2021;81(9):2566-2573. doi:10.1158/0008-5472.CAN-20-3991

26. Laughery MF, Brown AJ, Bohm KA, et al. Atypical UV Photoproducts Induce Non-canonical Mutation Classes Associated with Driver Mutations in Melanoma. *Cell Reports*. 2020;33(7):108401. doi:10.1016/j.celrep.2020.108401

27. Di Federico A, Angelicola S, Frascino M, et al. Clinical and Preclinical Activity of EGFR Tyrosine Kinase Inhibitors in Non-Small-Cell Lung Cancer Harboring BRAF Class 3 Mutations. *JCO Precis Oncol*. 2024;8:e2400240. doi:10.1200/PO.24.00240

28. Laguna JC, Tagliamento M, Lambertini M, Hiznay J, Mezquita L. Tackling Non-Small Cell Lung Cancer in Young Adults: From Risk Factors and Genetic Susceptibility to Lung Cancer Profile and Outcomes. *Am Soc Clin Oncol Educ Book*. 2024;44(3):e432488. doi:10.1200/EDBK_432488

29. Mendoza DP, Dagogo-Jack I, Chen T, et al. Imaging characteristics of BRAF-mutant non-small cell lung cancer by functional class. *Lung Cancer*. 2019;129:80-84. doi:10.1016/j.lungcan.2019.01.007

30. Murciano-Goroff YR, Pak T, Mondaca S, et al. Immune biomarkers and response to checkpoint inhibition of BRAFV600 and BRAF non-V600 altered lung cancers. *Br J Cancer*. 2022;126(6):889-898. doi:10.1038/s41416-021-01679-1

31. Lavoie H, Therrien M. Regulation of RAF protein kinases in ERK signalling. *Nat Rev Mol Cell Biol*. 2015;16(5):281-298. doi:10.1038/nrm3979

32. Martinez Fiesco JA, Durrant DE, Morrison DK, Zhang P. Structural insights into the BRAF monomer-to-dimer transition mediated by RAS binding. *Nat Commun*. 2022;13(1):486. doi:10.1038/s41467-022-28084-3

33. Nieto P, Ambrogio C, Esteban-Burgos L, et al. A Braf kinase-inactive mutant induces lung adenocarcinoma. *Nature*. 2017;548(7666):239-243. doi:10.1038/nature23297

34. Okimoto R, Lin L, Olivas V, et al. Preclinical efficacy of a RAF inhibitor that evades paradoxical MAPK pathway activation in protein kinase BRAF -mutant lung cancer. *Proceedings of the National Academy of Sciences*. 2016;113:201610456. doi:10.1073/pnas.1610456113

35. Lavoie H, Jin T, Lajoie D, et al. BRAF oncogenic mutants evade autoinhibition through a common mechanism. *Science*. 2025;388(6750):eadp2742. doi:10.1126/science.adp2742

36. Yao Z, Torres NM, Tao A, et al. BRAF mutants evade ERK dependent feedback by different mechanisms that determine their sensitivity to pharmacologic inhibition. *Cancer Cell*. 2015;28(3):370-383. doi:10.1016/j.ccell.2015.08.001

37. Yao Z, Yaeger R, Rodrik-Outmezguine VS, et al. Tumours with class 3 BRAF mutants are sensitive to the inhibition of activated RAS. *Nature*. 2017;548(7666):234-238. doi:10.1038/nature23291

38. Tabbò F, Pisano C, Mazieres J, et al. How far we have come targeting BRAF-mutant non-small cell lung cancer (NSCLC). *Cancer Treatment Reviews*. 2022;103:102335. doi:10.1016/j.ctrv.2021.102335

39. Noeparast A, Teugels E, Giron P, et al. Non-V600 BRAF mutations recurrently found in lung cancer predict sensitivity to the combination of Trametinib and Dabrafenib. *Oncotarget*. 2017;8(36):60094-60108. doi:10.18632/oncotarget.11635

40. Dankner M, Lajoie M, Moldoveanu D, et al. Dual MAPK Inhibition Is an Effective Therapeutic Strategy for a Subset of Class II BRAF Mutant Melanomas. *Clinical Cancer Research*. 2018;24(24):6483-6494. doi:10.1158/1078-0432.CCR-17-3384

41. Yang Q, Wang B, Meng X, et al. Unveiling the BRAF fusion structure variations through DNA and RNA sequencing. *Br J Cancer*. Published online April 19, 2025. doi:10.1038/s41416-025-02998-3

42. Schrock AB, Zhu VW, Hsieh WS, et al. Receptor Tyrosine Kinase Fusions and BRAF Kinase Fusions are Rare but Actionable Resistance Mechanisms to EGFR Tyrosine Kinase Inhibitors. *J Thorac Oncol*. 2018;13(9):1312-1323. doi:10.1016/j.jtho.2018.05.027

43. Vojnic M, Kubota D, Kurzatkowski C, et al. Acquired BRAF Rearrangements Induce Secondary Resistance to EGFR therapy in EGFR-Mutated Lung Cancers. *J Thorac Oncol*. 2019;14(5):802-815. doi:10.1016/j.jtho.2018.12.038

44. Dagogo-Jack I, Piotrowska Z, Cobb R, et al. Response to the Combination of Osimertinib and Trametinib in a Patient With EGFR-Mutant NSCLC Harboring an Acquired BRAF Fusion. *J Thorac Oncol*. 2019;14(10):e226-e228. doi:10.1016/j.jtho.2019.05.046

45. Wang CY, Hsia JY, Li CH, Ho CC, Chao WR, Wu MF. Lung Adenocarcinoma With Primary LIMD1-BRAF Fusion Treated With MEK Inhibitor: A Case Report. *Clin Lung Cancer*. 2021;22(6):e878-e880. doi:10.1016/j.cllc.2021.05.003

46. Piotrowska Z, Isozaki H, Lennerz JK, et al. Landscape of acquired resistance to osimertinib in EGFR-mutant NSCLC and clinical validation of combined EGFR and RET inhibition with osimertinib and BLU-667 for acquired RET fusion. *Cancer Discov*. 2018;8(12):1529-1539. doi:10.1158/2159-8290.CD-18-1022

47. Horn L, Bauml J, Forde PM, et al. Real-world treatment patterns and survival of patients with BRAF V600-mutated metastatic non-small cell lung cancer. *Lung Cancer*. 2019;128:74-90. doi:10.1016/j.lungcan.2018.12.003

48. Planchard D, Besse B, Groen HJM, et al. Phase 2 Study of Dabrafenib Plus Trametinib in Patients With BRAF V600E-Mutant Metastatic NSCLC: Updated 5-Year Survival Rates and Genomic Analysis. *J Thorac Oncol*. 2022;17(1):103-115. doi:10.1016/j.jtho.2021.08.011

49. Riely GJ, Smit EF, Ahn MJ, et al. Phase II, Open-Label Study of Encorafenib Plus Binimetinib in Patients With BRAFV600-Mutant Metastatic Non-Small-Cell Lung Cancer. *J Clin Oncol*. 2023;41(21):3700-3711. doi:10.1200/JCO.23.00774

50. Swalduz A, Beau-Faller M, Planchard D, et al. Real-world efficacy of the dabrafenib-trametinib (D-T) combination in BRAF V600E-mutated metastatic non-small cell lung cancer (NSCLC): Results from the IFCT-2004 BLaDE cohort. *Lung Cancer*. 2025;199:108038. doi:10.1016/j.lungcan.2024.108038

51. Mazieres J, Cropet C, Montané L, et al. Vemurafenib in non-small-cell lung cancer patients with BRAFV600 and BRAFnonV600 mutations. *Ann Oncol*. 2020;31(2):289-294. doi:10.1016/j.annonc.2019.10.022

52. Dankner M, Wang Y, Fazelzad R, et al. Clinical Activity of Mitogen-Activated Protein Kinase-Targeted Therapies in Patients With Non-V600 BRAF-Mutant Tumors. *JCO Precis Oncol*. 2022;6:e2200107. doi:10.1200/PO.22.00107

53. Rustgi N, Maria A, Toumbacaris N, et al. Combined RAF and MEK Inhibition to Treat Activated Non-V600 BRAF-Altered Advanced Cancers. *The Oncologist*. 2024;29(1):15-24. doi:10.1093/oncolo/oyad247

54. Yang H, Higgins B, Kolinsky K, et al. RG7204 (PLX4032), a selective BRAFV600E inhibitor, displays potent antitumor activity in preclinical melanoma models. *Cancer Res*. 2010;70(13):5518-5527. doi:10.1158/0008-5472.CAN-10-0646

55. Karoulia Z, Wu Y, Ahmed TA, et al. An Integrated Model of RAF Inhibitor Action Predicts Inhibitor Activity against Oncogenic BRAF Signaling. *Cancer Cell*. 2016;30(3):485-498. doi:10.1016/j.ccell.2016.06.024

56. Gautschi O, Milia J, Cabarrou B, et al. Targeted Therapy for Patients with BRAF-Mutant Lung Cancer: Results from the European EURAF Cohort. *J Thorac Oncol*. 2015;10(10):1451-1457. doi:10.1097/JTO.0000000000000625

57. Lito P, Rosen N, Solit DB. Tumor adaptation and resistance to RAF inhibitors. *Nat Med*. 2013;19(11):1401-1409. doi:10.1038/nm.3392

58. Hatzivassiliou G, Song K, Yen I, et al. RAF inhibitors prime wild-type RAF to activate the MAPK pathway and enhance growth. *Nature*. 2010;464(7287):431-435. doi:10.1038/nature08833

59. Poulikakos PI, Zhang C, Bollag G, Shokat KM, Rosen N. RAF inhibitors transactivate RAF dimers and ERK signalling in cells with wild-type BRAF. *Nature*. 2010;464(7287):427-430. doi:10.1038/nature08902

60. Zhang C, Spevak W, Zhang Y, et al. RAF inhibitors that evade paradoxical MAPK pathway activation. *Nature*. 2015;526(7574):583-586. doi:10.1038/nature14982

61. Yao Z, Gao Y, Su W, et al. RAF inhibitor PLX8394 selectively disrupts BRAF dimers and RAS-independent BRAF-mutant-driven signaling. *Nat Med*. 2019;25(2):284-291. doi:10.1038/s41591-018-0274-5

62. Hanrahan AJ, Chen Z, Rosen N, Solit DB. BRAF - a tumour-agnostic drug target with lineage-specific dependencies. *Nat Rev Clin Oncol*. 2024;21(3):224-247. doi:10.1038/s41571-023-00852-0

63. De La Fuente MI, Rodon Ahnert J, Yaeger R, et al. Safety and efficacy of the novel BRAF inhibitor FORE8394 in patients with advanced solid and CNS tumors: Results from a phase 1/2a study. *JCO*. 2023;41(16_suppl):3006-3006. doi:10.1200/JCO.2023.41.16_suppl.3006

64. Yaeger R, Sherman EJ, Fuente M de la, et al. Abstract 3248: Circulating tumor DNA analysis of patients with BRAF-mutated advanced unresectable solid tumors treated with plixorafenib (FORE8394/PLX8394) in phase 1/2a study. *Cancer Res*. 2025;85(8_Supplement_1):3248. doi:10.1158/1538-7445.AM2025-3248

65. FORTE: A phase 2 master protocol assessing plixorafenib for BRAF-altered cancers. - ASCO. Accessed October 30, 2025. https://www.asco.org/abstracts-presentations/ABSTRACT488346

66. Chen YK, Kanouni T, Arnold LD, et al. The Discovery of Exarafenib (KIN-2787): Overcoming the Challenges of Pan-RAF Kinase Inhibition. *J Med Chem*. 2024;67(3):1747-1757. doi:10.1021/acs.jmedchem.3c01830

67. Spira A, Gambardella V, Kato S, et al. Abstract CT032: Trials in progress: a global phase 1/1b clinical trial evaluating exarafenib (KIN-2787), a highly selective pan-RAF inhibitor, in adult patients with BRAF-altered solid tumors and NRAS mutant melanoma. *Cancer Research*. 2023;83(8_Supplement):CT032. doi:10.1158/1538-7445.AM2023-CT032

68. Cassier P, Mehnert JM, Kim R, et al. 613MO A phase I clinical trial evaluating exarafenib, a selective pan-RAF inhibitor in combination with binimetinib in NRAS-mutant (NRASMut) melanoma (Mel) & BRAF-altered solid tumors. *Annals of Oncology*. 2024;35:S491-S492. doi:10.1016/j.annonc.2024.08.680

69. Elkholi IE, Fraser S, Biondini M, et al. Exploring the efficacy and mechanism of action of combined pan-Raf and MEK inhibition in halting the growth of non-V600 BRAF mutated tumors. *JCO*. 2025;43(16_suppl):3117-3117. doi:10.1200/JCO.2025.43.16_suppl.3117

70. Ng PY, Han YC, Ogawa LS, et al. Preclinical efficacy of BDTX-4933, a brain penetrant MasterKey inhibitor targeting oncogenic BRAF Class I/II/III mutations. *European Journal of Cancer*. 2022;174:S86. doi:10.1016/S0959-8049(22)01027-9

71. Monaco KA, Delach S, Yuan J, et al. LXH254, a Potent and Selective ARAF-Sparing Inhibitor of BRAF and CRAF for the Treatment of MAPK-Driven Tumors. *Clin Cancer Res*. 2021;27(7):2061-2073. doi:10.1158/1078-0432.CCR-20-2563

72. Janku F, Kim TM, Iyer G, et al. First-in-human study of naporafenib (LXH254) with or without spartalizumab in adult patients with advanced solid tumors harboring MAPK signaling pathway alterations. *Eur J Cancer*. 2024;196:113458. doi:10.1016/j.ejca.2023.113458

73. Planchard D, Wolf J, Solomon B, et al. A phase Ib study of the combination of naporafenib with rineterkib or trametinib in patients with advanced and metastatic KRAS- or BRAF-mutant non-small cell lung cancer. *Lung Cancer*. 2024;197:107964. doi:10.1016/j.lungcan.2024.107964

74. Peng SB, Henry JR, Kaufman MD, et al. Inhibition of RAF Isoforms and Active Dimers by LY3009120 Leads to Anti-tumor Activities in RAS or BRAF Mutant Cancers. *Cancer Cell*. 2015;28(3):384-398. doi:10.1016/j.ccell.2015.08.002

75. Miyauchi S, Shien K, Takeda T, et al. Antitumor Effects of Pan-RAF Inhibitor LY3009120 Against Lung Cancer Cells Harboring Oncogenic BRAF Mutation. *Anticancer Res*. 2020;40(5):2667-2673. doi:10.21873/anticanres.14237

76. Chen SH, Zhang Y, Van Horn RD, et al. Oncogenic BRAF Deletions That Function as Homodimers and Are Sensitive to Inhibition by RAF Dimer Inhibitor LY3009120. *Cancer Discov*. 2016;6(3):300-315. doi:10.1158/2159-8290.CD-15-0896

77. Sullivan RJ, Hollebecque A, Flaherty KT, et al. A Phase I Study of LY3009120, a Pan-RAF Inhibitor, in Patients with Advanced or Metastatic Cancer. *Mol Cancer Ther*. 2020;19(2):460-467. doi:10.1158/1535-7163.MCT-19-0681

78. Al-Ani G, Bulfer SL, Zwicker JD, et al. Abstract 593: DCC-3084, a brain penetrant RAF dimer inhibitor, broadly inhibits BRAF class I, II, and III alterations leading to growth inhibition of intracranially implanted tumors in preclinical models. *Cancer Research*. 2024;84(6_Supplement):593. doi:10.1158/1538-7445.AM2024-593

79. Bulfer SL, Bourdonnec BL, Zwicker JD, et al. Abstract 4045: DCC-3084, a RAF dimer inhibitor, broadly inhibits BRAF class I, II, III, BRAF fusions, and RAS-driven solid tumors leading to tumor regression in preclinical models. *Cancer Research*. 2023;83(7_Supplement):4045. doi:10.1158/1538-7445.AM2023-4045

80. Cotto-Rios XM, Agianian B, Gitego N, et al. Inhibitors of BRAF dimers using an allosteric site. *Nat Commun*. 2020;11(1):4370. doi:10.1038/s41467-020-18123-2

81. Choi SH, Shin I, Kim N, Nam Y, Sim T. The first small molecules capable of strongly suppressing proliferation of cancer cells harboring BRAF class I/II/III mutations. *Biochemical and Biophysical Research Communications*. 2020;532(2):315-320. doi:10.1016/j.bbrc.2020.07.110

82. Rutherford KA, McManus KJ. PROTACs: Current and Future Potential as a Precision Medicine Strategy to Combat Cancer. *Mol Cancer Ther*. 2024;23(4):454-463. doi:10.1158/1535-7163.MCT-23-0747

83. Alabi S, Jaime-Figueroa S, Yao Z, et al. Mutant-selective degradation by BRAF-targeting PROTACs. *Nat Commun*. 2021;12(1):920. doi:10.1038/s41467-021-21159-7

84. Gunderwala AY, Nimbvikar AA, Cope NJ, Li Z, Wang Z. Development of Allosteric BRAF Peptide Inhibitors Targeting the Dimer Interface of BRAF. *ACS Chem Biol*. 2019;14(7):1471-1480. doi:10.1021/acschembio.9b00191

85. Cope NJ, Novak B, Liu Z, et al. Analyses of the oncogenic BRAFD594G variant reveal a kinase-independent function of BRAF in activating MAPK signaling. *J Biol Chem*. 2020;295(8):2407-2420. doi:10.1074/jbc.RA119.011536

86. Noeparast A, Giron P, De Brakeleer S, et al. Type II RAF inhibitor causes superior ERK pathway suppression compared to type I RAF inhibitor in cells expressing different BRAF mutant types recurrently found in lung cancer. *Oncotarget*. 2018;9(22):16110-16123. doi:10.18632/oncotarget.24576

87. Pratilas CA, Hanrahan AJ, Halilovic E, et al. Genetic predictors of MEK-dependence in non-small cell lung cancer. *Cancer Res*. 2008;68(22):9375-9383. doi:10.1158/0008-5472.CAN-08-2223

88. Su PL, Lin CY, Chen YL, Chen WL, Lin CC, Su WC. Durable Response to Combined Dabrafenib and Trametinib in a Patient With BRAF K601E Mutation-Positive Lung Adenocarcinoma: A Case Report. *JTO Clin Res Rep*. 2021;2(8):100202. doi:10.1016/j.jtocrr.2021.100202

89. Jiang L, Yang P, Liu Y, Li J. BRAF/MEK-targeted therapy in BRAF ex15 p.T599dup mutation-driven NSCLC: a case report. *J Cancer Res Clin Oncol*. 2024;150(3):162. doi:10.1007/s00432-024-05675-9

90. Turshudzhyan A, Vredenburgh J. A Rare p.T599dup BRAF Mutant NSCLC in a Non-Smoker. *Current Oncology*. 2021;28(1):196-202. doi:10.3390/curroncol28010021

91. Dagogo-Jack I. Durable Response to Dabrafenib Combined With Trametinib in a Patient With NSCLC Harboring a BRAF G469A Mutation. *Journal of Thoracic Oncology*. 2020;15(10):e174-e176. doi:10.1016/j.jtho.2020.07.007

92. Reyes R, Mayo-de-las-Casas C, Teixidó C, et al. Clinical Benefit From BRAF/MEK Inhibition in a Double Non-V600E BRAF Mutant Lung Adenocarcinoma: A Case Report. *Clinical Lung Cancer*. 2019;20(3):e219-e223. doi:10.1016/j.cllc.2019.02.022

93. Fang R, Xu S, Gong J, Liao Z. Clinical Response of Advanced Lung Adenocarcinoma with Class III BRAF G466V Missense Mutation to Dabrafenib and Trametinib: A Case Report. *Onco Targets Ther*. 2024;17:27-31. doi:10.2147/OTT.S448132

94. Liu Y, Zeng H, Wang K, Li Y, Tian P, Li W. Acquired *BRAF* N581S mutation mediated resistance to gefitinib and responded to dabrafenib plus trametinib. *Lung Cancer*. 2020;146:355-357. doi:10.1016/j.lungcan.2020.06.004

95. Grenda A, Krawczyk P, Targowska-Duda KM, Kieszko R, Paśnik I, Milanowski J. Efficacy of Dabrafenib and Trametinib in a Patient with Squamous-Cell Carcinoma, with Mutation p.D594G in BRAF and p.R461* in NF1 Genes-A Case Report with Literature Review. *Int J Mol Sci*. 2023;24(2):1195. doi:10.3390/ijms24021195

96. Liu L, Soler J, Reckamp KL, Sankar K. Emerging Targets in Non-Small Cell Lung Cancer. *International Journal of Molecular Sciences*. 2024;25(18):10046. doi:10.3390/ijms251810046

97. Kotani H, Adachi Y, Kitai H, et al. Distinct dependencies on receptor tyrosine kinases in the regulation of MAPK signaling between BRAF V600E and non-V600E mutant lung cancers. *Oncogene*. 2018;37(13):1775-1787. doi:10.1038/s41388-017-0035-9

98. Holderfield M, Lee BJ, Jiang J, et al. Concurrent inhibition of oncogenic and wild-type RAS-GTP for cancer therapy. *Nature*. 2024;629(8013):919-926. doi:10.1038/s41586-024-07205-6

99. Huo KG, Notsuda H, Fang Z, et al. Lung Cancer Driven by BRAFG469V Mutation Is Targetable by EGFR Kinase Inhibitors. *J Thorac Oncol*. 2022;17(2):277-288. doi:10.1016/j.jtho.2021.09.008

100. Imbody D, Arce K, Solanki HS, Haura EB, Pellini B. Targeting SHP2 Signaling in Lung Cancer. *J Thorac Oncol*. 2024;19(1):18-24. doi:10.1016/j.jtho.2023.08.009

101. Nichols RJ, Haderk F, Stahlhut C, et al. RAS nucleotide cycling underlies the SHP2 phosphatase dependence of mutant BRAF-, NF1- and RAS-driven cancers. *Nat Cell Biol*. 2018;20(9):1064-1073. doi:10.1038/s41556-018-0169-1

102. Bracht JWP, Karachaliou N, Bivona T, et al. BRAF Mutations Classes I, II, and III in NSCLC Patients Included in the SLLIP Trial: The Need for a New Pre-Clinical Treatment Rationale. *Cancers*. 2019;11(9):1381. doi:10.3390/cancers11091381

103. Marin-Acevedo JA, Hicks JK, Thapa R, Chen DT, Kimbrough E, Gray JE. Assessment of BRAF class I/II/III mutations, demographics, and treatment outcomes in NSCLC. *JCO*. 2021;39(15_suppl):e21016-e21016. doi:10.1200/JCO.2021.39.15_suppl.e21016

104. Lin Q, Zhang H, Ding H, et al. The association between BRAF mutation class and clinical features in BRAF-mutant Chinese non-small cell lung cancer patients. *J Transl Med*. 2019;17(1):298. doi:10.1186/s12967-019-2036-7

105. Li H, Zhang Y, Xu Y, et al. Tumor immune microenvironment and immunotherapy efficacy in BRAF mutation non-small-cell lung cancer. *Cell Death Dis*. 2022;13(12):1064. doi:10.1038/s41419-022-05510-4

106. Mazieres J, Drilon A, Lusque A, et al. Immune checkpoint inhibitors for patients with advanced lung cancer and oncogenic driver alterations: results from the IMMUNOTARGET registry. *Ann Oncol*. 2019;30(8):1321-1328. doi:10.1093/annonc/mdz167

107. Herbst RS, Baas P, Kim DW, et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. *Lancet*. 2016;387(10027):1540-1550. doi:10.1016/S0140-6736(15)01281-7

108. Dudnik E, Peled N, Nechushtan H, et al. BRAF Mutant Lung Cancer: Programmed Death Ligand 1 Expression, Tumor Mutational Burden, Microsatellite Instability Status, and Response to Immune Check-Point Inhibitors. *J Thorac Oncol*. 2018;13(8):1128-1137. doi:10.1016/j.jtho.2018.04.024

109. Otano I, Ucero AC, Zugazagoitia J, Paz-Ares L. At the crossroads of immunotherapy for oncogene-addicted subsets of NSCLC. *Nat Rev Clin Oncol*. 2023;20(3):143-159. doi:10.1038/s41571-022-00718-x

110. Gibson A, Pabani A, Dean M, Martos G, Cheung W, Navani V. Real-World Treatment Patterns and Effectiveness of Targeted and Immune Checkpoint Inhibitor Based Systemic Therapy in BRAF-Mutation Positive Non-Small Cell Lung Cancer. *JTO Clinical and Research Reports*. 2023;4:100460. doi:10.1016/j.jtocrr.2022.100460

111. Guisier F, Dubos-Arvis C, Viñas F, et al. Efficacy and Safety of Anti-PD-1 Immunotherapy in Patients With Advanced NSCLC With BRAF, HER2, or MET Mutations or RET Translocation: GFPC 01-2018. *J Thorac Oncol*. 2020;15(4):628-636. doi:10.1016/j.jtho.2019.12.129

112. Tan I, Stinchcombe TE, Ready NE, et al. Therapeutic outcomes in non-small cell lung cancer with BRAF mutations: a single institution, retrospective cohort study. *Transl Lung Cancer Res*. 2019;8(3):258-267. doi:10.21037/tlcr.2019.04.03

113. Addeo A, Passaro A, Malapelle U, Banna GL, Subbiah V, Friedlaender A. Immunotherapy in non-small cell lung cancer harbouring driver mutations. *Cancer Treat Rev*. 2021;96:102179. doi:10.1016/j.ctrv.2021.102179

114. Ahn MJ, Tanaka K, Paz-Ares L, et al. Datopotamab Deruxtecan Versus Docetaxel for Previously Treated Advanced or Metastatic Non-Small Cell Lung Cancer: The Randomized, Open-Label Phase III TROPION-Lung01 Study. *J Clin Oncol*. 2025;43(3):260-272. doi:10.1200/JCO-24-01544

115. Sands J, Ahn MJ, Lisberg A, et al. Datopotamab Deruxtecan in Advanced or Metastatic Non–Small Cell Lung Cancer With Actionable Genomic Alterations: Results From the Phase II TROPION-Lung05 Study. *JCO*. 2025;43(10):1254-1265. doi:10.1200/JCO-24-01349

116. Creelan BC, Wang C, Teer JK, et al. Tumor-infiltrating lymphocyte treatment for anti-PD-1-resistant metastatic lung cancer: a phase 1 trial. *Nat Med*. 2021;27(8):1410-1418. doi:10.1038/s41591-021-01462-y

117. Kong WM, Guo YJ, Ma J, Shi C. BTN2A1-BRAF fusion may be a novel mechanism of resistance to osimertinib in lung adenocarcinoma: a case report. *Transl Cancer Res*. 2023;12(1):186-193. doi:10.21037/tcr-22-2060

118. Wei XW, Deng JY, Xu CR, et al. Characteristics of and Treatment Strategies for Advanced EGFR-Mutant NSCLC With Concomitant BRAF Variations. *JTO Clin Res Rep*. 2022;3(7):100348. doi:10.1016/j.jtocrr.2022.100348

119. Giustini NP, Patel SP, Myall NJ, et al. Resistance to EGFR Tyrosine Kinase Inhibitor Therapy in Non–Small-Cell Lung Cancer via Newly Acquired Targetable Oncogenic Driver Alterations With an Emphasis on BRAF: Case Series and Literature Review of Treatment. *JCO Precis Oncol*. 2022;(6):e2100551. doi:10.1200/PO.21.00551

120. Schaufler D, Ast DF, Tumbrink HL, et al. Clonal dynamics of BRAF-driven drug resistance in EGFR-mutant lung cancer. *npj Precis Onc*. 2021;5(1):1-12. doi:10.1038/s41698-021-00241-9

121. Awad MM, Liu S, Rybkin II, et al. Acquired Resistance to KRASG12C Inhibition in Cancer. *N Engl J Med*. 2021;384(25):2382-2393. doi:10.1056/NEJMoa2105281

122. Zhao Y, Murciano-Goroff YR, Xue JY, et al. Diverse alterations associated with resistance to KRAS(G12C) inhibition. *Nature*. 2021;599(7886):679-683. doi:10.1038/s41586-021-04065-2

123. Peng P, Lv G, Hu J, Wang K, Lv J, Guo G. Co-mutations of epidermal growth factor receptor and BRAF in Chinese non-small cell lung cancer patients. *Ann Transl Med*. 2021;9(16):1321. doi:10.21037/atm-21-3570

124. Chen J, Facchinetti F, Braye F, et al. Single-cell DNA-seq depicts clonal evolution of multiple driver alterations in osimertinib-resistant patients. *Ann Oncol*. 2022;33(4):434-444. doi:10.1016/j.annonc.2022.01.004