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REVIEW

Advances in anti-BRAF therapies for lung cancer

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Summary
Non-small cell lung cancer (NSCLC) is one of the most frequent causes of mortality in the western world. v-raf murine sarcoma viral oncogene homolog B (BRAF) is a member of the Raf kinase family and plays a critical role in cellular growth, proliferation, and differentiation through the mitogen-activated protein kinase pathway. The incidence of BRAF mutations in NSCLC is low, accounting for 0–3% of all cases of lung cancer. Given the results obtained in metastatic melanoma, several studies have reported the efficacy of anti-BRAF therapies in NSCLC treatment. In this review, we describe changes in the landscape of BRAF-mutated lung cancer treatment and analyze insights from major clinical trials in the context of future therapeutic prospects.

Keywords BRAF · Lung cancer · V600

Introduction

Lung cancer is the most frequent cause of mortality worldwide, with an estimated 40% of cancer-related deaths [1]. Non-small lung cancer (NSCLC) accounts for approximately 85% of lung cancer cases and is divided into nonsquamous (70%), squamous (25%), and unspecified (5%) histological characteristics [2]. Between 2008 and 2014, the median overall survival (OS) of patients with NSCLC has been historically poor, with a 5-year survival of 24% for all patients and 5.5% for those with distant metastases [3]. Lung cancer is a heterogeneous disease composed of different clonal sub-populations harboring different molecular characteristics [4]. Over the past two decades, important advances in the treatment of NSCLC have been achieved, thereby increasing our understanding of the disease biology and tumor progression mechanisms. A better understanding of the biology of lung cancer has led to the development of novel biomarker-targeted therapies and heralded the era of precision medicine.

The most common genetic alteration in NSCLCs is associated with the epidermal growth factor receptor (*EGFR*) genes. This mutation, present in approximately 10–13% of Caucasian patients with lung cancer, has a role in the early phase of tumor initiation and represents a potential target for targeted therapy [5]. Other important mutations are related to anaplastic lymphoma kinase gene (*ALK*) rearrangements and the c-ros oncogene 1 (ROS1). Recently, additional molecular alterations have been identified, including amplification and mutations in the hepatocyte growth factor receptor (*MET*) and human epidermal growth factor receptor 2 (*HER2*) genes, rearrangements in the rearranged during transfection gene (*RET*), mutations in v-raf murine sarcoma viral oncogene homolog B (BRAF), and, according to the latest reports, alterations in the neurotrophic tropomyosin receptor kinase gene (*NTRK*). All these mutations are potentially druggable targets [6]. BRAF is a member of the Raf kinase family and plays a critical role in cellular growth, proliferation, and differentiation through the mitogen-activated protein kinase (MAPK) pathway [7]. This gene is altered with high mutational rates in various types of cancer, such as hairy cell leukemia (100%), melanoma (>60%), and papillary thyroid cancer (>50%) [8]. The most common BRAF mutation occurs at the level of

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T1799 transversion in exon 15, leading to the substitution of valine for glutamic acid (V600E) [9]. This alteration leads to constitutive activation of B-Raf kinase and the subsequent signal transduction to the MAPK/extracellular signal-regulated kinase (ERK) cascade, resulting in a 10-fold increase in BRAF activity compared to that in the wild-type (WT) protein [10]. Mutant BRAF is a prototype of the driver oncogene; its inactivation leads to cancer cell apoptosis, thereby indicating the existence of an acquired dependency of tumor cells on this mutant form of BRAF [11]. Given the results obtained in metastatic melanoma, Planchard et al. assessed the antitumor activity of dabrafenib monotherapy in BRAF (V600E)-mutant NSCLC in a study published in 2016. In this phase II open-label study, 84 patients were enrolled, of which 78 were treated and 6 were untreated. With a median follow-up of 10.7 months, an overall response rate (ORR) of 33% with a disease control rate of 58% was reported for dabrafenib monotherapy in the 78 previously treated patients with metastatic $BRAF^{V600E}$-mutated NSCLC. In patients treated with one to three previous lines of therapy, median progression-free survival (PFS) and duration of response were 5.5 and 9.6 months, respectively, and median the preliminary median OS was 12.7 months [12]. This was the first prospective trial of BRAF inhibition to focus on $BRAF^{V600E}$-mutated NSCLC. Primarily, antitumor activity of BRAF inhibitors (BRAFi) was shown only in some isolated case reports [13]. Human et al. studied the activity of vemurafenib in patients with $BRAF^{V600E}$ mutation in a phase II basket trial in 2015, and reported a ORR response rate of 42% and a median PFS of 7.3 months in the cohort with NSCLC [14]. Given these results, in 2016, the Food and Drug Administration (FDA) approved this drug for treatment of advanced NSCLC in patients harboring $BRAF^{V600E}$ mutation [15], and a molecular-level targeted approach was adopted for BRAF-mutant NSCLCs. In this review, we describe the changes in the landscape of BRAF-mutated lung cancer treatment and provide insights and future perspectives by analyzing major clinical trials.

Clinical characteristics of BRAF-mutated NSCLC

In NSCLC, the incidence of BRAF mutations is low, accounting for 0–3% of all cases of lung cancer. Since BRAF acts as an oncogene in NSCLC, driver mutations in *BRAF* are mutually exclusive from *EGFR* mutations or *ALK* rearrangements [16]. Even if The Cancer Genome Atlas reported a 3% mutation rate in squamous cell lung cancer, this alteration is almost completely confined to the adenocarcinoma subtype [17]. $BRAF^{V600E}$ mutation represents the most frequent BRAF mutation in lung cancer, accounting for approximately 50% of BRAF-mutant NSCLC, whereas fewer mutations have been identified at the G469A and G594G sites [18]. The occurrence of BRAF mutations has been reported to be lower in Asian (1.3%) populations than in Caucasian ones (3%), probably because of ethnic differences and the high frequency of EGFR mutations in Asian women with lung adenocarcinoma [19]. This mutation does not result in specific differentiation with respect to clinical features. However, some differences do exist between V600E and non-V600E mutants. $BRAF^{V600E}$ mutations have been reported to be more frequent in female patients and could not be correlated to smoking history, whereas non-V600E mutations were more likely to arise in males with a history of smoking [20]. Concerning the prognostic significance of BRAF mutations in NSCLC, as in colorectal [21] and papillary thyroid cancer [22], $BRAF^{V600E}$-mutant tumors are associated with poor prognosis compared to the non-V600E ones [23]. Specifically, Marchetti et al. in his retrospective study of 1046 NSCLCs harboring BRAF mutations indicated an association between V600E mutations and T status, N status, and pathological state, and a significantly shorter patient DFS and OS than those without these mutations [24]. This prognostic impact could be attributed to an aggressive histologic architecture such as non-mucinous adenocarcinoma, showing micropapillary features, acinar growth, and solid growth [25]. In contrast, non-V600E mutations in BRAF were not related to any specific histological features or prognosis [26].

Molecular pathway of BRAF

BRAF belongs to the family of serine-threonine protein kinases, and is an important effector molecule for the MAPK/ERK signaling pathway (Fig. 1). Somatic mutations in BRAF, leading to the V600E variant, alter two main regions of the peptide, by disrupting the glycine-rich P loop and its variant domain of the kinase segment. The conversion between active and inactive states in WT BRAF is carried out through activation of the inhibitory effect triggered by the glycine-rich P loop, which is extremely important for the incorporation of signal transduction provided by RAS [27, 28]. Because of the phosphomimetic characteristics of the V600E mutation of BRAF, the glutamate residue interacts directly with the glycine-rich P loop by blocking its function as an inhibitory regulator, thereby leading to a constant state of spontaneous activation of BRAF and initiating a cascade of events that requires no signalization from external stimuli on EGFR (Fig. 1). The resulting over-stimulated proliferation of the cell renders it independent of the influence of external growth factors [29, 30]. The inactivation of the control mechanism significantly increases the basal activity of the cell (up to 10 fold) and is carried over to the next cell division cycles, leading to a strong oncogenic pattern [8, 31]. Figure 1 demonstrates the mechanism by which V600E mutation overloads the MAPK pathway, decreasing the activation of apoptotic mechanism regulated by BAD and through caspase cascade events. High activity of ERK leads to an increase in the proliferation process and an increase in cellular migration,

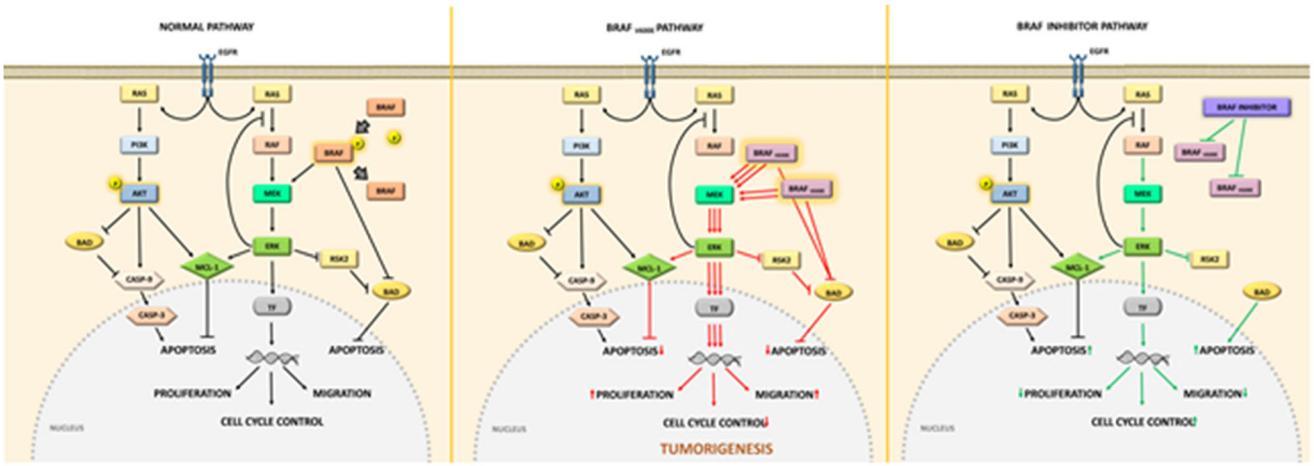

Fig. 1 Normal functional MAPK/ERK pathway in wild-type (WT) BRAF and its alteration in the presence of $BRAF^{V600E}$ mutation and the mechanism of action of drugs targeting the mutation. Normal functional MAPK/ERK pathway is activated after extracellular signaling, leading to a response in cell cycle control, proliferation, and cellular migration. The $BRAF^{V600E}$ mutation induces a self-sustained constant activation of the MAPK/ERK pathway, thereby inhibiting controlled cellular death by apoptosis via indirect regulation of BAD. The $BRAF^{V600E}$ pathway drastically increases the basal levels of proliferation, consistent with oncogenic development. Therefore, the $BRAF^{V600E}$ mutation could be targeted for treatment with specific inhibitors, which results in an increase in apoptotic activity

without EGFR-mediated initiation triggered by the presence of external stimuli [32]. This robust pathway also implies that selective inhibition of $BRAF^{V600E}$ disrupts the over-stimulated pathway, allowing the repair mechanisms associated with damaged cyclins to trigger apoptosis [33].

BRAF mutations

Approximately 200 BRAF-mutant alleles have been identified in human tumors, which could be further classified into three classes based on kinase activity, RAS-dependence, and dimerization status. Class 1 BRAF mutations (V600E/D/K/R) are the most frequently identified in solid tumors; these mutations lead to a strong activation of BRAF kinase activity and a constitutive activation of the MAPK pathway [34]. Class 2 BRAF mutations are divided into high or intermediate kinase activity based on MAPK pathway activation, and these mutants signal as constitutively active dimers. Both class 1 and class 2 BRAF mutations are RAS-independent and, therefore, resistant to RAF inhibitors [35]. Class 3 BRAF mutations have low or absent kinase activity; they are related to ERK-mediated feedback, and their activation is RAS-dependent. These mutants are not independent drivers and require upstream activation of the MAPK pathway; therefore, this class of mutations frequently coexist with RAS mutations or NF1 loss. Hence, a potential therapeutic strategy is the blocking of upstream RAS signaling [36].

A recent publication showed that, in addition to these three class of mutations, missense mutations, deletions, and a number of BRAF fusions called "mutations of unknown functions" have been identified [37]. For example, the BRAF fusion gene has been found in melanomas, prostate and gastric cancer, and in 85% of astrocytic pilomcytomas [38]. These mutations are single events, not evidenced in the largest database of solid tumors, and frequently coexist with other driver mutations [39]. Due to their low frequency of occurrence, these could be passenger mutations, thereby accounting for their lack of response to MAPK therapies. Therefore, further investigation of these rare BRAF mutants is warranted to distinguish BRAF drivers from passenger mutations.

FDA-approved anti-BRAF inhibitors

PLX4032, also known as vemurafenib, is a potent inhibitor of the BRAF mutant family. The name "vemurafenib" is derived from its ability to inhibit V600E-mutated BRAF [40]. It was approved by the FDA in 2011 after results from a phase III trial (BRIM-3), which showed improved OS and PFS rates in patients with $BRAF^{V600E}$-mutated melanoma [41]. In addition, vemurafenib has been approved by the FDA for the treatment of unresectable advanced melanoma [42]. Since 2017, it has also been approved for the Erdheim-Chester disease containing the $BRAF^{V600E}$ mutation [43]. PLX4032 is known to promote the apoptosis of mutated cells in a dose-dependent manner. Specifically, it interrupts the BRAF/MEK step in the BRAF/MEK/ERK pathway. PLX4032 is specifically active in BRAF-mutated cell lines [44]. The most common side effects in patients receiving vemurafenib are arthralgia, skin rash, nausea, photosensitivity, fatigue, pruritus, palmar-plantar dysesthesia, and cutaneous squamous cell carcinoma. As described by Flaherty et al., most PLX4032-related side effects appear to be proportional to the dosage used and the time of exposure [45].

Dabrafenib (GSK21188436) is a small molecule inhibitor of the BRAF-mutant kinase family. It is used in monotherapy or in combination with trametinib for the treatment of unresectable or metastatic $BRAF^{V600E}$-mutated melanoma, advanced $BRAF^{V600E}$-mutated NSCLC, and $BRAF^{V600E}$-mutated locally advanced or metastatic ATC [46]. Dabrafenib is a potent ATP-competitive inhibitor of BRAF kinase and is highly selective for mutant BRAF in kinase panel screening, cell lines, and xenografts [47]. Since 2013, it has been approved by the FDA for the treatment of advanced melanoma, on the basis of the results of a phase III trial (NCT01227889), in which an improved PFS compared to that with dacarbazine was reported [48]. The most common side effects seen with dabrafenib monotherapy are hyperkeratosis, headache, pyrexia, and arthralgia [49].

Trametinib (GSK1120212) is a type III, reversible allosteric, non-ATP competitive inhibitor of both MEK1 and MEK2. It binds within the cleft between the small and large lobes of the kinase, adjacent to the ATP binding pocket, so that both the ATP and the allosteric inhibitor can bind simultaneously to the kinase [50]. Trametinib can be used for monotherapy; however, in most cases, it is used in combination with dabrafenib for the treatment of adults with unresectable or metastatic melanoma harboring $BRAF^{V600E}$ mutation [51]. Trametinib is well tolerated, and the spectrum of side effects is consistent with that of MEK inhibitors. Skin-related toxicity, diarrhea, and most common side effects such as arthralgia, rash, headache, and fatigue are adequately managed with supportive care alone. Cardiac, ophthalmologic, and hepatic events are uncommon, and these have been reported to be reversible on interruption of trametinib treatment [52].

Encorafenib (LGX818), a new generation BRAFi, targets key enzymes in the MAPK signaling pathway [53]. It acts as an ATP-competitive RAF kinase inhibitor, decreasing ERK phosphorylation and downregulating cyclin D1 [54]. Encorafenib exhibits a more prolonged pharmacodynamic activity than other approved BRAFi molecules [55]. On June 27, 2018, FDA approved the combination of encorafenib and binimetinib (an anti-MEK1/2 protein kinase inhibitor) for treatment of patients with unresectable or metastatic melanoma with a $BRAF^{V600E}$ or $BRAF^{V600K}$ mutation, based on insights from an FDA-approved test. The approval was based on a phase III randomized, active-controlled, open-label, multicenter trial (COLUMBUS), which enrolled 577 $BRAF^{V600E}$ or $BRAF^{V600K}$ mutation-positive patients with unresectable or metastatic melanoma [56]. In this trial, the combination of encorafenib and binimetinib resulted in a longer PFS than vemurafenib. The most common (≥25%) adverse reactions in patients receiving the combination were fatigue, nausea, diarrhea, vomiting, abdominal pain, and arthralgia [56]. Subsequently, in 2020, FDA approved a combination of cetuximab (anti-EGFR monoclonal antibody) and binimetinib for the treatment of patients with adult metastatic colorectal cancer (CRC) harboring a $BRAF^{V600E}$ mutation. This was based on an FDA-approved test, after prior therapy, where the efficacy of treatment was evaluated in a randomized, active-controlled, open-label, multicenter trial (BEACON CRC) on 665 patients with metastatic CRC and $BRAF^{V600E}$ mutation who had shown disease progression after one or two previous treatment regimens [57]. This combination led to a significantly longer OS and a higher ORR response rate than standard therapy. The most common adverse reactions (≥25%) for the combinatorial treatment with encorafenib and cetuximab were fatigue, nausea, diarrhea, acneiform dermatitis, abdominal pain, decreased appetite, arthralgia, and rash [57]. Moreover, unlike the other BRAF inhibitors, this modality could trigger cellular senescence in $BRAF^{V600E}$ melanoma cells [58].

Mechanisms of anti-BRAF resistance

Potent inhibitors of the $BRAF^{V600E}$ mutant protein, such as dabrafenib, vemurafenib, and trametinib, have produced ORR response rates of 50–60% and shown enhanced PFS and OS rates in patients with $BRAF^{V600E}$ mutations, as compared to dacarbazine [48, 59]. Despite this promising activity, 50% of patients treated with these drugs developed disease progression after several months of treatment. This differential response of patients to these drugs is attributed to BRAF resistance mechanisms. One of the resistance mechanisms identified in the study by Montagut et al. is the expression of CRAF kinases. According to this study, it is possible that increased CRAF protein levels decrease the bioavailability of drugs within mutant cells [60]. These authors also indicated that elevated CRAF protein levels may similarly contribute to primary insensitivity to inhibition in a subset of BRAF-mutant cancer cells [60].

Elevated expression levels of COT represent another resistance mechanism of BRAF. COT is hypothesized to drive resistance to BRAF inhibition predominantly through the reactivation of MAPK signaling [61].

In addition, Shi et al. demonstrated that $BRAF^{V600E}$ amplification results in $BRAF^{V600E}$ overexpression, which is necessary and sufficient for acquired resistance to BRAFi [62].

Yet another mechanism of resistance to BRAFi involves N-RAS upregulation. High levels of activated N-RAS resulting from mutations lead to significant reactivation of the MAPK pathway [63].

Aberrant splicing of BRAF is also known to trigger resistance to BRAFi molecules. A new mechanism of acquired resistance has been identified in patients, where expressed BRAF splice isoforms (such as V600E) dimerize in a RAS-independent manner; the generation of splice variants is likely because of a mutational or epigenetic change affecting BRAF [64].

A notable study conducted by Paraiso et al. addressed the role of PTEN loss in intrinsic resistance to BRAFi. They have shown, for the first time, that loss of PTEN contributes to intrinsic BRAFi resistance via the suppression of BIM-mediated apoptosis [65].

Additional resistance mechanisms include the persistent activation of receptor tyrosine kinases, including platelet-derived growth factor receptor, insulin-like growth factor 1 receptor (IGF-1R), and EGFR. The EGFR/SFK pathway was found to mediate resistance to vemurafenib treatment in BRAF-mutant melanoma, and BRAF and EGFR/SFK inhibition was reported to block the proliferation and invasion of these tumors, providing potentially effective therapeutic options for these patients [66]. As described by Villanueva et al., an increase in IGFR-1R and pAKT levels in a post-relapse human tumor sample is consistent with a role of IGF-1R/PI3K-dependent survival in conferring resistance to BRAFi and could be a plausible explanation of the death of BRAFi-resistant cells upon combined treatment with IGF-1R/PI3K and MEK inhibitors [67].

Clinical development of anti-BRAF drugs for lung cancer treatment

BRAF is a serine/threonine kinase located inside the cell and is activated by RAS. In turn, BRAF activates downstream kinases, such as MEK and ERK (MAPK). BRAF mutations have been found in half of melanomas, mainly as a V600E mutation [68]. In metastatic non-small cell lung carcinoma (mNSCLC), mutations in BRAF are detected in 2–5% of cases, and the V600E mutant generally occurs in 1–2% of cases. The current data indicate that BRAF mutation in NSCLC is not indicative of any correlation with improved survival or any benefit to chemotherapy in clinical settings, except for the fact that 20–30% of patients with the V600E mutation are non-smokers, whereas the non-V600E subtypes are chain smokers [20, 24, 69–74]. A phase II clinical trial of 36 patients with NSCLC from 19 centers has shown that the presence of $BRAF^{V600E}$ mutation could be associated with increased responsiveness to combination therapy of dabrafenib and trametinib, which act as oral inhibitors of BRAF and MEK, respectively [75].

Increasing our understanding of BRAF biology has enabled the identification of new small molecule inhibitors of the catalytic activity of BRAF.

Dabrafenib and trametinib

BRAFi molecules including vemurafenib and dabrafenib as the second line of monotherapy in BRAF-mutant NSCLC showed an ORR of 33–42% and median PFS of 5.5–7.3 months. This combination was further investigated in first ($n = 36$) [75] and second line ($n = 57$) [76] treatments of $BRAF^{V600E}$ positive mNSCLCs. The ORRs were 64% and 63.2%, respectively. The respective median PFS was estimated at 14.6 and 9.7 months. Interim results from the MyPathway study, investigating the efficacy of vemurafenib against $BRAF^{V600E}$ mutation and other BRAF mutations, showed an ORR of 43 ($n = 14$) and 0 ($n = 7$) %, respectively [77].

BRAF and CRAF monomers, heterodimers, and homodimers might improve the efficacy of treatment [78–81]. In fact, BGB-293 is a novel inhibitor of WT BRAF, ARAF, CRAF, EGFR, and $BRAF^{V600E}$. The recommended phase II dose was 40 mg in patients with cancer harboring BRAF or KRAS/NRAS mutations. Of note, there was a partial response in one patient diagnosed with BRAF/MEK inhibitor-näive KRAS-mutated mNSCLC. The major dose-limiting toxicity (DLT) was caused by thrombocytopenia (observed in 13% of patients) [82]. Janku et al. showed that PLX8394 monotherapy or that in combination with a CYP3A4 inhibitor cobicistat increased the efficacy of PLX8394 in refractory solid cancers. The DLT for this combination was observed with respect to elevated levels of aspartate transaminase and alanine transaminase.

Circulating tumor BRAF as a prognostic marker

The decision to administer targeted therapies in NSCLC is sometimes limited because of unavailable or inadequate biopsies, owing to the difficulty of reaching the tumor region and unknown mutational status in many patients. Liquid biopsy could be a promising way to solve this issue through the detection of mutations in cell-free DNA (cfDNA). $BRAF^{V600E}$ mutations are frequently found in metastatic melanoma. Most of the studies are testing for cfDNA in such clinical settings. Since this mutation is less frequent in lung cancers (1–5%), cfDNA has been studied less frequently in this tumor. However, similarities in responses of EGFR and BRAF in cfDNA toward BRAFi molecules has been observed in two different NSCLC studies [83, 84]. Yang et al. used an innovative competitive allele-specific TaqMan polymerase chain reaction (CastPCR) method to detect driver mutations in cfDNA from 107 lung adenocarcinoma patients. Specificity, sensitivity, concordance values, PPV, and NPV of CastPCR-based detection of EGFR mutations in cfDNA were 94.2% (49/52), 56.4% (31/55), 74.8% (80/107), 91.2% (31/34), and 67.1% (49/73), respectively. Notably, both the specificity value and PPV for p.T790M reached 100% for EGFR. As for BRAF, the CastPCR approach yielded respective values of 28.6% (2/7), 93.0% (93/100), 88.8% (95/107), 22.2% (2/9), and 94.9% (93/98), respectively, which is indicative of good specificity [83]. Similarly, Guilbert et al. observed a good correlation between variations in plasma BRAF mutants in cfDNA and response to BRAF inhibitors in their case study [84]. Ahlborn et al. evaluated the longitudinal tracking of

$BRAF^{V600E}$ cfDNA as a marker for responses to BRAFi treatment in non-melanoma tumors. Tumor response was evaluated in half of the patients (8/16), and the median OS and PFS were 15 and 4.8 months, respectively. An increase in longitudinal measurements of $BRAF^{V600E}$ mutant cfDNA indicated disease progression before radiological evaluation, and a reduction of more than 50% of mutations after 4 and 12 weeks of therapy was shown to be significantly associated with prolonged PFS ($p = 0.003$ and $p = 0.029$, respectively) and OS ($p = 0.029$ and $p = 0.017$, respectively) [85]. Therefore, BRAFi combination therapies showed a ORR response rate of 50% in $BRAF^{V600E}$-mutated non-melanoma tumors.Li et al. demonstrated the reliability of cfDNA against standard immunohistochemistry (IHC) of tissue samples in a study of 190 Chinese patients with lung adenocarcinoma. For the $BRAF^{V600E}$ testing, these authors used the amplification refractory mutation system, based on a fluorescence PCR kit) to analyze the distribution and prognostic role of the mutation. They observed that 5/8 patients with $BRAF^{V600E}$ mutations in IHC matched the plasma DNA samples. The frequency of $BRAF^{V600E}$ mutation in this study of Chinese patients with lung adenocarcinoma was 4.2%, and cfDNA showed good potential for use in $BRAF^{V600E}$ mutational analysis of patients with lung adenocarcinoma [86].

Ongoing clinical trials

A phase II, open-label clinical study investigating the combined use of debrafenib and trametinib as a second line therapy in 27 patients with $BRAF^{V600E}$-mutated NSCLC is currently ongoing in South Korea (NCT03543306). A further study using this specific combination of drugs has been planned on a larger scale, with 174 participants diagnosed with the same mutated form of cancer (NCT01336634). Investigation of the effect of vemurafenib as a first-line therapeutic for $BRAF^{V600E}$ NSCLC in 60 patients is also imminent (NCT04302025). Use of this drug as both a second- and third-line therapy for the same purpose will also be investigated, using PFS as a primary endpoint in 119 patients. Furthermore, a future phase IV clinical trial is planned to assess the potential adverse effects (AE) of the combination of dabrafenib and trametinib as first-line treatment in 100 patients with $BRAF^{V600E}$-mutated NSCLC (NCT03340506). The full list of ongoing clinical trials investigating BRAFi efficacy in NSCLC treatment is summarized in Table 1.

Previously, a phase II study investigated the use of dabrafenib monotherapy twice daily in combination with trametinib once daily in stage IV NSCLC in patients harboring a V600E mutation (NCT01336634). The main outcomes of this study were the ORR (primary outcome) and OS and PFS (secondary outcomes). Another phase II clinical trial comprising 27 patients with lung cancer has been testing the efficacy of debrafenib combined with trametinib with the same primary and secondary outcomes as defined previously (NCT03543306).

A phase I study was conducted in 145 patients with either melanoma, head and neck cancer, NSCLC, or urothelial carcinoma to identify AEs to the first-line therapy. This was initiated as a two-arm clinical study: one arm was treated with enoblituzumab (anti-B7-H3 monoclonal antibody) and pembrolizumab (anti-PD-1 antibody) along with BRAFi if V600 mutations were detected, whereas the second arm was treated with enoblituzumab and an anti-PD-1 molecule (MGA012) (NCT02475213). A phase II clinical study of 60 patients with NSCLC investigated the use of vemurafenib, alectinib, entrectinib, cobimetinib, radiotherapy, or chemotherapy as first-line therapy. The aim of this study was to identify the major pathological responses of the first-line efficacy of these drugs (NCT04302025).

Another phase II clinical trial that enrolled 119 patients investigated the BRAFi molecules vemurafenib, anti-PD-L1, atezolizumab (MPDL3280A), alectinib, or trastuzumab emtansine (T-DM1). The primary outcome of the study was to observe how PFS differed when patients received each one of the monotherapies (NCT02314481).

A new phase I clinical investigation of 27 participants has been testing the maximum tolerable dose of a small molecule BRAFi (ABM-1310) in advanced solid tumors bearing the $BRAF^{V600}$ mutation (NCT04190628). The novelty of this small molecule is exhibited by its affinity binding to the target receptor in the tumor.

Encorafenib is a BRAFi that targets key enzymes of the MAPK signaling pathway. It has been evaluated in combination with binimetinib (anti-MEK1/2 inhibitors) in a randomized, open-label phase II clinical trial, which enrolled 144 patients with $BRAF^{V600E}$-positive NSCLC, with ORR as the primary outcome (NCT04526782).

Another notable therapy that has been investigated together with BRAFi is the tumor infiltrating lymphocyte (TIL) LN-144. A phase II clinical trial of 75 participants has been evaluating this TIL as a monotherapy or in combination with BRAFi and/or MEKi in patients with the $BRAF^{V600}$ mutation. The study also has additional arms that investigate the efficacy of the LN-144 TIL combined with pembrolizumab or as a monotherapy. The primary outcome of the investigation is in terms of ORR and treatment emergent adverse events, with OS and PFS as secondary outcomes (NCT03645928).

A late phase IV clinical trial is currently investigating the possible AEs of debrafenib in combination with trametinib in 100 patients with NSCLC, melanoma, solid tumors, rare cancers, and high-grade glioma (NCT03340506). It is important to note that targeting BRAF as an anticancer therapy has also been investigated in other solid cancers.

Menzer et al. reported the efficacy of combined BRAFi/MEKi vs. BRAFi monotherapy in a study of 103 patients with metastatic melanoma. Of the 58 patients bearing V600

Table 1 Selected ongoing trials with BRAF Inhibitors for NSCLC

Clinical Trial Identifier	Study Design	Intervention/s	Setting	Primary Endpoint	Phase	Status
NCT03645928	75 Participants, Non-Randomized, Parallel Assignment, Open Label	TIL LN-144; TIL LN-144 and if BRAF V600 mutation positive BRAFi or BRAFi with MEKi; TIL LN-144 with pembrolizumab for HNSCC; TIL LN-144 with pembrolizumab for NSCLC; TIL LN-144 single agent for NSCLC	Second or later line	ORR, TEAEs	2	Recruiting
NCT02475213	145 Participants, Non-Randomized, Sequential Assignment, Open Label	Enoblituzumab plus pembrolizumab and BRAFi if V600 mutation positive; enoblituzumab plus MGA012.	First line	AEs	1	Active, not recruiting
NCT03543306	27 Participants, Single group assignment, Non-Randomized, Open label	Dabrafenib plus Trametinib	Second or third line	ORR	2	Recruiting
NCT04302025	60 Participants, Single group assignment, Non-Randomized, Open label	Alectinib; Entrectinib; Vemurafenib; Cobimetinib; Radiotherapy; Chemotherapy	First line	MPR	2	Not yet recruiting
NCT02974725	195 Participants Single group assignment Non Randomized Open Laberl	LXH254x,LTT462, Trametinib,Ribociclib	Second line	AEs	1	Recruiting
NCT01336634	174 Participants, Single group assignment, Non-Radomized, Open label	Dabrafenib plus Trametinib	First line	ORR	2	Active, not recruiting
NCT02314481	119 Participants Single group assignment, Non-randomized Open Label	MPDL3280A, Vemurafenib, Alectinib, Trastuzumab emtansine	Second or third line	PFS	2	Recruiting
NCT03340506	100 Participants, Single group assignment, Interventional Open Label	Dabrafenib plus Trametinib	First line	AEs	4	Recruiting
NCT04190628	27 Participants Sequential Assignment, Non randomized Open Label	ABM-1310	Third and Fourth line therapy	MTD	1	Not yet recruiting
NCT04526782	144 Participants, Interventional, Crossover Assignment Randomized, Open Label	Encorafenib plus Binimetinib	First and Second line therapy	ORR	2	Not yet recruiting
NCT01543698 [1]	179 participants, Single group assignment, Non randomized, Open label	LGX818 in combination with MEK162 in patients with advanced solid tumors	Second or third line	MTD	1b/2	Active, not recruiting

Abbreviations: Adverse Events, AE; Complete Response, CR; Cytokine Release Syndrome, CRS; Dose Limiting Toxicity, DLT; Maximum Tolerated Dose, MTD; Objective Response, OR; Overall Response Rate, ORR; Pharmacodynamics, PD; Partial Response, PR; Progression Free Survival, PFS; Serious Adverse Events, SAEs; Major Pathological Response, MPR; Stable Disease, SD. The information was extracted from www.clinicaltrials.gov.

mutation, the ORR to BRAFi monotherapy and combined BRAFi/MEKi treatment was 27% (6/22) and 56% (20/36), respectively, whereas PFS was 3.7 and 8.0 months, respectively ($p = 0.002$) [87].

The DESCRIBE II trial has evaluated the effectiveness of BRAFi dabrafenib and trametinib in the treatment of $BRAF^{V600}$-mutated metastatic melanoma patients, in a compassionate use setting [88]. This retrospective trial showed substantial clinical activity with dabrafenib plus trametinib in patients with unresectable or metastatic melanoma, similar to what has been assessed in previous prospective controlled trials [89–91]. Furthermore, the analysis of treatment patterns demonstrated the effectiveness of the combinatorial treatment in patients with brain metastases and across lines of therapy with a manageable and well-tolerated safety profile.

Conclusions

The presence of the $BRAF^{V600E}$ mutation has been found to be associated with increased responsiveness to combined therapy with oral inhibitors of BRAF and MEK. Moreover, the $BRAF^{V600E}$ mutation can occur as a resistance mutation for EGFR-TKI therapy. Currently, the role of BRAF mutations in response to therapy has not yet been understood. The use of liquid biopsy by NGS and targeted real-time PCR could help in rapid identification of BRAF mutational status. The discovery of new technologies that could further improve the sensitivity of detection of mutated BRAF from liquid biopsy would be an exciting frontier in this case, as has been the case for EGFR. FDA approval for anti-BRAF therapy should be further investigated in combination with other drugs that could enhance the immune system, such as checkpoint inhibitors or CAR-T therapies. In fact, these mutations could make the cancers more susceptible to immunotherapies as well as to anti-BRAF therapies. Finally, in addition to the $BRAF^{V600E}$ mutation, novel surrogate biomarkers should be investigated to predict the efficacy of BRAFi for the purpose of stratification of patients in such a way that the best treatment could be given to those who would most likely respond.

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Compliance with ethical standards

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