BRAF Alterations as Therapeutic Targets in Non–Small-Cell Lung Cancer

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Background: Several subsets of non–small-cell lung cancer (NSCLC) are defined by molecular alterations acting as tumor drivers, some of them being currently therapeutically actionable. The rat sarcoma (RAS)–rapidly accelerated fibrosarcoma (RAF)–mitogen-activated protein/extracellular signal-regulated kinase kinase (MEK)–extracellular signal-regulated kinase (ERK) pathway constitutes an attractive potential target, as v-Raf murine sarcoma viral oncogene homolog B (*BRAF*) mutations occur in 2–4% of NSCLC adenocarcinoma.

Methods: Here, we review the latest clinical data on BRAF serine/threonine kinase inhibitors in NSCLC.

Results: Treatment of V600E *BRAF*-mutated NSCLC with BRAF inhibitor monotherapy demonstrated encouraging antitumor activity. Combination of BRAF and MEK inhibitors using dabrafenib and trametinib is under evaluation. Preliminary data suggest superior efficacy compared with BRAF inhibitor monotherapy.

Conclusion: Targeting BRAF alterations represents a promising new therapeutic approach for a restricted subset of oncogene-addicted NSCLC. Prospect ive trials refining this strategy are ongoing. A next step will probably aim at combining BRAF inhibitors and immunotherapy or alternatively improve a multilevel mitogen-activated protein kinase (MAPK) pathway blockade by combining with ERK inhibitors.

Key Words: BRAF, V600E, MEK, Vemurafenib, Dabrafenib, Trametinib, Non–small-cell lung cancer.

(*J Thorac Oncol.* 2015;10: 1396–1403)

Lung cancer is a leading cause of cancer death worldwide,[1,2] with 1.6 million deaths annually. Environmental factors and genetic alterations seem to contribute to increase lung cancer risk.[3–6] Although the majority of lung cancer cases is related to tobacco smoking,[7] 10–30% occurs in light-smoker or never-smoker patients, with a higher proportion of light-smoker or never-smokers in Asian countries.[8] Therapeutic decisions have traditionally been based on tumor histology. Non–small-cell lung cancer (NSCLC) accounts for 85% to 90% of lung cancers, with over 60% diagnosed at an advanced stage.[9] Advanced NSCLC is usually treated with platinum-based doublet chemotherapy, with inevitable subsequent relapse.

In NSCLC, numerous molecular alterations have been recently reported and defined as driver oncogenes following their role in transforming and maintaining cancer cells in preclinical models, such as epidermal growth factor receptor (*EGFR*), human epidermal growth factor receptor 2 (*HER2*), V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (*KRAS*), v-Raf murine sarcoma viral oncogene homolog B (*BRAF*), MNNG HOS transforming gene (MET) and phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (*PIK3CA*) mutations, anaplastic lymphoma kinase (*ALK*), v-ros avian UR2 sarcoma virus oncogene homolog 1 (*ROS1*) and rearranged during transfection (*RET*) chromosomal rearrangements, and *PIK3CA*, *MET*, and *HER2* amplifications. Most molecular aberrations seem to occur mainly in adenocarcinoma and are more frequently encountered in never smokers.[5,6,10,11] These mutations offer promising new therapeutic approaches, and the use of specific targeted agents has changed the landscape of lung cancer treatment. Agents targeting the abnormal activity of EGFR and ALK kinases have been validated as first-line treatment for advanced NSCLC harboring theses molecular alterations, with a better overall response rate (ORR) and progression-free survival (PFS) compared with standard chemotherapy.[12–16]

BRAF Mutations

The rat sarcoma (RAS)–rapidly accelerated fibrosarcoma (RAF)–mitogen-activated protein/extracellular signal-regulated kinase kinase (MEK)–extracellular signal-regulated kinase (ERK) mitogen-activated protein kinase pathway is one of the most important canonical cancer signaling pathways, mediating cellular responses to growth signals essential for cell proliferation and survival (Fig. 1).[17,18] Its role in carcinogenesis was initially proposed based on its frequent dysregulation in human cancer. BRAF is a member of the serine/threonine kinase RAF family (including the isoforms v-Raf murine sarcoma viral oncogene homolog A [ARAF], BRAF, v-Raf murine sarcoma viral oncogene homolog C [CRAF]) that is regulated by binding to RAS and directly activating MEK1/2, which can further phosphorylate ERK1/2. *RAS* and *BRAF* oncogenic mutations

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Disclosure: No financial support was provided. The authors declare no conflict of interest. No writing assistance was utilized in the production of this manuscript.

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DOI: 10.1097/JTO.0000000000000644

ISSN: 1556-0864/15/1010-1396

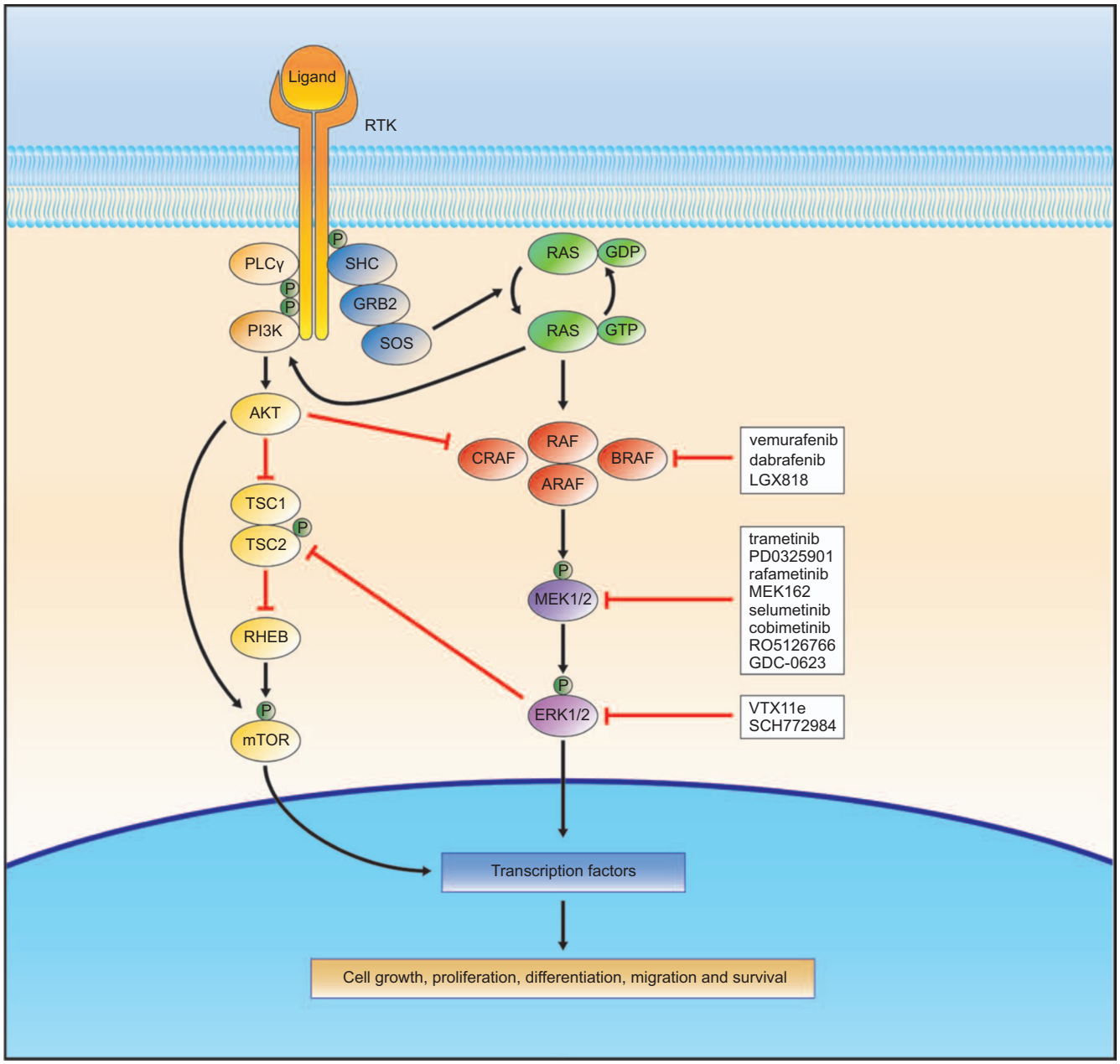

FIGURE 1. Illustration of RAS–RAF–MEK–ERK MAP kinase signaling pathway. Current inhibitors targeting BRAF, MEK, and ERK are depicted. AKT, Ak strain transforming; ARAF, v-Raf murine sarcoma viral oncogene homolog A; BRAF, v-Raf murine sarcoma viral oncogene homolog B; CRAF, v-Raf murine sarcoma viral oncogene homolog C; ERK-1/2, extracellular signal-regulated kinase 1/2; GDP, guanosine diphosphate; GRB2, growth factor receptor-bound protein 2; GTP, guanosine triphosphate; MAP, mitogen-activated protein; MEK1/2, mitogen-activated protein/extracellular signal-regulated kinase kinase 1/2; mTOR, mammalian target of rapamycin; P, phosphate; PI3K, phosphatidylinositol 3-kinase; PLCγ, phospholipase Cγ; RAF, rapidly accelerated fibrosarcoma; RAS, rat sarcoma; RHEB, Ras homolog enriched in brain; RTK, receptor tyrosine kinase; SHC, Src homology 2 domain-containing-transforming protein; SOS, son of sevenless; TSC1/2, tuberous sclerosis protein 1/2.

are usually mutually exclusive events.[5,10,19] *BRAF* is mutated in 8% of all human cancers, predominately in hairy cell leukemia (100%), melanoma (50%), papillary thyroid carcinoma (45%), colorectal (10%), and rarely in ovarian and lung cancer.[5,10,19] Almost all nonsynonymous activating and nonactivating *BRAF* mutations have been identified in exon 11 and exon 15.[19–21] A T1799 point mutation in exon 15 of the *BRAF* gene, resulting in a valine to glutamate substitution at codon 600 (V600E) affecting the kinase domain of the BRAF protein, is the most common oncogenic driver mutation in melanoma (90%).[19,22]

The *BRAF* V600E mutation results in constitutive activation of its serine/threonine kinase, with a high dependency on downstream MEK signaling, and represents an actionable component of this pathway.[23,24] BRAF inhibitors have been

TABLE 1. Clinical Features and Outcome of *BRAF*-Mutated NSCLC Patients

First author	Paik[20]	Marchetti[37]	Ilie[38]	Cardarella[39]	Luk[21]	Litvak[40]	Brustugun[41]	Villaruz[42]
Country	United States	Italy	France	United States	Australia	United States	Norway	United States
Year	2011	2011	2013	2013	2014	2014	2014	2015
Patients (n)	697	1046	450	883	273	63	979	951
BRAF (n)	18 (2.6%)	37 (3.5%)	40 (8.9%)	36 (4.1%)	7 (2.6%)	63 (NA)	17 (1.7%)	21 (2.2%)
V600E (%)	50	57	52	50	57	57	NA (100)	81
Smokers (%)								
V600E	100	52[a]	57	72	100	57	71	76
Non-V600E	100	100[a]	89	89	100	43	NA	100
Female (%)								
V600E	78	76[a]	52	56	75	53	59	53
Non-V600E	44	7[a]	26	50	33	56	NA	25
V600E (n)								
Early stage (I and II)	3 (33%)[b]	11 (52%)[a]	6 (29%)	3 (17%)	NA	11 (31%)	6 (35%)	5 (31%)[c]
Advanced stage (III and IV)	6 (67%)[b]	10 (48%)[a]	15 (71%)	15 (83%)	NA	25 (69%)	11 (65%)	11 (69%)[c]
Non-V600E (n)								
Early stage (I and II)	5 (56%)[b]	12 (80%)[a]	8 (42%)	2 (11%)	NA	10 (37%)	NA	1 (25%)
Advanced stage (III and IV)	4 (44%)[b]	3 (20%)[a]	11 (58%)	16 (89%)	NA	17 (63%)	NA	3 (75%)
Survival outcome[d]								
BRAF mutant vs. wt	Same[e]	Same	NA	Same	NA	Same[f]	NA	Same[g]
V600E vs. wt	NA	Worse	NA	NA	NA	NA	Same	NA
Non-V600E vs. wt	NA	Same	NA	NA	NA	NA	NA	NA
V600E vs. non-V600E	NA	Worse	Worse	Same[h]	NA	Better[i]	NA	NA

[a]The clinical features of one patient with non-V600E-mutated squamous cell carcinoma were not reported by Marchetti et al[37] and were note taken into account in this table.
[b]Paik et al[20] defined early stage as I to IIIA and advanced stage as IIIB to IV.
[c]The staging of one patient with *BRAF* V600E mutant was not reported by Villaruz et al[42] and was not taken into account in this table.
[d]Survival outcome and comparison between studies have to be taken with precaution, as the analyses were conducted over small numbers and different tumor stages.
[e]OS was calculated for advanced stage *BRAF* mutants versus other driver mutations (*EGFR*, *KRAS*, and *ALK*).
[f]OS was compared for early and advanced *BRAF* mutants versus *KRAS* or *EFGR* mutations.
[g]OS was compared between *BRAF* mutants and other driver mutants (*EGFR*, *KRAS*, *ALK*) or wild-type.
[h]Not statistically significant.
[i]Better OS was found with advanced IIIB to IV stage *BRAF* V600E mutants, but OS was the same in early I to IIIA stage between V600E and non-V600E mutants.
BRAF, v-Raf murine sarcoma viral oncogene homolog B; NSCLC, non–small-cell lung cancer; NA, not applicable; V600E, valine to glutamate substitution at codon 600; OS, overall survival; wt, wild-type; EGFR, epidermal growth factor receptor; KRAS, V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; ALK, anaplastic lymphoma kinase.

extensively studied in melanoma, where vemurafenib and dabrafenib have been approved (in 2011 and 2013, respectively) as single agents for the treatment of patients with advanced melanoma with *BRAF* V600E mutation.[25–27] Vemurafenib and dabrafenib are type I BRAF serine/threonine kinase inhibitors that have higher selectivity for BRAF V600E mutant protein than wild-type BRAF,[28,29] with essentially no activity against other human kinases. They preferentially bind the active site of the serine/threonine kinase domain of BRAF, which is constitutively activated in V600E mutants, in contrast to type II BRAF inhibitors such as sorafenib, which preferentially bind the inactive conformation. They are very potent against preclinical BRAF V600E mutants, with a half maximum inhibitory concentration (IC_{50}) of 31 nM for vemurafenib and 0.65 nM for dabrafenib. The MEK inhibitor trametinib has also been approved (in 2013) as monotherapy for patients with advanced melanoma with either a *BRAF* V600E or V600K mutation.[30] Trametinib is a MEK1/2 inhibitor with a favorable pharmacokinetic profile and a long circulating half-life. The drug inhibits MEK1/2 kinase activity and prevents RAF-dependent MEK phosphorylation.[18,31] Recently, the combination of dabrafenib and trametinib has shown superior efficacy compared with BRAF inhibitor monotherapy in *BRAF* V600E-mutated or V600K-mutated advanced melanomas,[32–35] hence becoming the new standard therapy for this indication.

BRAF Mutations in Lung Cancer

BRAF mutations are detected in approximately 2% to 4% of lung cancer, hence occurring at a lower frequency than *EGFR* mutations (10–15%) and probably in a slightly smaller subpopulation than *ALK* rearrangements (3–5%). These mutations have been predominantly diagnosed in the adenocarcinoma histological subtype, without obvious ethnicity or gender predominance.[5,19] Preclinical data in mice suggested a potential oncogenic role of *BRAF* mutations in the development of lung adenocarcinoma.[36] In contrast to melanoma where V600 mutations represent the vast majority of encountered *BRAF* mutations, V600E mutations represent only about half of *BRAF* mutations detected in NSCLC. Although V600E mutations are related to an elevated basal kinase activity compared with wild-type *BRAF,* non-V600E *BRAF* mutations occur often in the phosphate-binding loop (P-loop), which is the adenosine triphosphate (ATP) binding site.[19]

The biological and prognostic impact of *BRAF* mutations in NSCLC have been reported in several retrospective studies, all limited by the small patient numbers (Table 1[20,21,37–42]). The prognostic impact of V600E and non-V600E mutations remain contradictory in the reported series available.[37,40] Paik et al[20] performed an institutional analysis at Memorial Sloan-Kettering Cancer Center on 697 consecutive early-stage and late-stage lung adenocarcinoma. *BRAF* mutations occurred in 3% of patients, and the mutations identified were V600E (50%), G469A, and D594G. All patients were current or former smokers, and there was no difference in overall survival (OS) for *BRAF*-mutated patients when compared with other *EGFR*-mutated, *ALK*-mutated, or *KRAS*-mutated subpopulations.

Marchetti et al[37] reported the results of a retrospective series of 1046 surgically resected NSCLC (squamous and nonsquamous cell) patients. *BRAF* mutations were found in 3.5% of the tumors and in 4.9% of lung adenocarcinoma. The V600E mutation occurred in 2.8% of adenocarcinoma, more often among never-smokers, females, and aggressive histological types (micropapillary) and represented 58% of all *BRAF* mutations documented. Patients with the *BRAF* V600E mutation had shorter disease-free survival (DFS) and OS compared with wild-type and non-V600E mutations. In contrast, non-V600E mutations were found in current or former smokers. There was no difference in disease-free survival or OS observed in patients with or without non-V600E mutations. In this analysis, all *BRAF*-mutated tumors were found to be *KRAS* negative. Luk et al[21] conducted a mutation analysis for BRAF, *EGFR*, and *KRAS* in 273 advanced NSCLC patients and found that *BRAF* mutations occurred in 2.6% of cases. All patients had a smoking history in contrast to the *EGFR*-mutated population, and there was no difference in sex distribution between *BRAF* mutant and wild-type patients. The V600E mutation accounted in 58% of patients and was associated with the aggressive micropapillary subtype.

According to Cardarella et al,[39] *BRAF* mutations were identified in 4% of lung adenocarcinoma in a retrospective analysis of 883 patients with NSCLC, half of whom had V600E mutations. There were no differences in clinical features between *BRAF*-mutated and wild-type patients. However, the analysis suggested that patients with the V600E mutation seem to have shorter PFS after platinum-based chemotherapy. Recently, the Lung Cancer Mutation Consortium reported on a series of 63 patients with *BRAF* mutations in advanced stage adenocarcinoma and found a high incidence of V600E mutations (80%).[42] *BRAF* mutations were more likely to occur in current or former smokers with no gender predominance. There was no difference in OS between *BRAF* mutant and wild-type patients.

Therapy of BRAF Mutant NSCLC

In vitro preclinical models of NSCLC demonstrated that both vemurafenib and trametinib were effective as single agents in *BRAF* V600E mutant cells.[43] Moreover, trametinib was also effective in non-V600E mutants. The combination of vemurafenib and trametinib increased tumor cell death, suggesting that the combination should be more effective.[43] Two other MEK inhibitors (PD0325901 and CI-1040) have also shown activity in in vitro and in vivo preclinical models of NSCLC with BRAF V600E or non-V600E mutations.[44–46]

In patients with NSCLC harboring the *BRAF* V600E mutation, partial responses (PR) or complete responses (CR) have been reported for vemurafenib[47–50] and dabrafenib monotherapies.[51–53] However, the responses were not durable.[49,50,52] Sorafenib has also been reported to be active in patients with *BRAF* mutant NSCLC.[54] An update from the European EURAF cohort study was presented at the European Lung Cancer Conference 2015.[55] This retrospective multicenter study collected data from patients with advanced NSCLC with *BRAF* mutations, who were treated with at least one BRAF inhibitor outside of a dedicated clinical trial. Out of 35 patients, all with adenocarcinoma, 83% had *BRAF* V600E mutations, and 17% had non-V600E mutations (such as G466V, G469A, G469L, G596V, V600K, and K601E). Seventy-four percent patients received vemurafenib, 23% dabrafenib, and 3% sorafenib. Most patients received one line of BRAF inhibitors, whereas 11% patients received two lines of BRAF inhibitors. Although most BRAF inhibitors (86%) were used after at least one line of chemotherapy, five patients received BRAF inhibitors as first line, among who three achieved a PR. ORR was 53%, with 85% disease control rate (DCR), including 6% CR, 47% PR, and 32% stable disease. The planned subgroup analysis of BRAF V600E patients receiving vemurafenib showed 54% ORR and 96% DCR. PFS with first-line therapy (including chemotherapy) was 9.3 months for V600E and 1.5 months for non-V600E, and OS was 25.2 and 11.8 months, respectively. Overall, PFS and OS using a BRAF inhibitor for V600E mutants were 5 and 10.8 months, respectively. The duration of BRAF therapy was 4.3 months, ranging from 0.5 to 41 months, with some patients having durable responses. Most patients with non-V600E mutations did not respond to BRAF inhibitors and had a significantly poorer outcome, although CR was observed with one *BRAF* G596V-mutated NSCLC patient treated with vemurafenib. The mechanism of non-V600E *BRAF*-mutated NSCLC primary resistance to BRAF inhibitors is not fully understood. Three-dimension structural modeling of BRAF G469L suggested that it induces a conformational change impairing the binding of vemurafenib and dabrafenib.[56]

Prospective Studies

There are very few completed or ongoing prospective clinical trials in *BRAF*-mutated NSCLC, undoubtedly related to the rarity of these alterations and subsequent difficulty in enrolling and randomizing these patients. The multicenter VE-BASKET (NCT01524978) phase II study is assessing vemurafenib in *BRAF* V600-mutated nonmelanoma solid tumors, including NSCLC.[57] Out of 19 patients with NSCLC, 42% had unconfirmed PR and 42% had stable disease. The study is still recruiting. AcSé (NCT02304809) is a French phase II study evaluating vemurafenib monotherapy in patients with *BRAF* V600-mutated nonmelanoma solid tumors, including NSCLC. Results are pending.

The interim results of the BRF113928 (NCT01336634) prospective study was recently presented at the 2015 American Society of Clinical Oncology annual meeting.[58] This multicenter, single-arm, two-stage, open-label phase II study assessed the safety and efficacy of dabrafenib and trametinib in patients with stage IV NSCLC with *BRAF* V600E mutation,

after failure of at least one line of chemotherapy. The first stage of the study evaluated the safety of dabrafenib as monotherapy.[53] In 78 patients who received at least one prior line of treatment, ORR was 32%, DCR for more than 3 months was 56%, duration of response was 11.8 months, and 48% of responders had progressive disease. Among the six patients who received dabrafenib as first-line treatment, three achieved a PR. The most common adverse events (AEs) were similar to dabrafenib treatment in melanoma. Serious AEs occurred in 45%, with one death from intracranial hemorrhage. The second stage of the study evaluated the safety and efficacy of dabrafenib combined with trametinib.[58] Out of 33 treated patients, 82% remained on therapy at the time of the interim analysis, 12% had progressive disease, and 6% stopped because of AEs. Out of 24 evaluable patients, ORR was 63%, and DCR for more than 3 months was 88%, suggesting a superior antitumor activity than dabrafenib monotherapy. Toxicities were manageable and similar to those seen in previous studies in melanoma. Since the study passed the futility interim analyses, recruitment is ongoing. A third cohort assessing the combination treatment in previously untreated BRAF V600E NSCLC is currently recruiting.

A novel BRAF inhibitor LGX818 is currently being evaluated as monotherapy in an open-label, multicenter, single-arm phase II study in *BRAF* V600-mutated advanced NSCLC (NCT02109653). LGX818 is also being evaluated either in combination with the MEK inhibitor MEK162 or as a triplet combination with MEK162 and the cyclin-dependent kinase inhibitor LEE011 in an open-label, multicenter, phase Ib/II study in *BRAF*-mutated advanced solid tumors (NCT01543698). Other interesting ongoing studies are the phase Ib/II studies assessing the phosphatidylinositol 3-kinase (PI3K) inhibitor BKM120 either in combination with trametinib (NCT01155453) or the MEK inhibitor MEK162 (NCT01363232) in selected advanced solid tumors, including *BRAF*-mutated NSCLC. The MEK inhibitor selumetinib is also being assessed in a phase II study in nonmelanoma tumors with *BRAF* mutations (NCT00888134).

DISCUSSION

BRAF mutations represent rare driver alterations found in lung adenocarcinoma. Beyond this histological subtype, there is to date no clear clinical or pathological parameter that correlates with a higher prevalence of *BRAF* mutations. These mutations have been reported in heavy smokers, regardless of ethnicity and gender. The prognostic impact of *BRAF* mutations remains unclear to date, limited by the small numbers studied, with some series suggesting a worse outcome in this patient population.

Single-agent BRAF inhibitor therapy demonstrates activity in NSCLC; however, this activity is inferior to previous observations using ALK or EGFR tyrosine kinase inhibitors (TKIs) in related oncogene-addicted NSCLC. It appears that the type of *BRAF* mutation observed is predictive of response to BRAF inhibitor therapy, with V600E mutations presenting with higher response rates and PFS. However, the numbers of non-V600E mutations treated prospectively is still very low, and no conclusion can be drawn yet. Furthermore, some specific non-600E mutations might confer sensitivity to BRAF inhibitors, such as G596V mutation.[56] Of interest, a complete response was achieved with the TKI dasatinib in a unique case of NSCLC harboring *BRAF* Y472C.[59] Further characterization of non-V600E mutations is required.

Importantly, all patients treated with BRAF inhibitors will eventually demonstrate tumor progression. Resistance mechanisms to BRAF inhibitors are being thoroughly characterized in melanoma and consist of elevated expression of the kinases CRAF, COT or mutant BRAF, activating mutations in neuroblastoma RAS viral (v-ras) oncogene homolog (*NRAS*), *MEK1* or Ak strain *AKT1*, aberrant splicing of *BRAF*, activation of PI3K through the loss of phosphatase and tensin homolog (PTEN), and persistent activation of receptor tyrosine kinases, including platelet-derived growth factor receptor β (PDGFRβ), insulin-like growth factor 1 receptor (IGF-1R), and EGFR.[60–66] However, resistance mechanisms to BRAF inhibitors in NSCLC have not been characterized yet. In a single case of *BRAF* V600E-mutated NSCLC treated with dabrafenib, molecular profiling revealed three new acquired mutations in *KRAS*, *TP53*, and *CDKN2A*.[52] This highlights the need to conduct serial biopsies and molecular profiling at baseline and at progression to gain insight into BRAF inhibitor resistance mechanisms.

A combination of BRAF and MEK inhibitors may delay the time to resistance/progression, similar to the situation in preclinical models and in melanoma. The dabrafenib and trametinib combination in *BRAF* V600E-mutated NSCLC demonstrated very promising response rates that surpass those of dabrafenib monotherapy, with safety profiles similar to those found in melanoma. Results from the combination of LGX818 and MEK162 are awaited.

Future Strategies for *BRAF*-Mutated NSCLC

BRAF mutations have been identified in a variety of cancers, including NSCLC. Identifying the mechanisms of resistance to BRAF inhibitors in *BRAF*-mutated NSCLC will undoubtedly help in refining treatment strategies in the future. Moreover, it will guide strategies for front-line RAF targeted treatment or treatment at resistance. The role of dual BRAF/MEK inhibition could also be better defined. Some mechanisms of resistance reported in melanoma may be relevant in NSCLC. Recently, mechanisms of resistance to combined BRAF/MEK inhibition in melanoma have also been proposed.[67–69] These comprise *BRAF* alterations, *BRAF* amplifications, *MEK* mutations, PI3K–AKT–mammalian target of rapamycin (mTOR) pathway alterations, *PTEN* loss, cell cycle protein alteration, and *NRAS* mutations. One strategy to circumvent resistance to BRAF/MEK inhibitors is to combine other pathway inhibitors, such as PI3K–AKT–mTOR inhibitors,[70] ERK inhibitors,[69–71] or CDK4/CDK6 inhibitors. Tumor biopsies and analysis at progression is going to be necessary for full elucidation of resistance mechanisms. This will allow for the development of rational, evidence-based strategies for the next generation of clinical trials.

Non-V600E mutations should be better characterized in NSCLC to find appropriate agents for therapy. A better description of the genomic landscape of such tumors would be of great interest. Interestingly, some non-V600E mutations seem to have variable degrees of kinase activity and might even confer resistance to BRAF inhibitors but are possibly sensitive to MEK inhibitors through transactivation of

CRAF.[23] Thus, combinations of BRAF with MEK inhibitors should also be a potential option in this scenario.

Recently, with the emergence of immunotherapy and the recently reported striking benefit of checkpoint inhibitors in unselected patients with NSCLC in second-line therapy when compared with chemotherapy,[72–76] more treatments can be offered to patients, and the opportunity for drug combinations as well as the sequence of different compounds become a field of debate. Obviously, the right sequence of treatment—including chemotherapy, targeted agents and immunotherapy—should be rigorously tested in clinical trials.

Combining BRAF and/or MEK inhibitors and immunotherapy is tempting, and clinical trials testing these combinations are ongoing in melanoma but not in NSCLC yet. In favor of this strategy, recent studies demonstrated that high loads of mutations and tumor heterogeneity can increase the chance of benefiting from anti-CTLA-4 or programmed cell death protein 1 (PD-1) immunotherapy by generating tumor neoepitopes that are used as neoantigens and bind to major histocompatibility complex (MHC) class I molecules with great affinity.[77–80] A higher mutational load has been reported in patients with a smoking history,[81] and *BRAF* mutations are often found in smokers. In addition, there are several small series suggesting tolerability and promising activity of combining TKIs and checkpoint inhibitors in NSCLC, suggesting that the combination of BRAF/MEK inhibitors with immunotherapy merits further research. As a direct consequence, *BRAF*-mutated patients should not be excluded from immunotherapy treatment strategies.

CONCLUSION

The landscape of NSCLC treatment has dramatically changed since the emergence of targeted agents against actionable driver mutations, among which *BRAF* mutations belong. With immunotherapy soon becoming standard treatment after platinum-based chemotherapy, the complexity and diversity of treatment options is broadening. Finding the right personalized biology-driven treatment for each patient at baseline and at recurrence is the quest for the next years to come.

There is a strong need to better characterize resistance mechanisms and validate predictive and prognostic biomarkers that will help treatment decision. To this end, molecular profiling of tumors at diagnosis and at subsequent relapse is crucial. The ability to molecularly characterize tumors through the use of circulating tumor cells or tumor DNA (so called liquid biopsies) will be a significant advance. It appears that this approach may indeed be feasible.[82,83]

Although such questions will always remain restricted to relatively small clinical trials, an international collaborative effort will be needed to accurately define standards of care in *BRAF*-mutated NSCLC and allow for the registration of new treatment options for this tiny patient population.

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