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Review Article

BRAF Inhibitors in Carcinogenesis and their Clinical Implications: A Review

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ABSTRACT

Mutant BRAF is a silent activator and driver of carcinogenesis. This gene is less predominant compared to frequently mutated genes implicated in cancer development. However, its occurrence in cancer has been attributed to aggressive oncogenic growth following their role in activating key oncogenic pathways, BRAF/MEK/ERK and PI3K/AKT/MTOR pathways which play crucial roles in carcinogenesis. BRAF can self-activate and drive cancer growth as a single monomer or as a dimer independent of RAS activation. A complete blockade in oncogenic BRAF will serve as a target for the development of potential therapeutic agents with no side effects. The use of BRAF inhibitors in targeting oncogenic signaling pathways has however proven inefficient due to side effects, drug resistance and relapse of the disease. The current treatment in targeting BRAF-driven oncogenesis involve the combination of BRAF inhibitors, MEK inhibitors, and immunotherapy. Resistance to BRAF inhibitors have been a serious challenge to the treatment of BRAF-linked carcinogenesis. Although, current research is targeting the use of immunotherapy as a single therapy. Other therapies with ongoing research include the use of nanotechnology for effective drug targeting and delivery at a high concentration; as well as ongoing pre-clinical trials to overcome BRAF resistance to treatment which include pre-mRNA splicing, BCL2 inhibitors, tubulin inhibitors, mitochondrial-targeted agents, polo-like kinase inhibitors and many others. This review discussed different treatment strategies for mutant BRAF, their mode of action and the specific cancers treated as well as current trends for mutant BRAF induced cancer.

Keywords: BRAF mutation, Mutant BRAF, Cancer, BRAF inhibitors, Oncogenic pathway.

Introduction

V-Raf Murine Sarcoma Viral Oncogene (otherwise known as BRAF oncogene) is a driver mutation in the process of carcinogenesis. It belongs to the Rapidly Accelerated Fibrosarcoma (RAF) kinase family which comprises of A-RAF, BRAF and CRAF. BRAF gene encodes a serine-threonine protein kinase that plays an important role in driving cancer growth and metastasis through a complex signaling cascade, which is known as the Rat sarcoma virus (RAS) /V-Raf Murine Sarcoma Viral Oncogene (BRAF)/Mitogen-activated protein kinase/ERK kinase (MEK) / Extracellular-signal-regulated kinase (ERK) pathway (RAS/BRAF/MEK/ERK). BRAF mutations in cancer cells are usually acquired during carcinogenesis. BRAF "activating mutations" lead to the continuous production of the BRAF protein. The class I BRAF mutation (V600E mutant) is the most dominant, RAS independent, and can signal cancer growth as a single monomer. BRAF is a major kinase and a key activator of the RAF/MEK/ERK signaling cascade.[1] Cancer cells that harbor oncogenic BRAF are more likely to respond differently to treatment options, such as chemotherapy and immunotherapy, and targeted therapies which are used to control cancer growth in BRAF positive tumors.[2]

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Tumours with BRAF mutations are clinically distinct from other tumors and this can give information on the prognosis of a particular tumor. Studies have indicated that the primary mechanism of resistance to BRAF inhibitors (BRAFi) is a result of the abnormal reactivation of the MAPK pathway. This led to the emergence of both dual (BRAFi and MEK inhibitors (MEKi)) and triple (BRAFi, MEKi, and immunotherapy) combined therapy which is also limited due to lack of efficacy and high level of toxicity. The occurrence of BRAF mutations in cancer disease and implications for treatment are herein discussed and new trends in combating BRAF - associated cancer burden are evaluated in this study.

Materials and Methods

The studies included were identified by computer aided literature search with limitation on date of publication (2011- 2022). The search was conducted across PUBMED, Science Direct and Google Scholar. The search keywords were 'BRAF Inhibitors in Carcinogenesis and their Clinical Implications' and Boolean expression were used for this search. Studies involving case study, clinical trials, review report and original articles on BRAF inhibitors in cancer were included in this study while studies on other RAF subtypes and non-related studies to BRAF mutation and inhibitors were excluded from this study. The Flow chart diagram (Figure 1) below illustrates the selection for this study.

Results and Discussion

BRAF Mutation and its Signaling Pathways

BRAF protein is a serine/ threonine-protein kinase that is involved in the downstream signaling of the MEK and ERK pathways and thus drives oncogenic proliferation in the process. BRAF mutation is

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responsible for the initiation of cellular growth, proliferation, and differentiation by driving the BRAF/MEK/ERK pathway.[3] The BRAF gene is located on chromosome 7(7q34). It can be activated by RAS-GTP (the active conformation of RAS) or by self-activation either as a monomer or dimer independent of RAS. BRAF has been categorized into three distinct classes with each class marked with its distinctive features. Each of the classes has varying clinical characteristics, tumor behavior, and response to therapy. The class 1 BRAF is the most aggressive form of BRAF mutation which is located at position 600 exon 15 of the BRAF gene. The most frequent in this class is V600E which involves a single point substitution of valine with glutamate (GTG to GAG) at position 600. Another more commonly mutant member of this group is the V600K (which occurs in 5-30% of cases) in which valine undergoes two-point mutation to lysine (GTG to AAG).[3] The Class I are V600E mutant groups existing as monomers and are capable of self-activation independent of RAS signaling. They require high kinase activity and include the V6OOE, V600D, V600K, and V600R subgroups. Class 2 are V600 mutant, exist as dimers, independent of RAS activation and require intermediate kinase activity. Examples of this group include K601T, K601N, K601D, fusion protein, L597V, G469A, and G469V. However, class 3 are non-V600 mutant, activated by RAS, and exists as a heterodimer (BRAF and CRAF). They have a low level of kinase activity with a longer survival period compared to classes 1 and 2 respectively.[4] Examples include G466V, G466A, G596R, D594H, F595L, D287H, N581S, D594N, and many others that directly depend on RAS signaling.

There are different techniques for testing BRAF mutation in a patient, which include: next-generation sequencing (NGS) which reveals other genetic mutations such as c-KIT as well as NRAS;[5] sanger sequencing which shows the nucleotide changes in the sequence, and immunohistochemistry which is a more sensitive and a specific test for BRAF V600E but non-specific for other BRAF subgroups and need further confirmatory test using NGS or Polymerase Chain Reaction (PCR) test.[5] A more novel technique for detecting and monitoring the therapeutic response of tumors harboring BRAF involves the use of liquid biopsy such as the plasma sample of patients.[6]

BRAF Signaling Pathways

Studies have clearly illustrated that BRAF drives carcinogenesis through downstream signaling of a cascade of protein kinases, the BRAF/MEK/ERK kinase. BRAF upon activation drives the downstream signaling of these pathways. We have been able to illustrate that BRAF can exist as a monomer or dimer independent of RAS activation. BRAF can also be dependent on the activated conformation of RAS (RAS-GTP). Upon activation of BRAF, MEK1/2 are activated simultaneously which further signals the activation of ERK, the last member of the signaling cascade. The phosphorylation of ERK upon activation also causes further phosphorylation of both cytoplasmic and nuclear transcription factors (TFs) which induces cancer by altering crucial genes that regulate normal cell growth and differentiation.

Oncogenic BRAF can drive two pathways in the process of carcinogenesis, the BRAF/MEK/ERK and BRAF induced Phosphoinositide 3-kinase (PI3K)/ Protein Kinase B (PKB also known as Akt)/ Mammalian target of repamycin (mTOR) (BRAF/P13K/Akt/mTOR) pathways respectively as shown in Figure 2. This process can occur through an onset upstream activation of the RAS oncogene which further drives the cascade for the two pathways or through an independent BRAF activation which signals the pathways. Targeted inhibition of BRAF, MEK, ERK, PI3K, Akt, and mTOR will block and prevent further signaling of these pathways which will serve as a potential therapy for cancer management. The activation and amplification of BRAF can drive carcinogenesis either through the signaling of the BRAF/MEK/ERK pathways or the PI3K/Akt/mTOR pathways or activation of both occurring simultaneously. BRAF can signal these pathways following an upstream activation by RAS oncogene or independent of RAS activation.

BRAF Inhibitors (BRAFi)

BRAF inhibitors are medications employed to effectively target pathways of tumors with mutant BRAF which drives cancer growth and progression.

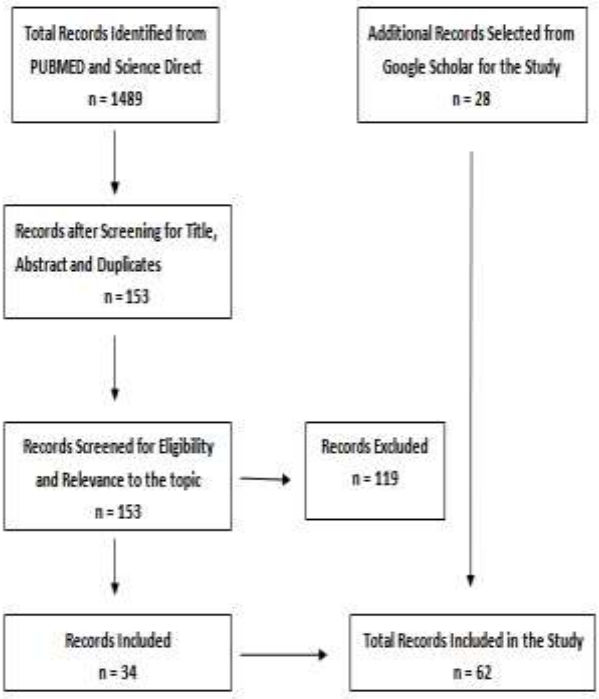

Figure 1: Flow Chart Diagram showing Records included in the Study.

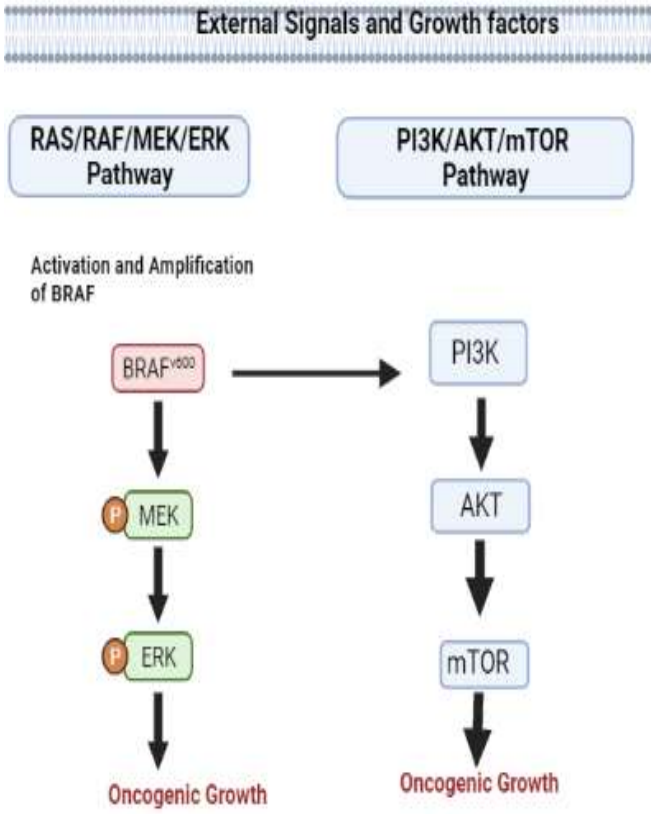

Figure 2: Mutant BRAF Signaling pathways for BRAF/MEK/ERK and PI3K/Akt/mTOR pathways respectively (Designed with Biorender.com).

The primary action of these medications is to interrupt the complex signaling pathways involved in cancer growth and progression and not necessarily to kill cancer cells like chemotherapy medications.[2] The inhibitors help in controlling cancer growth for a specific period but do not cure cancer. This targeted blockade in the signaling pathway prevents further downstream activation and finally execute cell cycle arrest. These small molecule inhibitors inactivate the catalytic activity of BRAF, thereby interrupting the complex signaling pathways involved in cancer growth and progression. BRAF inhibitors can be combined with MEK inhibitors which generate a blockade point in the Mitogen-Activated Protein Kinase (MAPK) pathway at two different levels, inhibiting oncogenic downstream signaling and causing cell cycle arrest as shown in Figure 3. Kinases are encoded in the human genome and they play a vital role in the normal body functioning and processes. A lot of diseases such as cancer emanate as a result of dysfunction or deregulation of kinase activities and as such kinases can be used as important therapeutic targets for many diseases of man. G-protein coupled receptors are primary pharmaceutical targets followed by protein kinases,[7] and more than 25% of medications have been produced to target protein kinases. BRAF inhibitors are kinase inhibitors for mutant BRAF and they can be combined with MEK inhibitors which act to inhibit tumor growth at other points in the RAF/MEK/ERK signaling pathway. The inhibition of BRAF has been reported to be associated with MEK-dependent signaling activation, and thus the need for combined inhibition using both BRAF inhibitors and MEK inhibitors for more effective therapeutic targeting.[8] This combined therapy provides a more effective treatment option for a longer duration with fewer side effects compared to using BRAF inhibitors alone. Grassi et al.[9] reported the use of a combined triple therapy consisting BRAF inhibitors, MEK inhibitors, and EGFR inhibitors to give more efficient treatment for BRAF than using only BRAF inhibitors. It was reported that the rare class 1 BRAF V600 mutations such as V600R, V600D, and V600M show a more progressive response to BRAF inhibitor treatment and increased overall survival (OS) compared to the most frequent V600E and V600K in melanoma patients.[10] BRAF non-V600 subgroup are less common, with a yet to be identified prognostic value as indices for cancer progression; however future studies might be able to unravel the prognostic value of this BRAF subgroup.

Mechanism of Action of BRAF Inhibitors: A Clinical Overview

These medications (BRAFi) are in oral forms and are selective inhibitors of BRAF kinase. These small molecule inhibitors inactivate the catalytic activity of BRAF.[11] Through their inhibitory effect on BRAF, the MAPK signaling pathway which regulates cancer growth and survival is interrupted. These medications are selectively specific for V600 mutant BRAF due to their competitive inhibition of its ATP binding pocket and stabilizing the kinase in its active conformation.[12] According to pre-clinical studies on BRAF inhibitors, vemurafenib and dabrafenib were evaluated to be selective inhibitors of kinase activity in melanoma patients harboring mutant BRAF; thereby blocking ERK phosphorylation and subsequent cell growth, and further inducing G1 cell-cycle arrest and programmed cell death.[13,14]

It has been clinically evaluated that vemurafenib inhibits V600E, V600D, and V600R mutant cell lines,[15] while dabrafenib exhibit inhibitory action against V600E, V600D, V600R, and V600K.[13,16] There is no inhibition of vemurafenib or dabrafenib on non-V600 mutations or wild type BRAF;[13,15] however, the action of encorafenib is in contrast as this drug targets both V600E and V600K and equally exhibit an inhibitory effect on wild type BRAF.[12,17] These three drugs (vemurafenib, dabrafenib, and encorafenib) can activate the MAPK pathway through a RAS-dependent mechanism, especially in cells with preexisting RAS mutations.[16,18,19] This may lead to drug resistance when the drugs select for the survival of non-BRAF V600 cells.[18] A combination of both BRAF inhibitors with MEK inhibitors leads to decreased MAPK signaling and provides a more potent and longer inhibition of the ERK signaling pathways.[16,20] This is as a result of a further inhibitory action of MEK inhibitors downstream of the RAF/MEK/ERK pathway, thereby providing better management for tumors associated with BRAF mutations such as in melanoma cell lines, breast, and other cancer types. BRAF and MEK inhibitors block the MAPK pathway and execute two key functions which are; inhibition of oncogenic downstream signaling and cell cycle arrest.

The use of EGFR inhibitors (e.g cetuximab) has been reported to be efficient in targeting the Class 3 BRAF,[21] which depends on RAS activation that is triggered by EGFR mutation. This study was carried out in colorectal cancer patients harboring BRAF in which class 3 BRAF responded to anti-EGFR treatment compared to class 2. This study is however contrary to two other distinct studies carried out by Johnson et al.[22] and Shinozaki et al.[23] who both reported that EGFR inhibitors are unable to alleviate BRAF non-V600 mutant (class 3). There is thus a gap in the literature that needs to be filled by evaluating if the inhibition of EGFR has a positive impact to decrease tumors harboring non-V600 BRAF mutant.

Classifications of BRAF inhibitors

There are two basic classifications of BRAF inhibitors; type 1 and type 2 inhibitors.[24] The type 1 inhibitors bind to the active conformation of the protein kinases leading to the formation of ~1-3 hydrogen bonds with the kinase hinge residues through an interaction with the hinge region of BRAF. These inhibitors interact with the protein kinases at the ATP binding site and form hydrophobic bonds in and around adenine regions.[7,25] On the contrary, type-II inhibitors act by binding at the inactive conformation of the protein kinases. Hydrogen bonds are formed between BRAF inhibitors and the residues in the αC-helix and DFG motif in the allosteric site.[7,24,25]

Type I BRAF Inhibitors

Vemurafenib (PLX4032/RG7204/RO5185426)

Vemurafenib is an active inhibitor of BRAF V600E, V600D, V600K, and V600R mutants, but does not inhibit wild-type BRAF in melanoma tumors; however, it has a potent inhibitory effect at a higher concentration in other cell lines. This kinase inhibitor has a low molecular weight and is potent in the inhibition of oncogenic BRAF and acts through a selective binding at the BRAF V600E ATP binding site and inhibiting its oncogenic drive. It has been reported to show its inhibitory action in different cancer types such as melanoma, papillary thyroid carcinoma, colorectal cancer, ovarian cancer, and more recently in the inhibition of BRAF-linked metastatic Triple Negative Breast Cancer (TNBC).[26] This drug was approved by the U.S Food and Drug Administration in 2011 for the treatment of metastatic melanoma and other tumors harboring BRAF V600E mutant.[24] This drug is associated with severe symptoms and skin toxicities.[27]

Dabrafenib (GSK2118436)

Dabrafenib exhibits an inhibitory effect on both mutated BRAF V600E (with IC50= 0.8 nM),[13] and a few other kinases at very high concentrations. This drug can inhibit growth activities in other mutated forms of BRAF such as V600D and V600R.[13] This drug was approved by FDA in 2013 for BRAF V600E-related cancer types.[28] It is associated with various skin toxicities and side effects.[29]

LGX818

LGX818 has an inhibitory action on mutated BRAF V600E. It only inhibits the V600E but does not have an inhibitory action on wild-type BRAF following a clinical trial on more than 4000 cell lines of wild-type BRAF.[30] This drug was tested to inhibit both rat and mice multiple mutated BRAF xenograft at a low dosage of 1 mg/kg but did not inhibit the wild type BRAF at a very high dose of 300 mg/kg administered twice.[30]

PLX4720

PLX4720 is very potent for V600E mutated BRAF at lower concentrations without inhibiting wild-type BRAF. It inhibits the phosphorylation of the extracellular signaling receptor kinase (ERK) and induces cell cycle arrest and death for BRAF V600E mutant type. The IC50 for mutated BRAF V600E is 13nM and this drug has shown inhibitory activity on WT- BRAF (IC50 = 160 nM), CRAF (IC50 = 6.7 nM), and other kinases at very high doses.[31] PLX4720 also has inhibitory activity on mutated BRAF V600E anaplastic thyroid cancer and orthotopic thyroid cancer by downregulating certain genes involved in cancer growth and metastasis.[32]

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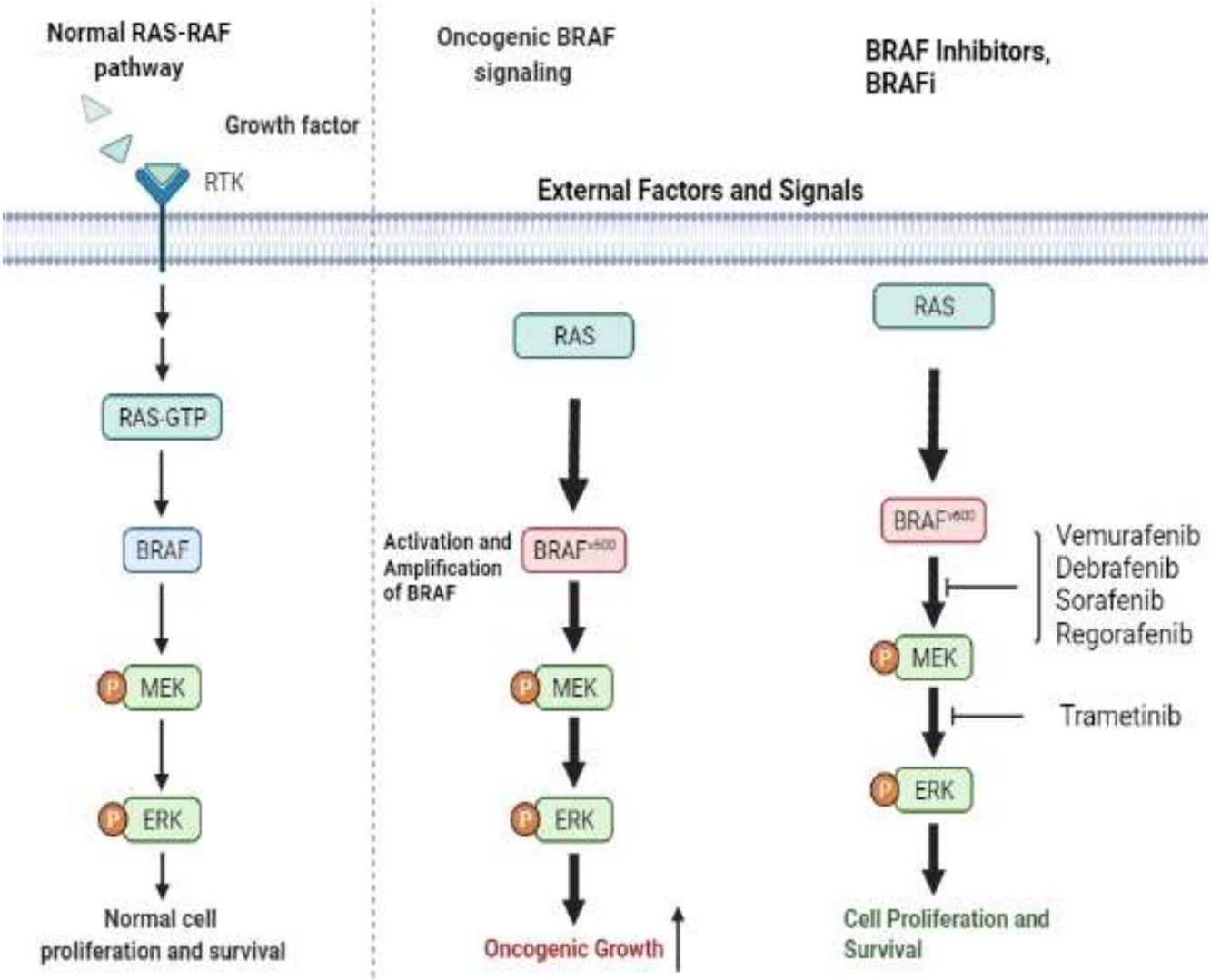

Figure 3: Mechanism of action of dabrafenib and trametinib (Designed by Biorender.com)

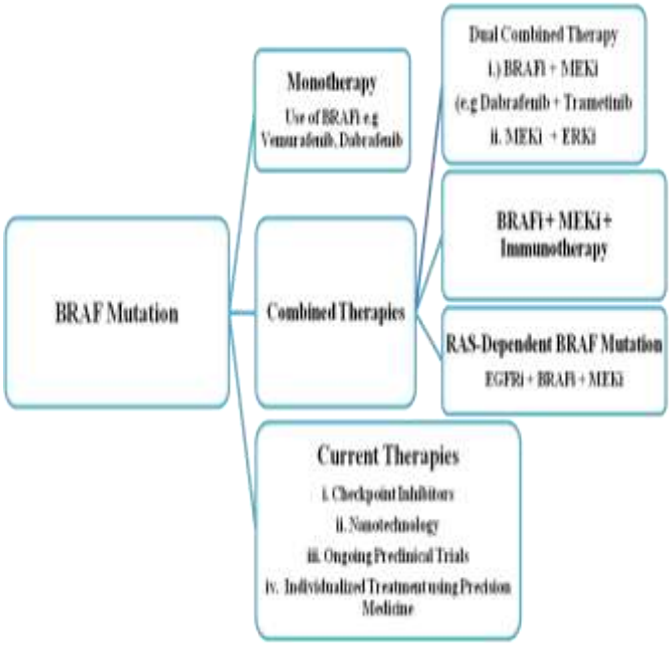

Figure 4: Illustrative Presentation of Therapies for Targeting BRAF Mutation.

SB-590885

SB-590885 is very active and selectively inhibits V600E mutated BRAF by inhibiting ERK phosphorylation,[33] and kinase activities in the active conformation of BRAF protein. Among 48 different kinases, SB-590885 showed selective inhibition for only BRAF and CRAF. Its inhibitory constant (Ki app) for BRAF is 0.16 nM,[33] while that of CRAF is 1.72 nM. SB-590885 is useful in treating mutated BRAF cell lines by decreasing the tumorigenic properties and cellular transformation of malignant cells.[33,34]

GDC-0879

GDC inhibits mutant BRAF in both cell-based assay (IC50 = 0.13nM) and in-vitro studies. It is a potent inhibitor for CRAF among 140 different kinases[35] at a concentration of 1.0 µM.

Type-II BRAF Inhibitors

Sorafenib (BAY 43-9006)

It inhibits different kinases such as RAF kinases and receptor tyrosine kinases (RTKs) which induces cancer. The FDA approved it for the treatment of unresectable hepatocellular carcinoma patients and metastatic renal cell carcinoma.[36] Treatment of cancer with Sorafenib is associated with severe toxicities.[37]

Regorafenib (BAY 73-4506)

It inhibits mutant BRAF V600E (IC50 = 19 nM), wild-type BRAF (IC_{50} = 28 nM), CRAF (IC_{50} = 2.5 nM) and other kinases.[12] The US FDA approved this drug for the treatment of metastatic colorectal cancer and unresectable metastatic GI stromal tumors.[38,39] The use of

this medication is associated with different adverse symptoms and toxicities.[40]

XL281 (BMS-908662)
XL281 is very active and selectively inhibit RAF; most especially the BRAF wild type (IC_{50} = 5nM), CRAF (IC_{50} = 3 nM) and BRAF V600E (IC_{50} = 6 nM) at a similar range of concentration for RAF.[24] Adverse effects include diarrhea, vomiting, fatigue, anorexia, nausea, and hypokalaemia.[41]

RAF265
RAF265 is capable of inhibiting various kinases such as mutated BRAF V600E (EC50 = 0.14 µM, CRAF (IC50 = 3-60 nM), and even the wild type BRAF. This drug also shows an inhibitory effect on c-Kit, VEGFR2 (EC_{50} = 0.19 µM, IC_{50} = 30 nM), and PDGFRβ.[24,42] In the pre-clinical trial, patients showed reduced cell proliferation and apoptosis, although this does not correlate with reduced phosphorylated ERK 1/2.[43] The toxicities associated with this drug include ataxia, diarrhea, thrombocytopenia, visual disturbance, hyperlipidemia, and pulmonary embolism.[42]

Unclassified BRAF inhibitor
ARQ736
ARQ736 is an active ATP-competitive that inhibits BRAF V600E. It has the potency to kill cancer cell lines and also exhibit an inhibitory effect on wild-type BRAF as well as CRAF on different cancer types such as colorectal cancer, thyroid cancer, and melanoma.[44,45] This drug is also potent in reducing angiogenesis in humans and cytokine-related proteins.[44,45]

Resistance to BRAF Inhibitors
Over the years, a lot of mechanisms have been established to explain resistance to BRAF inhibitors and this can be classified into two (2); namely i.) Intrinsic resistance (also known as primitive resistance), which explains an innate resistance to BRAF inhibitor treatments, and ii.) Secondary resistance (extrinsic resistance), explains the loss of drug efficacy during treatment.[12]

Intrinsic Resistance
Most cancer patients with oncogenic BRAF do not respond to treatment with BRAF inhibitors and are best defined as intrinsically, innately, or primarily resistant to targeted treatments designed to downregulate the MAPK signaling pathway. Unresponsive BRAF inhibitor patients were first evaluated during the phase II clinical trials of vemurafenib, in which 47% of patients were unresponsive to treatment and were later confirmed in phase III clinical trial.[46] Several mutated genes tend to contribute to this; such as COT, NF1, RTK, RAC1, CDKN2A/CDK4, and PTEN which can increase the survival of cancer cells, its promotion, and migration thus leading to insensitivity to BRAF inhibitors.[47,48] Also, the tumor microenvironment is an important factor that should be considered in intrinsic resistance. The stromal secretion of Hepatocyte Growth Factor (HGF) triggers the activation of P13K and MAPK which induces resistance to BRAF inhibitors.[49]

Extrinsic Resistance
Extrinsic resistance is attributed to the reactivation of the MAPK pathway, which can occur downstream, upstream, or even at the BRAF level.[12] One of the possible mechanisms that explain this is the presence of activated RAS which induces resistance to BRAF inhibitors; NRAS mutations were reported in 8-20% of patients who developed resistance after taking BRAF inhibitors.[50,51] Also, high CRAF levels and ectopic CRAF expression are associated with BRAF resistance.[52] Activation of MEK 1 oncogene observed in 7% of progressed patients,[51] is linked to the resistance of both BRAF inhibitors and MEK inhibitors which reduces the efficacy of the combined therapy; although sensitive to ERK inhibitors.[12]
Other mechanisms linked to acquired resistance to BRAF inhibitors include: BRAF gene amplification,[50,51] acquisition of mutations such as BRAFL514V mutation[53] and BRAF L50H mutation,[54] intratumoral and intertumoral heterogenicity[55] and mutations of several other genes such as RTK, ERK1/2, PTEN, and PI3K/AKT are associated with extrinsic resistance. The mechanism of resistance is as follows (1) RTK is upregulated; (2) Increase (amplification) in BRAF ; (3) Alternative splicing of BRAF gene; (4) Loss in the function of NF1; (6) Activation of ERK; (7) Loss of Phosphatase tensin homologue (PTEN); and (8) Activation of alternative signaling pathways. BRAF, v-Raf murine sarcoma viral oncogene; ERK, extracellular signal-regulated kinase; GFR, growth factor receptor; mTOR, mammalian target of rapamycin; NF1, neurofibromin 1; PTEN, phosphatase and tensin homolog; RTK, receptor tyrosine kinase.[56]

Overcoming BRAF Resistance
Studies have indicated that the primary mechanism of resistance to BRAF inhibitors is as a result of the abnormal reactivation of the MAPK pathway.[57] Therefore, the most important way to overcome BRAF inhibitor resistance will involve a combined therapy of BRAF inhibitors and other kinase inhibitors (such as MEK inhibitors and ERK inhibitors) downstream in the MAPK phosphorylation pathway. This combined therapy is an active treatment that gives a lasting clinical response among patients[58,59] and also has a reduced level of toxicity compared to using only BRAF inhibitors.[12] Also, MEK inhibitor/ ERK inhibitor is an effective combined therapy useful in the management of resistance to BRAF inhibitors.[60] BRAF inhibitors and MEK inhibitors can be combined to serve as a standard care therapy for treating mutant BRAF in cancer patients. In addition to the role of BRAF inhibitors, MEK inhibitors act by blocking the MEK/ERK signaling pathway which is usually activated when a patient undergoes treatment with BRAF inhibitors; this delays drug resistance and promotes long-lasting response.[61] MEK inhibitors also inhibit the synthesis of programmed death ligand-1,[62] and also help to inhibit the activation of the immune system which occurs during treatment with BRAF inhibitors. Some of the well-researched and often combined BRAF/MEK inhibitors include; encorafenib + binimetinib,[63] vemurafenib + cobimetinib,[59] and dabrafenib + trametinib.[64] In addition, the combination of MEKi and ERKi is efficient in the treatment of BRAF inhibitor resistance.[65]

Current Clinical Implications for BRAF Treatment
Recent advances in research have developed immunotherapy as an additional treatment option for BRAF mutation and blockade of the MEK/ERK signaling pathway. Immunotherapy proofs to be a useful treatment option as the immune system is effectively utilized to fight cancer cells most especially the checkpoint inhibitor antibodies which are used in combination with other kinase inhibitors. These include the monoclonal antibody called ipilimumab which was approved by the FDA for the management of malignant melanoma in 2011;[66] programmed cell death protein 1 (PD 1), pembrolizumab, and checkmate 067 are checkpoint molecules which were subsequently approved by the FDA.[66] The ongoing research on the dual combination of different kinases with immunotherapy (checkpoint inhibitors) has provided hope that this combined therapy will be tolerated with greater efficiency.[67] However, one of the major challenges encountered is the high toxicity profile posed by treatment such as bowel perforation and liver toxicity which may undermine the efficacy of the therapy in the long term.[68,69] Also, the response rate of the triple effect of combined BRAFi/MEki and immunotherapies appeared to be less compared to treatment with only BRAF/MEK inhibitors.[70] A clear knowledge of the mechanism of generation and metastasis of tumour cells is crucial for the development of novel and effective therapies to induce cell death in cancer cells.[71] In an attempt to remedy the rate of toxicities and efficacy of the triple therapy, future studies are keen on understanding the various mechanisms of resistance to the triple therapy to develop better therapeutic products with a high level of efficacy on the long term and no observable side effects. There are series of pre-clinical trials ongoing to develop novel targets for overcoming BRAF resistance such as pre-mRNA splicing, BCL2 inhibitors, tubulin inhibitors, mitochondrial-targeted agents, polo-like kinase inhibitors, and many others,[72] as shown in figure 4 above. In addition, current research interest is exploring chemoprevention using food and their active ingredients as potential therapy for cancer prevention due to the high toxicity profiles and adverse effects of conventional treatments.[73]

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Table 1: Mutant BRAF Therapies and Mode of Action

BRAF Mutation Therapy	Mode of Action	Cancer Type(s) Targeted	References
Monotherapy	Actively inhibits BRAF kinases depending on the type of BRAF inhibitor used as described above.	Melanoma, papillary thyroid carcinoma, colorectal cancer, ovarian cancer, Triple negative breast cancer (TNBC); unresectable hepatocellular carcinoma patients and metastatic renal cell carcinoma.	26,36,68,69
i. BRAF Inhibitors (BRAFi)			
ii. EGFR Inhibitors (EGFRi)	Actively targets Class 3 BRAF mutation which depends on RAS signaling.	Metastatic Colorectal cancer, non-small cell lung cancer (NSCLC), locoregional advanced head and neck cancers.	21, 77,78
Combined Therapies			
Dual Therapy i. BRAF inhibitors (BRAFi) + MEK inhibitors (MEKi)	Inhibits both BRAF kinases and MEK kinases thereby blocking further signaling of the BRAF/MEK/ERK pathway.	Malignant Melanoma, NSCLC, Anaplastic thyroid cancer (ATC)	20,60,61,79 , 80
Other Combined Therapies ii. BRAFi + MEKi + Immunotherapy	Provides an effective inhibitory effect on both BRAF and MEK kinases with increased drug efficacy and reduced toxicities	Advanced melanoma	65,70
iii. RAS Dependent BRAF signaling BRAFi + MEKi + anti-EGFR	This combined therapy blocks EGFR activation of RAS oncogene which initiates the pathway; and it also blocks downstream signaling of the BRAF and MEK kinases.	Metastatic colorectal cancer	9, 21
Current Therapies			
i. Checkpoint inhibitors (Immunotherapy) and different kinases	Research is ongoing on the use of combined kinases and immunotherapy to provide a more efficient treatment with no side effects.	Wide range of cancers such as bladder, rectal, colon, head and neck, liver, stomach, breast, cervical, lung, renal cell cancers, skin cancer including melanoma, and any solid tumors.	66,67,80
ii. Nanotechnology	Current research is exploring effective drug targeting and delivery using nanotechnology.	Wide range of cancer through combination with other therapies such as gene therapy, chemotherapy, immunotherapy and radiotherapy.	74-75
iii. Ongoing pre-clinical trials	There are ongoing pre-clinical trials to overcome BRAF resistance such as pre-mRNA splicing, BCL2 inhibitors, tubulin inhibitors, mitochondrial targeted agents, polo-like kinase inhibitors and many others.	Different cancer types	72

Furthermore, research is currently ongoing to improve existing therapies such as the use of nanotechnology to effectively deliver drugs even at higher concentrations,[74-75] with minimal or no side effects. Also, due to the multiplicity of potential targets, research is currently in progress to evaluate the use of proteomic and genomic data for more personalized therapy for targeting the pathways which are activated during BRAF inhibition.[76] This personalized therapy will provide more targeted and efficient treatments to patients harboring BRAF.

Conclusion

The therapeutic benefits of BRAF inhibitors have been limited as a result of drug resistance. This led to the emergence of dual and other combined therapies which are associated with side effects and reduced efficacy on the long run. Current clinical trials are evaluating the use of immunotherapy and any of the kinase inhibitors to treat mutant BRAF and resistance to BRAF inhibitors. Future researches are exploring other novel therapies that will effectively target BRAF resistance and maintain a long-lasting treatment.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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