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Resistance mechanisms in BRAF^{V600E} paediatric high-grade glioma and current therapeutic approaches

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Paediatric high-grade gliomas (pHGG) are aggressive central nervous system tumours with a poor prognosis. BRAF^{V600E} mutant pHGGs can be treated with targeted BRAF inhibitors, which have shown both preclinical activity and potent clinical efficacy. Unfortunately, the development of drug resistance results in disease relapse or progression and is the primary cause of treatment failure. While there is a lot of data to explain mechanisms of resistance in other BRAF^{V600E} tumours, comparatively little is known about the mechanisms of BRAF inhibitor resistance in BRAF^{V600E} pHGG. Recent literature has identified aberrations in members of the RAS/RAF/ERK pathway, the PI3K/AKT/MTOR pathway and the cell cycle as major contributors to the resistance profile. A range of novel therapies have been suggested to overcome BRAF inhibitor drug resistance in BRAF^{V600E} pHGG. This review will discuss the current literature available for BRAF inhibitor resistant BRAF^{V600E} pHGGs and provide an overview of the currently available and proposed therapies.

KEYWORDS

high grade glioma (HGG), resistance, BRAF^{V600E}, MEK = mitogen extracellular kinase, pediatric

BRAF^{V600E} paediatric high-grade gliomas

Paediatric high-grade gliomas (pHGG) are a subset of central nervous system (CNS) tumours with a poor prognosis and low survival (1–4). For many pHGG subtypes there are few treatments available, with palliative treatments the only option for many. BRAF^{V600E} mutations are seen in both paediatric low-grade glioma (pLGG) and pHGG, with an overall prevalence in pHGG of approximately 6% across the tumour subtypes (5–9). In pLGG, prognosis is poorer for patients with BRAF^{V600E} mutant tumours, and these tumours have a higher likelihood of transforming to HGGs (10),

Indeed, $BRAF^{V600E}$ is the most common recurrent mutation in these secondary pHGG, occurring in 39% of cases (11). Interestingly, in pHGG $BRAF^{V600E}$ mutations confer an improved prognosis compared to wildtype tumours, with a 2 year survival of 67% (5). However, secondary mutations such as *CDKN2A/B* deletion or *TERT* promoter mutations, frequently co-occur with $BRAF^{V600E}$, with evidence demonstrating that these mutations lead to more aggressive tumours and a poorer prognosis (8, 12, 13). While most $BRAF^{V600E}$ mutant pHGG respond well to BRAF inhibition, the response is confounded by the rapid onset of drug resistance. Until recently, limited research has been performed on drug resistance in $BRAF^{V600E}$ pHGG, with most studies focussing on pLGG and adult HGG cell lines studies in place of pHGG (14, 15).

The therapeutic use of targeted BRAF inhibitors have shown improved survival in adults with $BRAF^{V600E}$ mutated melanoma in Phase 3 clinical trials and are now part of standard clinical care, with first generation BRAF inhibitors vemurafenib and dabrafenib approved by the FDA in 2011 and 2013 respectively (16, 17). This has informed several paediatric tumour studies (10, 18–20), with BRAF inhibitors since showing promise in pHGG (21, 22). Vemurafenib is currently being investigated through the Children's Oncology Group MATCH trial for its efficacy in treating paediatric tumours harbouring $BRAF^{V600}$ mutations, including pHGG (NCT03155620, NCT03220035). Unfortunately, the onset of resistance to first line BRAF inhibitors is a common outcome, thought to be due to reactivation of the MEK pathway, as occurs in other tumours (23). One strategy to mitigate against this phenomenon is to combine BRAF inhibitors with MEK inhibitors, which is being investigated in several ongoing clinical trials (NCT03919071, NCT04201457, NCT02684058) in $BRAF^{V600E}$ pHGG (24). Recent findings from one of these phase II clinical trials (NCT02684058) reported that the combination of dabrafenib and trametinib treatment led to a 56% response rate and 66% clinical benefit rate in children with recurrent or refractory pHGG (25). However, the majority of patients demonstrated tumour progression within 12 months, indicating that the development of resistance also occurs following treatment with combination BRAF/MEK inhibitor therapy, as seen in other tumour types (26, 27).

$BRAF^{V600E}$ pHGG resistance mechanisms

The most commonly identified mechanisms of resistance in $BRAF^{V600E}$ mutated tumours are due to reactivation of the RAS/RAF/ERK pathway (Figure 1). Much of the research into resistance mechanisms has been conducted in melanoma, due to the high prevalence of the $BRAF^{V600E}$ mutation in this disease (28). Common underlying mechanisms include upregulation of receptor tyrosine kinases (RTKs): EGFR, PDGF-β, and IGF1R,

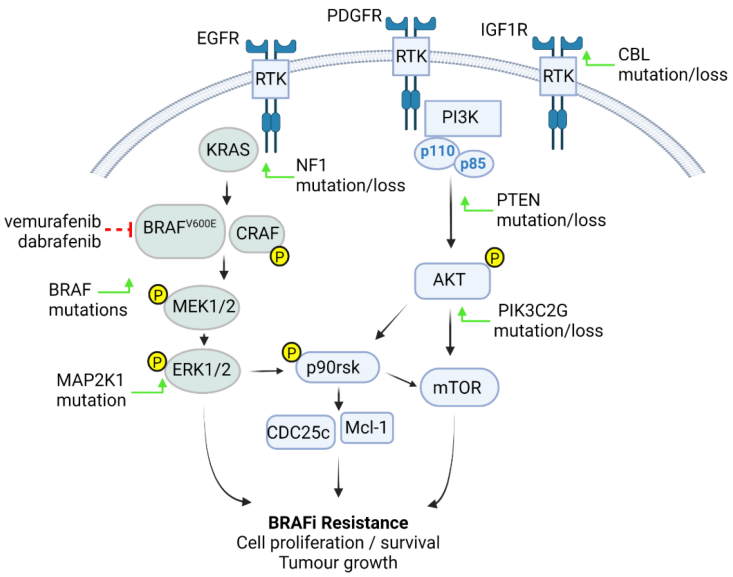

FIGURE 1
$BRAF^{V600E}$ pHGG resistance mechanisms. Tumour cells become resistant to BRAFi through increased activation of the RAS-RAF- MEK-ERK or PI3K-AKT pathways. These events are driven by upregulation or activation of receptor tyrosine kinases (RTK) such as EGFR, PDGFRβ and IGF1R through either BRAF mutations or CBL, NF1, PTEN, MAP2K1, or PIK3C2G mutation or loss (green arrows), resulting in pathway phosphorylation and activation of downstream p90rsk, Mcl-1 and cell cycle components, leading increased cell proliferation, survival and tumour growth (figure created with BioRender.com).

mutational activation of KRAS and NRAS, aberrant BRAF splicing, RAF isoform switching, $BRAF^{V600E}$ dimerisation and $BRAF^{V600E}$ amplification (29–34). Additionally, upregulated Wnt signalling, *Mcl*-1 overexpression (35, 36), as well as Sterol Regulatory Element-Binding Protein (SREBP1) activation and subsequent lipogenesis have been implicated in BRAF inhibitor resistance (37, 38).

In contrast to melanoma, there has been less investigation into the mechanisms of resistance in $BRAF^{V600E}$ pHGG. Consistent with the findings in melanoma, however, reactivation of the RAS/RAF/ERK has been implicated in resistance to BRAF inhibitors in $BRAF^{V600E}$ pHGG. Further, a secondary point mutation has been identified as a driver of resistance in a case study of $BRAF^{V600E}$ paediatric glioma (39). Through whole-exome sequencing Wang et al., identified a secondary $BRAF^{L514V}$ mutation, which was present following tumour progression, but not in the pre-dabrafenib treatment tumour. This mutation directly induced ERK signalling, promoted RAF dimer formation, and was sufficient to confer resistance to dabrafenib (39). Similarly, next generation sequencing has been used on both pre- and post-BRAF inhibitor treated $BRAF^{V600E}$ HGG and LGG from paediatric and young adult patients to identify novel mutations that act as putative drivers of resistance (13). These identified mutations in genes that modulate RTK activity, such as *CBL*, alterations in RAS/RAF signalling through *NF1* missense mutations, secondary point mutations of *BRAF*, activating mutations of *MAP2K1* and mutations in *PTEN*. Alterations in the PI3K/AKT/mTOR pathway have also been identified as a mechanism of resistance in pHGG, with mutations in *PIK3C2G* and upregulation of p-AKT identified (13, 40). Furthermore, mutations in *BAP* and *ANKHD1* in post-BRAF inhibitor treated tissues have also implicated cell cycle drivers as contributors towards BRAF inhibitor resistance (13). Further validation of *NF1, CBL* and *PTEN* loss, performed in several pLGG and adult HGG $BRAF^{V600E}$ cell lines (but not pHGG), confirmed their roles as drivers of BRAF inhibitor resistance. CRAF-dominated signalling and alterations in KRAS and EGFR signalling were also identified as drivers of resistance. Subsequent mechanistic analyses for vemurafenib resistance were undertaken using drug resistant lines developed from $BRAF^{V600E}$-mutant glioma cell lines AM38 ($BRAF^{V600E}$ adult HGG) and MAF-794 ($BRAF^{V600E}$ pLGG). Single-nucleotide variants conferring functional alterations in $ERRFI1^{S251*}$ and $TET2^{V1199E}$ were observed in each of the respective resistant cell lines, suggestive of possible mechanisms of BRAF inhibitor resistance *in vitro* (13). Baseline upregulation of several RTKs, RAS, p-CRAF and p-p90rsk were also seen in three $BRAF^{V600E}$ HGG tumours (including one paediatric and one adolescent sample) collected following relapse after BRAF inhibitor treatment (40). Interestingly, in contrast to Schreck et al. (13), no known mutations of RAS, MAPK or PI3K/AKT were identified in the resistant tumour samples and no novel driver mutations were able to be identified (40). Additionally, DNA methylation status was also largely unaffected in these patients following BRAF inhibition treatment, overall indicating that nongenomic and non-epigenomic events may also be involved in driving resistance mechanisms (40).

Treatments to overcome resistance

As discussed above, a common strategy used to overcome resistance to BRAF inhibitors is to use combination treatments (41). With MEK reactivation being the most common mechanism of drug resistance in $BRAF^{V600E}$ tumours, MEK inhibitors are a frequently used combination therapy, with several MEK inhibitors approved for clinical use. Dual BRAF and MEK inhibition has demonstrated promising results in several other $BRAF^{V600E}$ tumour types (42–44). The efficacy of these treatment regimens are now being tested within the setting of pHGG. Pre-clinical studies in a $BRAF^{V600E}$ expressing Ink4a-Arf knock-out mouse HGG model showed that dabrafenib monotherapy had no significant impact on survival. Further, *ex vivo* dabrafenib treatment of HGG cells derived from these mice resulted in activation of EGFR and AKT, that was concurrent with p-ERK increases (45). However, it should be noted that this was a model of intrinsic, rather than acquired resistance. Regardless, subsequent combination treatment with dabrafenib and the MEK inhibitor, trametinib, significantly improved survival and reduced Ki67 staining compared to the control group (45), mirroring the aforementioned preliminary phase II clinical trial findings (24, 25).

Despite these promising early results, it is well established in other tumour types that resistance to this combination therapy frequently develops (26, 27). In support of this, other combination therapies targeting multiple, rather than single pathways, have been trialled with some success in $BRAF^{V600E}$ pHGG preclinical models. For example, HSP90 is a chaperone protein, known to associate with RTKs and several proteins shared between the MAPK and AKT/mTOR pathways (46–49). In support of a potential role of this chaperone in resistance mechanisms, HSP90 inhibitors have been found to have a synergistic response in combination with either dabrafenib or trametinib in both primary HGGs, as well as in HGGs collected following relapse after BRAF inhibitor treatment. While these treatments did not completely negate the tumorgenicity of the resistant HGG phenotype, the combination therapies did significantly improve the response when compared to single agent treatments. Importantly, the addition of an HSP90 inhibitor, in combination with dabrafenib or trametinib was also effective in two $BRAF^{V600E}$ HGG BRAF/MEK inhibitor resistant PDX

models, with tumour regression most evident in the paediatric model (40).

A range of novel inhibitors have been developed that may overcome drug resistance in $BRAF^{V600E}$ pHGG. Targeted first generation BRAF inhibitors act upon BRAF monomers, preventing their dimerisation. As a resistance mechanism, reactivation of the RAS/RAF/ERK pathway frequently occurs through secondary mutations which promote RAF dimerisation, independent of RAS activation (29, 50). Therefore, dimer disrupters and pan-RAF inhibitors, which act upon RAF dimers as opposed to BRAF monomers, are being explored as novel therapies. Dimer disrupters prevent dimer formation, resulting in inhibition of downstream ERK signalling (51). Dimer disrupters have shown efficacy against $BRAF^{V600E}$ dimer forming melanoma and colorectal cancer cells and PDX models (51) and are currently in clinical trial for both paediatric and adult activating BRAF V600E and non-V600E mutant tumours, including brain tumours (NCT02428712). Pan-Raf inhibitors act by binding equally to both of the RAF monomers that form the RAF dimer, and therefore can inhibit RAF signalling in $BRAF^{V600E}$ tumours which exhibit RAF dimerisation (52). Pan-Raf inhibitors have been shown to display efficacy within the CNS and demonstrate blood brain barrier permeability properties (53, 54).The pan-RAF inhibitors, LY3009120 and belvarafenib, were efficacious in an intrinsically RAF inhibitor resistant glioma line. These cells were isolated from a tumour removed from a patient at the Children's Hospital Colorado and found to be resistant to BRAF inhibitors (13, 55). Interestingly, despite these cells demonstrating resistance to the BRAF inhibitor vemurafenib, combining the pan-RAF inhibitor LY3009120 with vemurafenib resulted in enhanced cytotoxicity, over LY3009120 treatment alone (13). These studies highlight the potential of combining pan-RAF and MEK inhibitor therapy in order to overcome drug resistance in pHGG and are supported by such treatment regimens displaying efficacy in drug resistant melanoma and colorectal cancer (56, 57).

Indeed, the identification of new pathways to target in combination with BRAF inhibition may be more effective than targeting a single pathway. Next generation sequencing is likely to contribute significantly towards identifying these targets. For example, inhibition of cellular autophagy pathways has already been identified as an area of interest for drug resistant $BRAF^{V600E}$ pHGG. Autophagy inhibition has been shown to reverse drug resistance in $BRAF^{V600E}$ glioma, with combined chloroquine and vemurafenib treatment reducing cell growth in vemurafenib resistant adult and paediatric glioma cell lines and cultures (58). A phase I/II trial (NCT04201457) is currently underway to ascertain the effectiveness of this approach, using hydroxychloroquine in combination with the BRAF inhibitor dabrafenib and/or the MEK inhibitor trametinib in $BRAF^{V600E}$ pHGG.

The future for $BRAF^{V600E}$ pHGG research

Promising results are beginning to emerge on overcoming drug resistance in $BRAF^{V600E}$ pHGG. However, future preclinical studies will need to have a greater focus on pHGG models to help elucidate the biology of this disease. To date there have been no reports using tissues and/or cells of pHGG origin to study the mechanisms of resistance, with both adult HGG or pLGG samples or cells lines being substituted. Due to distinct differences, both genomic and phenotypic, between HGG and LGG, as well as between paediatric and adult tumours, the development of specific pHGG models will help direct clinical trials and future treatment strategies.

Conclusions

$BRAF^{V600E}$ pHGG is a rare CNS tumour with a poor prognosis, which responds well to targeted therapy; however, treatment is limited by the rapid onset of resistance. Whilst significant progress has been made in determining the underlying mechanisms of resistance in other $BRAF^{V600E}$ cancers, drug resistance in $BRAF^{V600E}$ pHGG is less well understood. Recent studies have helped to begin to elucidate these mechanisms. Already, the identification of novel therapies such as dimer disrupters and pan-RAF inhibitors are an exciting development for the field. It is hoped that further identification of resistance mechanisms in $BRAF^{V600E}$ pHGG will facilitate further improvements in patient outcomes.

Author contributions

RL wrote the first draft of this manuscript and reviewed subsequent iterations, BR and DZ wrote and reviewed this manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

DZ declares consulting and advisory board fees from Bayer, Astra Zeneca, Accendatech, Novartis, Day One, FivePhusion, Amgen, Alexion, and Norgine.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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