The medical therapy of craniopharyngiomas

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Title Page

Full title: THE MEDICAL THERAPY OF CRANIOPHARYNGIOMAS: THE WAY AHEAD

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Abstract

Context: Craniopharyngiomas which are categorized as adamantinomatous (ACP) or papillary (PCP) have traditionally been treated with surgery and/or radiotherapy, although when the tumors progress or recur further therapeutic possibilities are very limited. ~~albeit with limited therapeutic options available for recurrent or progressive disease.~~ Following recent advances in their molecular pathogenesis, further medical therapeutic options have emerged.

Evidence Acquisition: The search strategy that we selected to identify the appropriate evidence involved the following mesh terms: ("Craniopharyngioma"[Mesh] AND "Craniopharyngioma/drug therapy"[Mesh] NOT ("review"[Publication Type] OR "review literature as topic"[MeSH Terms] OR "review"[All Fields]) AND ("2009/05/01"[PDat]: "2019/04/28"[PDat]).

Evidence Synthesis: Mutations of β-catenin causing Wnt activation with alterations of the MEK/ERK pathway are encountered in the great majority of patients with ACP; specific alterations also stratify patients to a more aggressive behavior. In most PCP there is primary activation of the Ras/Raf/MEK/ERK pathway secondary to *BRAF*-V600E mutations. BRAF inhibitors, such as dabrafenib or vemurafenib, either alone or in combination with the MEK inhibitors trametinib and cobimetinib, have been administered in patients with PCP producing clinically useful and, in some cases, sustained responses. In contrast to PCP, drugs targeting β-catenin and its downstream MAPK pathway in ACP have so far only been used in *in vitro* studies, but appear to be promising new targets clinically.

Conclusions: The identification of specific genetic alterations in patients with craniopharyngiomas has expanded the therapeutic options, providing evidence for a a customized approach using newer molecular agents. ~~more personalized approach with molecular targeted drugs.~~ More studies including a larger number of carefully selected patients are required to evaluate the response to currently available and evolving agents alone and in combination.

Précis

The presence of specific genetic alterations in craniopharyngiomas has important implications for the diagnosis and potential treatments of these neoplasms with molecular targeted agents.

Introduction

The title of this review may sound like an oxymoron, as until very recently the mainstays of treatment for craniopharyngiomas (CP) have been surgery and radiotherapy (RT). However, over the last few years there have been major developments in our understanding of the molecular pathology of these tumors that could set the scene for specific targeted therapy.

Craniopharyngiomas develop as malformations of embryonic remnants along the original ~~as embryonic malformations in the sellar and parasellar regions arising along the~~ pathway of the craniopharyngeal duct. They are characterized as mostly benign epithelial tumors according to the World Health Organization (WHO) classification of *Tumors of the Central Nervous System* (CNS), namely WHO grade I tumors (1-3). They are relatively rare, with an incidence of 0.5-2.5/ 10^6 cases/ year (2,4-7), and are divided into two distinct subtypes, adamantinomatous CP (ACP) and papillary CP (PCP), differing in both histological features and genetic alterations (8). ACP is more prevalent and displays a bimodal age distribution, with peaks between the ages 5-15 years and 45-60 years, but can occur at any age, even during the neonatal period (3,9,10); PCP has been classically considered as an adult entity peaking between 40 and 55 years (2,4,7,11), although pediatric cases have occasionally been reported (12).

Harvey Cushing not only suggested the term craniopharyngioma but also recognized the significant challenges in their management (13). Indeed, their anatomic vicinity to vital structures (hypothalamus, optic pathways, brain) and their intrinsic local infiltrative tendency render their growth pattern unpredictable and complete safe

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surgical removal problematic (14). Furthermore, malignant transformation may also rarely be seen and, overall, the long-term morbidities and mortality of patients with craniopharyngioma are disappointedly high, particularly compared to patients with treated pituitary neuroendocrine tumors (PitNETs) (15,16).

Current therapeutic options are relatively limited: surgery, either with the intention to treat by gross total resection (GTR) or with the intention to reduce their mass by subtotal/ partial resection (STR) (17,18), with or without post-operative radiotherapy (RT) (19), is the primary therapeutic modality (3). In a systematic review, STR with adjuvant RT, and GTR, showed similar rates of long-term tumor control (20). Currently, STR followed by RT is recommended, unless there is a clear margin between the tumor and surrounding vital structures allowing complete safe tumor removal. Whenever feasible, the tumours are approached by the transsphenoidal route ~~The trans-sphenoidal approach is used whenever possible~~ (17,18). The intra-cystic administration of sclerosing substances, mainly bleomycin and interferon-alpha, has been suggested in the past to attenuate fluid formation and diminish cyst size (21-23). Bleomycin use is limited by its toxicity (21,22), while interferon-alpha has shown some benefit with an acceptable safety profile (24); however, no prospective or randomized study has been performed and there are isolated reports of relatively poor responses and major adverse effects following interferon-alpha instillation, limiting its use (21).

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Recently, progress in molecular biology has unraveled novel potentially druggable molecular pathways in CPs that offer new promising therapeutic options. The use of such drugs is especially important to minimize the otherwise adverse effects

associated with surgery and RT, particularly in recurrent tumors where more there are few or no alternative options. ~~conventional options have been exhausted.~~ The purpose of this mini-review is to present the available literature on clinical studies and case reports describing targeted-treatments which are based on the recently-identified molecular alterations in both ACP and PCP. The search strategy that we selected to identify the appropriate evidence involved the following mesh terms: ("Craniopharyngioma"[Mesh] AND "Craniopharyngioma/drug therapy"[Mesh] NOT ("review"[Publication Type] OR "review literature as topic"[MeSH Terms] OR "review"[All Fields]) AND ("2009/05/01"[PDat]: "2019/04/28"[PDat]) (**Figure 1**).

Molecular biological advances in ACP and PCP

It has recently been shown that each CP histological subtype (Figures **2** and **3**) is characterized by alterations in oncogenic molecular signaling pathways, harboring distinct mutational, transcriptomic and epigenomic profiles as a result of different gene mutations, gene expression and methylation patterns (25-28). These findings have shed light on the identification of tumor-specific signaling pathway activation that may lead to CP-targeted therapies. An additional role of the different molecular profiles may be to differentiate pathologically indeterminate cases of CP (28-29).

Sekine *et al.* first described activating mutations of the β-catenin encoding gene *CTNNB1* in ACPs as early as 2002 (30). Whole-exome sequencing revealed mutations in *CTNNB1* in the majority of a small group of ACPs (11/12, 92%) (25). In addition, targeted genotyping revealed *CTNNB1* mutations in 96% of ACPs (51/53) (25). Next-generation panel sequencing did not identify any mutations other than those in *CTNNB1* (27). In 76% of the ACPs, a mutation in exon 3 of *CTNNB1* was found, and there was a trend towards a worse event-free survival in cases mutated at Thr41 (27). When primary and recurrent ACP tumor samples from the same patient were tested, the same *CTNNB1* mutation (S45P) was found without additional ones (28). No significant large chromosomal aberrations have been found, although a fraction of ACPs showed recurrent focal gains of chromosomal material, while in other cases there was loss of ~~showed loss in the~~ chromosomal region Xq28 (27).

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These *CTNNB1* mutations are exclusively found in exon 3 which encodes the destruction/degradation complex of β-catenin: β-catenin is usually held in a multi-

protein complex and, when phophorylated at specific residues, is subject to rapid degradation. Mutation leading to a loss of such phosphorylation sites results in aberrant β-catenin nucleo-cytoplasmic accumulation (30) (**Figure 3A**). This represents the most reliable immunohistochemical (IHC) marker for the confirmation of ACP, aiding in the differential diagnosis from other (para)sellar lesions (31,32); β–catenin is a component of the adherens junction complex and a central mediator of the canonical Wnt molecular signaling pathway (8). In the cytoplasm, β-catenin maintains a low concentration when phosphorylated within the multi-protein destruction-regulator complex which facilitates its ubiquitination and degradation by the proteasome. This process corresponds to inactive Wnt signaling (default "off" state) in the absence of any Wnt ligands (8,33-35) (**Figure 4A**). This regulator complex includes a number of proteins such as the scaffold proteins Axin and Axin2, the tumor suppressor protein adenomatosis polyposis coli (APC), and the phosphokinase glycogen synthase kinase-3β (GSK-3β), casein kinase 1α (CK1), protein phosphatase 2A (PP2A) or alternatively by I kappa B kinase alpha (IKK-α) (36). APC is phosphorylated by these kinases, resulting in recruitment of β-catenin to the complex (34,36), which is then tagged by these kinases for degradation through phosphorylation of the serine (Ser33, Ser37, Ser45) or threonine (Thr41) residues (of exon 3 on the *CTNNB1*) (34,36-39). This phosphorylation pattern is then recognized by multiple ubiquitin molecules, such as a component of E3 ubiquitin ligase, β-TrCP, ubiquitinating β-catenin, resulting in dissociation of α-catenin from β-catenin with concomitant loss of cadherin adhesion (34,35,37-41). Exogenous Wnt signaling ligands 'switch on' i.e., activate Wnt signaling (41,42). The Wnt protein family includes approximately 20 different proteins that bind to the Frizzled (Fz) family of receptors; the Wnt pathway is activated (34) resulting in an intracellular signaling

182 cascade that promotes dimerisation of Fz with LRP5/6 (34,43) The Fz/LRP5/6 dimer
183 can bind to Axin, GSK3β, and CK1, which facilitates their accumulation at the cell
184 membrane, preventing them from joining the β-catenin destruction complex (34-
185 36,43,44), i.e. disrupting the regulator complex via Dishevelled (Dvl) by preventing
186 the phosphorylation of β-catenin and its subsequent degradation (45).Therefore, β-
187 catenin protein accumulates and ultimately translocates to the nucleus, where it
188 activates factors allowing transcription of β-catenin target genes, such as CyclinD1,
189 c-Myc, CD44, Survivin, VEGF or fascin (26,46-49), resulting in stimulation of cellular
190 proliferation and other Wnt-regulated cellular processes (50). Cellular localisation of
191 β-catenin is not *per se* sufficient to promote target gene activation, as opposed to
192 *nuclear* β-catenin accumulation which is sufficient to induce Wnt signaling target
193 gene expression. Target genes (Axin2, bone morphogenetic protein (BMP)4 and
194 Fascin-1), stem cell markers (CD133 and CD44) and the cell cycle inhibitor
195 $p21^{Waf1/CIP1}$ co-localise in cells with nuclear β-catenin (8). Fascin (48) acts as a
196 facilitator of tumor cell migration, necessary for invasion and dissociation (36); β-
197 catenin binds fascin by its armadillo-repeat sequence (essential for the passive
198 nuclear-pore-complex translocation of β-catenin) (51) which promotes relocation of β-
199 catenin, changes cell adhesion properties, and reduces β-catenin destruction (36).
200 Inhibition of fascin or β-catenin expression has been shown to decrease the
201 migratory capacity of ACP tumor cells in culture (36). Other binding factors for the
202 armadillo-repeat sequences include the TCP-family transcription factors, Axin2, and
203 APC (52). In ACP, distinct β-catenin clusters also result by activation of other growth
204 factor pathways, such as epidermal growth factor receptor (EGFR), or src-driven
205 phosphorylation at the tyrosine of codon 654 (Y654) and sonic hedgehog (SHH)
206 signaling pathways (8,26,28,48,52-55), which may cause the dissociation of β-

catenin from the adherens complex by reducing its binding affinity to E-cadherin (40,56), and may facilitate tumor stem cell maintenance (57-59). In addition, Fyn, Fer or c-Met promote phosphorylation of β-catenin tyrosine residue 142, which results in dissociation of α-catenin from β-catenin (**Figure 4A**). RNA profiling (60) has confirmed that human ACP clusters express high levels of members of the fibroblast growth factor (FGF), BMP and Wnt families (50), representing paracrine growth-related signaling molecules that play a wide range of developmental and physiological roles. FGF-2 is expressed only in recurrent ACPs, while PDGFR-α expression is significantly higher in recurrent ACP compared with non-recurrent tumors, and ; VEGF and fibronectin are present in both ACP and PCP ~~were expressed in both types of tumors~~ (61). ACP is characterized by high gene expression of genes encoding both BMP2 and BMP4, which are downstream of the Wnt/β-catenin pathway (62,52,53). ~~The~~ EGFR is a member of the human epidermal growth factor receptor (HER) family; the principal ~~,~~ ~~while the major~~ downstream signal transduction pathways activated by receptors of the HER family are the Ras-Raf mitogen-activated protein kinase (MAPK) pathway (MAPK/ERK pathway) and the phosphatidylinositol 3-kinase (PI3K)/Akt pathway (8) (**Figure 4B**).

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The Ras-Raf-Mek-ERK1/2 is known to be one of the most dysregulated MAPK pathway signaling cascades in human cancer (63,64). Extracellular growth factors (FGFs, EGF, PDGF) bind to and activate receptor tyrosine kinases, causing a downstream phosphorylation cascade which eventually leads to transcription of target genes regulating cellular proliferation, differentiation, apoptosis and senescence (63). Downstream activation of the MAPK/ERK pathway, as evidenced by phosphorylation of ERK1/2, was identified by IHC around the clusters with

particularly prominent staining at the forefront, known as the leading edge, of the tumor ~~at the leading edge of tissue invasion~~ (50). Phospholipase Cγ (PLCγ), the signal transducer and activator of transcription (STAT) and src/FAK cascades, are also stimulated (8,65,66). SHH signaling pathway gene products, such as Gli2 and patched (Ptch)1, are over-expressed in ACP, and their cleaved active forms are especially highly expressed (8,26,28,53). Up-regulation of certain matrix metallopeptidases (MMPs) has also been reported in ACP transcriptome studies (23,50). A variable degree of co-localization between Ki-67 and pERK1/2 expression has also been also shown (60). In ACP, whole-exome sequencing showed additional mutations in genes listed in the Cancer Gene Census (67), although many of these may be 'passenger' mutations (25); there is also evidence for ~~,~~ recurrent copy number changes in some tumors ~~while some ACP showed recurrent focal copy-number aberrations at least in subgroups of cases~~ (27). Thus, mutation-produced loss of phosphorylation sites on β-catenin leads to a plethora of downstream changes initiating and augmenting tumor growth.

Considering PCPs, a *BRAF*-V600E mutation was documented (25) by targeted genotyping in 95% of PCPs (36/39), while whole-exome sequencing revealed recurrent mutations in *BRAF*-V600E in all PCPs (3/3) (25). In a parallel study, an analysis of known oncogenes revealed that *BRAF*-V600E mutations were seen in 81% (17/21) of PCPs by targeted Sanger sequencing and in 86% (18/21) of PCPs by IHC (68). Agreement between methods was seen in 95% (20/21) of cases; however, interpretation of anti-BRAF-V600E staining was challenging due to occasional non-specific reactivity (68). It is well established that the gain-of-function mutation *BRAF*-V600E, a critical serine/threonine kinase in the Ras-Raf-Mek-ERK1/2 pathway,

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renders it a potent oncogene leading to increased cell proliferation and survival, resulting in cell transformation and tumorigenesis (69,70) (**Figure 4B**). Such mutations render the BRAF kinase constitutively-active. ~~Mutations in *BRAF* transform the BRAF kinase into a constitutively-active kinase.~~ The MAPK pathway also plays a role in controlling stem-cell specification during development and perhaps during stem-cell homeostasis in the post-natal period (70), modulating the balance between the proliferation and differentiation of Sox2+ cells, implying that persistent proliferative capacity of Sox2+ cells may underlie the pathogenesis of PCP (70). Sox2+ progenitor cells appear to show tumor-inducing potential, driving tumor formation in a paracrine manner by inducing tumorigenic events in adjacent cells but without gains or losses of genetic material (53,54,71). ~~No gains or losses of genetic material were detected in the PCPs (27). I~~In PCPs (25), whole-exome sequencing with a recently described novel technique - *MuTect* – also showed isolated mutations of cancer-related genes encoding chromatin remodeling factors (CHD5, CHD6) and cell adhesion molecules (CDH26, PTPRT), and one (KIAA1549) that is fused to BRAF in most cases of pilocytic astrocytoma (72,73). However, it is presently unclear whether these mutations are pathogenetic or merely 'passenger' mutations (25).

~~Brastianos *et al* first postulated that~~ *CTNNB1* and *BRAF* mutations were mutually exclusive and clonal in ACP and PCP, according to an original observation by Brastianos et al, and there were no ~~each CP subtype, since they did not detect any~~ other recurrent mutations or genomic aberrations in either subtype (25) (**Figures 3A** and **3B**). Thus, the specific oncogenic change ~~The crucial pathogenetic event~~ appears to be Wnt activation in ACP, and activation of the Ras/Raf/MEK/ERK pathway by *BRAF*-V600E mutations in PCP (27). It was then suggested that one

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should classify CPs as ACP *CTNNB1*-mutated, ACP *CTNNB1*-wild-type, PCP *BRAF*-V600E-mutated and PCP *BRAF*-wild-type (74). Comparison microarray analysis of 18 ACP and 10 PCP samples revealed significant up-regulation of several direct targets of the Wnt/β-catenin signaling pathway in ACPs compared to PCP, including LEF1 and AXIN, while components of the SHH such as GLI2, PTCH1 and SHH were also over-expressed specifically in ACP in comparison to PCP (8,36,48,52,71,75,76). Unsupervised consensus clustering of the gene expression values of the 5000 most variable genes resulted in two distinct and stable clusters that perfectly separated ACP and PCP samples. Gene expression data showed increased expression of microtubule-associated protein 2 (MAP2), tenascin C (TNC) and the stem cell marker CD133 (PROM1) in ACPs. CD44 and claudin 1 (CLDN1) were significantly down-regulated in ACPs, but showed ~~a recently described distinguishing marker for the two variants, were significantly down-regulated in ACPs but exhibited significantly~~ higher expression in PCPs (28,52,75). Moreover, reduction of claudin-1 protein levels point towards an invasive growth pattern in ACP (75). Down-regulation of microRNA (miRNA) miR-132 was identified as a marker of aggressiveness, playing a role in epithelial–mesenchymal transition (EMT), in a study of 754 miRNAs from childhood CP (77). However, in contrast to the view of a complete molecular separation between these two tumor subtypes, Larkin *et al* in a small cohort of ACPs reported a dual aberration of *CTNNB1* mutated at Thr41 together with *BRAF*-V600E mutation in two tumors (68). This finding was validated by review of morphology and comparison with IHC findings. ~~Further validation was obtained by~~ Sequencing in forward and reverse directions ~~sequencing in forward and reverse directions~~ from two DNA samples extracted on different occasions confirmed these findings. Whether this represents the rare occurrence of a collision tumor needs to be established in future

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larger studies (68). The expression of additional stem cell markers SOX9, KLF4 and Okt4 was also identified by IHC in both ACPs and PCPs (8,78).

Other transcriptional molecular analyses of ACP have revealed the expression of immune-system gene expression, including Interleukin (IL)-1β, IL-6, IL-8 (CXCL-8), IL-10, IL-18 and TNF (TNF-α). In particular, there is a characteristic inflammatory cytokine/chemokine reaction in both ACP cyst fluid and sold tumor components. ~~ACP cyst fluid and solid tumor components are characterized by an inflammatory cytokine and chemokine expression pattern.~~ Cytokines and chemokines with elevated concentrations in ACPs included IL-6, CXCL-1 (GRO), CXCL-8 and the immunosuppressive cytokine IL-10, and their relevant receptors are also expressed in ACPs., as were IL-10, plus . ~~Most receptors for these cytokines and chemokines are also over-expressed in ACPs. In addition to IL-10,~~ the ~~established~~ immunosuppressive factor IDO-1 ~~was over-expressed by ACPs~~ at both mRNA and protein levels (79). This interaction between tumor cells and chemokines is not dissimilar to that seen in some PitNETs (80).

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Summing up, in the era of molecular targeted treatments, the molecular pathways that characterize either ACP or PCP are under vigorous investigation. Currently, ACP is characterized and identified by activating mutations of the β-catenin-encoding gene CTNNB1, while PCP are characterizedby the activating BRAF-V600E mutation. In both cases, there is up-regulation of the MAP-kinase pathway which is critically involved in cell proliferation.

Targeting molecular aberrations in craniopharyngiomas

Currently, there are no approved targeted or cytotoxic therapies available for the systemic treatment of CPs. The development of molecular targeted treatments in oncology has opened new horizons in the pharmacological treatment of tumors caused by genetic alterations. Recently, in cancers harboring *BRAF*-V600E alterations, V600E mutation-specific BRAF inhibitors, such as dabrafenib or vemurafenib (81,82), have been shown to be effective anti-tumor agents. In addition, the MEK inhibitors trametinib and cobimetinib have been administered in combination with BRAF inhibitors, since these compounds override any resistance to BRAF inhibition (81,83). These data have led to the use of BRAF inhibitors in patients with aggressive PCPs (see Table 1 for summary). Aylwin *et al.* (83) described the beneficial effect of the off-label administration of vemurafenib (960 mg twice daily) in a 41 year-old female with a 16-year history of a recurrent PCP (carrying a *BRAF*-V600E mutation) with progressive visual failure following three trans-sphenoidal operations. Magnetic resonance imaging (MRI) two weeks after starting treatment showed marked reduction in the size of the tumor with resolution of the surrounding edema, while three months later there was evidence of radiological improvement. ~~after starting vemurafenib radiological remission was seen.~~ The craniopharyngioma recurred six weeks later and vemuranefib was re-started with reduction of tumor size followed by stabilization for seven months but this was followed by further growth. In the same year, Brastianos *et al.* (84), reported on the use of dabrafenib (150mg twice daily) in a 39 year-old male with a PCP harboring a *BRAF*-V600E mutation. This patient had undergone four craniotomies along with cyst decompression over a period of 11 months: as early as four days after treatment, the tumor showed a ~~a~~ 23% size reduction while the cyst volume decreased by 32%. ~~in tumor size and a~~

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~~32% decrease in cyst volume were identified.~~ The beneficial effect was even more obvious 17 days after treatment, achieving a 52% reduction in tumor size and 70% decrease in the cyst volume. The MEK inhibitor trametinib (2mg, orally, twice daily) was added to the therapeutic scheme on day 21 aiming to enhance the effect. Fourteen days after this combined therapy, there was a 85% reduction in tumor size and 81% decrease in the cyst volume. On day 38 of treatment, the patient underwent endoscopic trans-sphenoidal surgery: two weeks later the combined treatment was discontinued, and a week later RT was offered (50.4 Gy in 28 fractions). Seven months later, the patient has remained free of symptomatic recurrence. In the following year, Roque & Odia (29) described a 47 year-old female with acute visual loss and a 4-month history of amenorrhea, cold intolerance and headache. A 2.7cm cystic lesion was identified in the suprasellar area harboring a *BRAF*-V600E mutation (29). Subsequently, she underwent right frontal craniotomy but the tumor regrew causing bilateral visual impairment necessitating an Ommaya catheter placement into the cyst and then RT (54 Gy in 30 fractions). Seven months later, the tumor regrew and dabrafenib (150mg twice daily) and trametinib (2-mg daily) were administered. After two months, a 52% reduction in tumor volume was documented, and after five months, the tumor size was reduced by 75%. The patient was continuing to improve on this treatment both radiologically and clinically 7 months post-treatment. Subsequently, Rostami *et al.* (85) reported a 65 year-old male with visual deficits who underwent a partial trans-sphenoidal resection of a PCP harbouring a *BRAF*-V600E mutation. After rapid tumor growth, dabrafenib (150mg twice daily) was initiated, followed by trametinib (2mg daily) three weeks later for a total of 7 weeks. An 11% tumor reduction was seen at four weeks and 91% by the 8th week.

Very recently, Himes *et al.* (86) presented the longer-term effects of monotherapy with dabrafenib in a patient with a history of non-Hodgkin's lymphoma and colon cancer who had a PCP with the *BRAF*-V600E mutation treated by carnial surgery and RT. ~~who had undergone craniotomy and RT for a PCP with the *BRAF*-V600E mutation.~~ Three years after the RT he developed tumor recurrence, and dabrafenib was initiated (150mg twice daily, reduced to 150mg daily due to joint pains but then increased to 225mg daily). Two months following treatment, an enlargement of the cystic component was documented in parallel with a reduction of the solid component. However, six months post-treatment significant reduction in the size of both components was identified, while by 9 months only minimal residual tumor was evident and this remained stable until 12 months; treatment was discontinued, and the tumor remained stable for further 12 months. A sixth case was recently reported at the *European Congress of Endocrinology 2019* (87) by Juratli and colleagues, who administered dabrafenib in a neo-adjuvant setting to a 21 year-old male prior to surgery and found a more than 80% reduction of tumor size. All these cases support the potential benefits of BRAF inhibitors, at least in achieving transient tumor responses, either as neo-adjuvant treatment or following recurrence (**Table 1**). What is unclear from these single reports relates to the efficacy of different inhibitors, the mechanisms of tumor resistance, and the means to overcome such resistance. An interesting observation from one of these case reports was the documentation of a circulating *BRAF*-V600E mutation in the peripheral blood of the patient during treatment with the BRAF-inhibitor (84). The authors stated that their finding of detectable *BRAF*V600E in peripheral blood was unexpected, since there was not any previously reported case of circulating tumor cells or cell free DNA in patients with

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intracranial benign tumors. However, if this is not a result of the surgical procedures or drug treatment, and the presence of *BRAF*V600E can be detected in the blood before any treatment, then this assessment may be of value as a selection criterion of the patients that may benefit from BRAF-inhibitor neoadjuvant therapy (84). Following these observations, an ongoing cooperative group trial (*Alliance A071601*) is testing the combination of vemurafenib and cobimetinib in an open-label, phase II study in patients with *BRAF*-V600E–mutant CP (88) to determine the frequency, durability, and extent of BRAF-treatment responses in patients with PCP. In this study, vemurafenib is administered twice daily on days 1-28, and cobimetinib four-times daily on days 1-21; treatment is repeated every 28 days to a maximum of 5 cycles as long as the disease shows a failure to progress and there is no unacceptable toxicity. ~~for up to 5 cycles in the absence of disease progression or unacceptable toxicity.~~ Patients may then be treated with RT, surgery, or to continue the combination of medical treatment. In another published series, a patient in whom GTR was achieved had a recurrence of a cystic tumor component after two years, and has been referred to a phase-II trial of combined BRAF/MEK inhibitors (NCT03224767) (88,89).

Adverse effects of compounds currently used in the management of craniopharyngiomas

Data on the adverse effects profile of these treatments in patient with CP are limited. The phenomenon of “pseudo-progression” with dabrafenib, defined as early initial cystic enlargement together with solid component reduction, followed by lesion shrinkage, has been observed, similar to that seen in gliomas after RT or chemotherapy (86). In addition, transient fever has been described with BRAF

inhibitors (29,84,85). Finally, the addition of the MEK inhibitor to BRAF inhibition may play a role in reducing the incidence of secondary squamous cell skin carcinomas, as previously shown in patients with melanoma (90).

Other potential treatments targeting craniopharyingiomas

Despite the identification of the deranged molecular pathways in ACP pathogenesis, drugs targeting β-catenin and its downstream MAPK pathway have been used only in *in vitro* studies. A transcriptome study used mRNA microarray gene expression analysis and unraveled several 'druggable' pharmaceutical targets that were consistently over-expressed in a panel of 15 ACP, compared to other brain tumors and normal brain tissue (26). LCK, EPHA2, SRC were identified as targets of the tyrosine kinase inhibitor (TKI) dasatinib. AREG, EGFR, ERBB-3 in the EGFR pathway may respond to inhibitors such as cetuximab, erlotinib, lapatinib; and MMP9 and MMP12 could potentially respond to the oral MMP9/12 inhibitor, AZD1236 (26,50). Recurrence in ACP has been associated with increased VEGF expression suggesting a possible therapeutic role for the VEGF inhibitor bevacizumab (49,61). Indeed, some of these targets can be shown to be expressed in the primary ACP tumor using Western blot analysis ~~Western blot analysis confirmed that a subset of these targets is highly expressed in ACP primary tumor samples~~ (26). On the other hand, although the SHH pathway may be implicated in the pathogenesis of ACP, inhibition of this pathway may be tumorigenic (91). This relies on data showing that vismodegib, a well-established inhibitor of SHH, results in premature tumor formation and increased tumor cell proliferation in both genetically-engineered and patient-derived xenograft mouse models (91). Inhibition of EGFR signaling by the TKI, gefitinib, reduced ACP cell migration *in vitro* by decreasing Fascin expression; this

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was attributed to the finding of nuclear co-localization of activated EGFR, β-catenin and Fascin in ACP cell migration *in vitro* (48), implying a crosstalk of these pathways in ACP. Since Annexin A2 expression, a Ca2+-regulated binding protein, correlates with gefitinib sensitivity *in vitro*, this may serve as a biomarker of a response to treatment (92). Small samples of human ACP tumors were cultured with and without trametinib, a specific MEK inhibitor; the inhibition of the MAPK/ERK pathway resulted in reduced proliferation along with a dose-dependent significant decrease in the Ki-67 proliferation index in the trametinib treated tumors, suggesting a possible role in the management of not only PCP but also ACP (91). Notably, the well-established synergistic effects of combined targeted therapies in other neuroendocrine tumor models provide support for the potential use of combinatorial targeted therapy in CPs (93,94).

Given the potential role of inflammation in the pathogenesis of craniopharyngioma and previous studies on intra-tumor administration of interferon-alpha in patients with CP (22), an open label, phase II study is underway aiming to estimate the one-year disease stabilization rate associated with the use of Peg-interferon alfa-2b in patients with progressive unresectable or recurrent CP after surgery alone (without adjuvant RT). The study will also estimate the sustained objective response rate (partial and complete response) to Peg-interferon alfa-2b in patients with CP which progress or recur following RT (95). Finally, noting the immune microenvironment in ACP, IL1R inhibitors, such as anakinra, may have a place in our therapeutic armamentarium (60).

Conclusions

The discovery of distinct oncogenic molecular signaling pathways in CPs has opened new avenues for the personalized treatment of patients with these challenging tumors. The key pathogenetic event in ACP appears to be Wnt activation along with alterations of the MEK/ERK pathway, whereas in PCP there is primary activation of the Ras/Raf/MEK/ERK pathway by *BRAF*-V600E mutations. Current literature on PCPs includes single case reports, with both BRAF and MAPK inhibitors, in patients with V600E mutations. Their results are promising but well-designed studies are needed aiming to provide robust data on treatment regimens and protocols, as well as on efficacy and safety. It also remains unclear whether single or combination therapies will be necessary, although *in vitro* data from other endocrine tumors have highlighted the use of multiple simultaneous combination treatments. The use of MAPK inhibitors for the more common ACPs also requires assessment in clinical trials, particularly in view of the risk of acquired drug resistance. The value of combination therapies aiming to target multiple molecular mediators implicated in the pathogenesis of ACP and possibly minimizing drug toxicities also requires further investigation.

~~needs to be evaluated.~~

Nevertheless, the recently expanding data on the pathogenesis of CPs offer substantial ground for the translation of molecular insights into practical therapies for primary, recurrent or very aggressive tumors which will, hopefully, improve the prognosis of the patients. The eventual use of neo-adjuvant medical treatment or even an entirely non-operative treatment strategy for CP should improve the quality of life of these patients, minimizing the neurological and endocrinological sequelae

caused either by the tumor extension or by the neurosurgical or radiotherapeutic complications. Combination therapy strategies could be also be effective in cases of ACP in order to target the multiple molecular mediators that are implicated in ACP pathogenesis and to mimimize any adverse treatment effects. ~~and minimizing the side effects of treatment.~~

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Table 1. Case reports and current clinical trials of patients with papillary craniopharyngioma (PCP) and BRAF-V600E alterations treated with V600E mutation-specific BRAF inhibitors (dabrafenib or vemurafenib) with or without combined treatment with MEK inhibitors (trametinib or cobimetinib).

Case series/ reports	CP	Previous treatment	Histopathological and molecular genetic analysis	Novel treatment	Duration of treatment	Outcome (mean of measurement)	Comments
Aylwin et al, 2015	PCP	TSSx2, RT.	Pyrosequencing analysis indicated the presence of the BRAF mutation c.1799T[A (p.Val600Glu).	Vemurafenib 960 mg bd.	3 months.	Near complete resolution (MRI.)	Recurrence 6 weeks off treatment; restart of treatment and stabilization for 7 months with a regrowth.
Brastianos et al, 2015	PCP	Craniotomy x3.	Uniform staining of the BRAFV600E protein (VE1 antibody) by IHC; V600E mutation confirmed by allele-specific genetic testing. The mutation was detectable in peripheral blood during treatment. Absence of nuclear staining for β-catenin by IHC.	Dabrafenib 150 mg bd + after 3 weeks trametinib 2 mg od.	35 days (followed by other treatments).	85% reduction tumor volume and 81% reduction tumor-associated Cyst (MRI).	
Roque et al, 2016	PCP	Craniotomy, Ommaya catheter with percutaneous aspiration of cyst fluid, RT 54 Gy in 30 fractions.	(+)ve staining for BRAFV600E by IHC; next-generation sequencing confirmed BRAFV600E mutation and FGFR3 splice site (437_445+3del12) with two variants of unknown significance (BRCA2-G1771D and FGFR4-S551F).	Dabrafenib 150 mg orally twice daily and trametinib 2-mg orally od.	7 months	Almost disappearance of 2.6 × 2.3 × 3.2-cm tumour (MRI); reduced volume and intensity of FDG uptake on PET.	
Rostami et al, 2017	PCP	TSS	Weak staining of mutated BRAFV600E (VE1 antibody) by IHC; BRAFV600E genotype confirmed by pyrosequencing mutational analysis.	Dabrafenib 150 mg bd + after 3 weeks trametinib 2 mg od	7 weeks	91% reduction tumour size (MRI).	Combined BRAF and MEK-targeted therapy.
Himes et al, 2019	PCP	Craniotomy, RT 36 Gy in 12 fractions.	BRAF V600E mutation was confirmed in specimens from his original resection.	Dabrafenib 150 mg bd (shortly)→ od (several weeks)→ 225 mg od	1 year under treatment and follow-up for 1 year off treatment.	Minimal residual tumor.	
Jurati et al, 2019	PCP			Dabrafenib 150 mg bd + trametinib 2 mg od.		>80% reduction of tumour size (MRI).	
NCT03224767	PCP	Cohort A: ± surgery Cohort B: RT ± other treatment (except for BRAF or MEK inhibitors)	IHC for BRAF V600E mutation (VE1) and β-catenin IHC (membranous, non-nuclear pattern).	Vemurafenib and Cobimetinib.	Vemurafenib bd day 1-28 + cobimetinib QD days 1-21.	Every 28 days for up to 5 courses in the absence of disease progression or unacceptable toxicity (MRI).	Patients may then receive RT, surgery, or continued treatment. .

bd: bis die (twice a day); CP: craniopharyngioma; IHC: immunohistochemistry; MRI: magnetic resonance imaging; od: once a day; PCP: papillary craniopharyngioma; RT: radiation therapy; TSS: trans-sphenoidal surgery; ^{18}F-FDG PET/CT: ^{18}fluoro- D-glucose Positron emission tomography/ computed tomography; RT: radiotherapy; (+)ve: positive

FIGURE 1. The mesh terms used to the search strategy : ("Craniopharyngioma"[Mesh] AND "Craniopharyngioma/drug therapy"[Mesh] NOT ("review"[Publication Type] OR "review literature as topic"[MeSH Terms] OR "review"[All Fields]) AND ("2009/05/01"[PDat]: "2019/04/28"[PDat])

FIGURE 2. Photomicrograph of a representative case of a Papillary Craniopharyngioma. Hematoxylin and eosin staining, x100 magnification.

(Dr A. Ramanathan and Professor A.B.Grossman, unpublished data)

FIGURE 3A. Histology of adamantinomatous craniopharyngioma. Basal epithelium demonstrating the aberrant nuclear accumulation of beta-catenin in nodular whorls (arrowheads) due to beta-catenin mutation; Anti-beta-catenin, x400 magnification.

FIGURE 3B. Histology of papillary craniopharyngioma. Squamous epithelium showing membranous immunoreactivity of beta-catenin, lacking clusters with aberrant nuclear accumulation, x400 magnification.

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FIGURE 4A . Schematic presentation of adherens junction complex (including β- and α-catetin and E-cadherin) and the molecular signaling pathways that may crosstalk in both, ACP or PCP. In ACP, β-catenin clusters cells show an activation of the Wnt, SHH and EGFR pathway, which are reported to crosstalk with each other. **2A.** When Wnt signaling is inactive, axin recruits and binds to GSK3β, CK1α, and PP2A. APC is phosphorylated by these kinases, recruit β-catenin to the complex and tag it for degradation through phosphorylation of the serine (S33, S37, S45) or threonine (T41) residues of exon 3 on the CTNNB1 gene. This phosphorylation pattern is recognised by β-TrCP, which ubiquitinates β-catenin, When Wnt signaling is active, by the activation of a Wnt ligand or when extracellular SHH binds to the transmembrane receptor PTCH1, the protein degradation complex is inhibited and β-catenin is accumulated firstly in cytoplasm and then in the nucleus. There, β-catenin represents a transcription co-factor by interacting with transcription factors of the TCF family TCF/LEF1, initiating the target genes expression.

FIGURE 4B. In ACP, paracrine growth-related signaling molecules such as SOX2. FGFs, EGF, PDGF bind to their receptors activating the Ras/Raf/MAPK/ERK1/2 pathway and the PI3K/Akt pathway. Similarly, in PCP the BRAF-V600E serine/threonine kinase is a critical component of the Ras-Raf-Mek-ERK1/2 pathway that has been mutated. Red lines show the inhibitors that may be used as molecular-targeted therapy for CP.

ACP: adamantinomatous craniopharyngioma; APC: adenomatosis polyposis coli; BMP: bone morphogenetic protein; CK1: casein kinase 1α; CP: craniopharyngiomas; Dvl: Dishevelled; EGFR: epidermal growth factor receptor; FGF: fibroblast growth factor; Fz: Frizzled; GSK-3β: glycogen synthase kinase-3β; IKK-α: I kappa B kinase alpha; MAPK: mitogen-activated protein kinase; PCP: papillary craniopharyngioma; PI3K: phosphatidylinositol 3-kinase; PP2A: protein phosphatase 2A; PTCH1: patched 1; SHH: sonic hedgehog; TCF: T-cell factor; LEF1: lymphocyte enhancer factor 1. VEGF-R: vascular endothelial growth factor receptor