Tumors of the Central Nervous System

Edited by
Antonio Meola

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Tumors of the Central Nervous System

Special Issue Editor

Antonio Meola
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About the Special Issue Editors

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Preface to “Tumors of the Central Nervous System”

For several decades, the classification of brain tumors was based on histogenesis. Brain tumors were classified on the basis of their presumed cell of origin and differentiation stage. From a practical viewpoint, the characterization of brain tumors was traditionally based on light field microscopy and, more recently, on immunohistochemical assays and ultrastructural studies.

The 2016 WHO classification of tumors of the central nervous system [1] radically changed the rationale behind brain tumor classification. The 2016 classification is based both on the histological as well as the genetic and molecular features of brain tumors. As a consequence, some neuro-oncological categories were completely restructured, such as glioblastoma, medulloblastoma and embryologic tumors. Moreover, some new neuro-oncological entities were created, while other were removed from the classification. Certainly, our new knowledge about brain tumor biology and the development of modern molecular and genetic tests, allowed transitioning from a purely histological classification of brain tumors to a mixed histological and genetic/molecular classification. The new genetic and molecular features of brain tumors can have a tremendous impact on clinical practice, determining appropriate treatment selection, and patients’ prognosis.

In this new era of brain tumors, imaging plays a pivotal role, as well. The recent advancements in imaging techniques (i.e. MR spectroscopy, PET/CT) allow detecting molecular markers of brain tumors, which are associated with different histotypes. Thus, imaging will provide the clinician with important information about brain tumor histology, biology and prognosis.

The special issue on “Tumors of the Central Nervous System” of OBM Neurobiology, includes several important contributions highlighting contemporary concepts of brain tumor biology derived from novel molecular, genetic and radiological studies.

References

Review

**Diffuse Intrinsic Pontine Glioma: Translation of Genomic Knowledge to Clinical Practice**

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**Abstract**

Pediatric brain tumors account for approximately 25% of all cancers in children and are currently the leading cause of cancer-related deaths in the pediatric population with an estimated incidence of 5.14 cases per 100,000 person years [1]. Up to 10-15% of all pediatric brain tumors arise in the brainstem, with the majority of these classified as the diffuse intrinsic pontine glioma (DIPG) subtype [2]. The outcome of children with DIPG remains dismal with a median survival of <1 year. Owing to the unique location of DIPGs, surgical intervention is unachievable due to difficulty of physical access and the diffuse infiltration of the tumor throughout the pons. Radiotherapy has thus far been the only method identified to provide transient benefit in the setting of a uniformly fatal outcome. Decades of laboratory studies and clinical trials have failed to identify any
chemotherapies delivering survival benefit, either alone or as an adjunct to radiotherapy. Advances in surgical biopsy techniques and next generation sequencing modalities have more recently provided a better understanding of the distinct pathophysiology of DIPGs and opened new windows of opportunity for the development of molecular-targeted therapies with hope of delivering more effective treatment for children suffering from DIPG whilst minimizing systemic toxicity. In this review, we will explore the recent advances in the genomic understanding and treatment of DIPG, and the resulting outcomes of the preclinical and clinical drug trials which are guiding the next wave of therapeutic discovery for this disease of unmet clinical need.

Keywords
Diffuse intrinsic pontine glioma; genetics; histone H3; epigenetic; targeted therapy; clinical trials

1. Introduction

DIPGs are aggressive high-grade gliomas (HGGs) derived from glial cells located in the pons of the brainstem with varying degrees of infiltration into neighboring structures. DIPG accounts for 80% of all brainstem gliomas and was traditionally classified based on anatomical location, radiographic location and where possible, by histopathology. The most recent edition of the World Health Organization (WHO) Classification of CNS Tumors published in 2016 uses molecular information in addition to histopathology and has redefined the majority of DIPGs as diffuse midline glioma, H3 K27M-mutant. Biopsied DIPGs have demonstrated the heterogenous histopathological spectrum of this tumor. They are usually high-grade astrocytoma’s with increased microvascular proliferation, and are often associated with increased mitosis and necrosis, although up to a quarter of those biopsied lack these features and appear to be low grade on histological examination. Regardless of its histopathological grade at diagnosis, tumors under the DIPG/diffuse midline glioma, H3 K27M-mutant classification are aggressive in nature and have a poor overall prognosis [3].

DIPGs most commonly affect younger children with a peak incidence between the ages of 6 to 9, and a male to female ratio of 1:1. Patients typically present with a brief history of clinical symptoms and signs consistent with brainstem dysfunction, supported by typical radiologic findings on magnetic resonance imaging (MRI) (Table 1). Clinical signs in over 50% of presentations involve the classical triad of: 1) cranial nerve deficits (most commonly affecting the sixth and seventh cranial nerves resulting in facial asymmetry and diplopia); 2) cerebellar and bulbar signs (ataxia, dysmetria, drooling and dysarthria); and 3) long tract signs (hyperreflexia, upward going Babinski reflex and weakness) [4, 5]. Once the diagnosis is confirmed, treatment options are limited. Patients with histopathologically confirmed DIPG on current treatment protocols have a median survival of just 8-10 months with virtually all children succumbing within 2 years of diagnosis [6]. A small number of long-term survivors (LTSs) have been reported in various studies and case series and were systematically reviewed by Hoffman et al in 2018 [7, 8]. Although this review excluded LTSs reported with MRI findings
atypical for DIPG, many still lacked tissue biopsy to prove the diagnosis. Intriguingly, it does suggest that children presenting under the age of 3 or over the age of 10 had a better prognosis, with cranial nerve palsy, ring enhancement, extrapontine extension and necrosis at presentation being negative prognostic factors. Owing to its aggressive nature, DIPG remains the primary cause of death from brain tumors diagnosed in children. As such, it poses a significant challenge to all clinicians involved in the care of these children.

DIPG was first described by Dr Harris and Dr Newcomb in a pediatric case report in 1926. The 14-year-old boy presented following a minor head injury, and over the ensuing weeks was reported to have rapidly progressive cranial nerve palsies and pyramidal tract dysfunction, and he died within two months of presentation. The diagnosis of a pontine glioma was made clinically and the diffuse nature was confirmed histologically at post mortem [9]. Until recently, research initiatives into DIPG have been limited to therapies (radiation and chemotherapy) with limited benefit in other forms of HGG [10, 11]. Owing to the anatomical position of the tumor in the ventral pons, surgical interventions have had little role to play in its management [12]. There is no role for chemotherapy. Steroids may alleviate symptoms transiently but have the burden of severe side effects. Radiation is used as a palliative measure for symptom control, and has demonstrated a minimal survival benefit of a few months in some studies [13-15].

Table 1 Clinical and radiological features of DIPG

<table>
<thead>
<tr>
<th>Common symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Eye movement abnormality.</td>
</tr>
<tr>
<td>- Diplopia.</td>
</tr>
<tr>
<td>- Facial weakness and asymmetry.</td>
</tr>
<tr>
<td>- Sudden onset hearing impairment.</td>
</tr>
<tr>
<td>- Limb weakness, difficulty standing/walking and abnormal gaits.</td>
</tr>
<tr>
<td>- Headache.</td>
</tr>
<tr>
<td>- Nausea and vomiting.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Clinical findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triad of</td>
</tr>
<tr>
<td>1. Cranial neuropathies (most frequently 6th and 7th cranial nerve palsies).</td>
</tr>
<tr>
<td>2. Cerebellar and Bulbar signs (ataxia, dysmetria, drooling, dysarthria).</td>
</tr>
<tr>
<td>3. Long tract signs (hyperreflexia, increased tone, clonus, weakness).</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Radiological findings (on MRI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Involvement of ventral pons, with or without an exophytic component into the prepontine cistern.</td>
</tr>
<tr>
<td>- Basilar artery Encasement/encroachment.</td>
</tr>
<tr>
<td>- Hypo/Isointense on T1-weighted imaging.</td>
</tr>
<tr>
<td>- Hyperintense on T2-weighted imaging and FLAIR sequences.</td>
</tr>
</tbody>
</table>

2. Treatment

2.1 Medical Therapy

Traditional chemotherapies have proven to be ineffective in the treatment of DIPG, presumably due to poor blood brain barrier permeability and/or distribution throughout the
tumor mass. Regardless, conventional cytotoxics have continued to be trialed, alone and in combination, but without effect. Interestingly, a limited study investigating neoadjuvant high-dose methotrexate, BCNU, cisplatin and tamoxifen administered to DIPG patients until progressive disease, then followed by radiotherapy, reported an encouraging median overall survival of 17 months [16]. Since survival following radiotherapy is comparable to historical controls, the overall improved survival duration is likely attributed to upfront chemotherapy. However, long-term survival remained poor and the use of multi-agent medical therapies has not been adopted into routine clinical care.

Corticosteroids are widely used as supportive treatment to manage symptoms but do not have any effect on the tumor tissue itself. Administration of dexamethasone at the time of diagnosis will decrease edema of the surrounding brainstem and pons, with often rapid improvement in symptoms and signs. However, the side-effects of prolonged administration of dexamethasone can be a clinical challenge resulting in a compromised quality of life made more difficult with the return of symptoms and signs once they are withdrawn [17].

2.2 Surgical Resection

DIPGs typically arise in the pons resulting in destruction of intrinsic nerve fiber tracts with spread to the adjacent midbrain, medulla and cerebellar peduncles. Cranial nerves VI and VII are most commonly affected, although cranial nerves V, X and XI can also be commonly affected as the disease progresses. Owing to the central brainstem being involved and the diffuse nature of the tumor, surgical resection is not recommended [12, 18-20].

2.2.1 Stereotactic Biopsy

It should be noted that surgical intervention may only be considered after referral to a highly specialized neurosurgical team, in conjunction with pediatric neuro-oncologists, before any treatment is initiated. In the past, the paucity of available surgical tissue has in part hindered the development of suitable preclinical models for research. Until recently, DIPG tissue acquisition was difficult due to the morbidity associated with the anatomical site of the tumor and tissue was only acquired at post mortem under consent and often following extensive treatment.

In 2007, Roujeau et al. published the first major report of upfront stereotactic biopsy of DIPG patients. The study found that of the twenty-four patients studied, only two suffered deficits from the procedure, with both being transient and lasting less than two months. Importantly, a histological diagnosis was made in all 24 patients, and that 22 had an infiltrative malignant glioma, with 1 having a pilocytic astrocytoma, and 1 having a low-grade glioma. This study concluded that tissue biopsies in DIPG have a high diagnostic yield associated with minimal morbidity and resulted in a consensus meeting in Paris in 2011 where it was agreed that biopsy of brain stem gliomas is technically safe with acceptable risks to inform on optimal therapeutic interventions [21]. Since then, similar clinical studies have continued to prove that there is minimal risk of persistent morbidity or mortality associated with the procedure whilst providing valuable pathologic and histologic insights [22-24].

This important innovation has led to increasing numbers of recent trials incorporating genomic analysis into their treatment protocols as a core indicator for potential targeted treatment options. With increased willingness to biopsy, the increased availability of tissue has led to an exponential increase in our understandings of the genomic, transcriptomic and epigenetic landscapes of DIPG, and an increase in pre-clinical models. Tumor biopsies will also
enable direct analysis of treatment naïve and treatment resistant tissues from longitudinal samples of the same patient. Collectively, these resulting resources are leading to the rationalized introduction of new clinical trials for targeted therapies, which will be discussed in more detail below.

2.2.2 Liquid Biopsy

Detection of the Histone H3 mutations in CSF-derived tumor DNA from patients with diffuse midline glioma was first described in 2017 by Huang et al [25]. In a small cohort of 6 diffuse midline glioma patients, sufficient CSF-derived tumor DNA was isolated from 5 of the patients and Histone H3 mutations were detected in 4 of these using Sanger sequencing. More recently, using digital droplet PCR, Histone H3 and additional driver mutations were detected in both CSF and plasma-derived tumor DNA from 48 diffuse midline glioma patients, all with matching tumor tissue [26]. Furthermore, this approach was applied to the longitudinal surveillance of patients during their clinical management and showed that changes in circulating tumor DNA mutational allele frequencies accurately reflected tumor response to therapy. This highlights the potential use of patient-derived liquid biopsy as both a clinically relevant diagnostic and predictor of therapy response and disease progression in patients with DIPG.

2.3 Radiation Therapy

To date, radiotherapy is the only therapeutic option to have demonstrated transient benefits in length of survival from diagnosis. Whilst not curative, and merely palliative, radiation therapy remains the standard of care treatment for children with DIPG. It is the only treatment repeatedly and reproducibly proven to provide temporary symptomatic relief and short-term progression control. Conventional radiotherapy targets the tumor volume plus a clear margin of 1 to 2 cm of adjacent neural tissue, accumulating a total dose between 54 Gy and 59.4 Gy over a period of 6 weeks with five treatment days per week, at a dose of approximately 1.8 Gy per fraction. However, despite significant radiological tumor shrinkage achieved with radiotherapy, the response is transient [27].

Furthermore, clinical trials have endeavored to improve survival rates through administration of higher doses of radiation and the use of adjunct chemotherapy and radiosensitizers, however survival and symptomatic benefits of these radiotherapy variations have shown no additional benefit [28, 29]. Recent studies of radiotherapy in DIPG have instead focused on identifying the lowest radiation dose required to attain the same therapeutic effect in order to maximize quality of life by reducing the burden of treatment. Indeed, a recent Phase III trial investigating hypofractionated radiotherapy, cumulative dose of 39 Gy in daily fractions of 3 Gy in thirteen treatments over 2.6 weeks, resulted in nearly comparable outcomes to conventional fractionation but with less burden on patients [30]. The potential for re-irradiation following disease progression has also become a recent area of interest, with several limited cohort studies reporting neurological improvement and improved median survival [31]. Upcoming phase II clinical trials of a prospective nature with increased target populations are investigating the reproducibility and morbidity associated with this method.

3. Genomics of DIPG

Recent advances in next generation sequencing techniques and stereotactic biopsies to access tissues, has enabled the in-depth exploration of the potential genomic and molecular
alterations underpinning DIPG tumorigenesis. Importantly, these studies have revealed distinct biological driver aberrations that could be therapeutically exploited. This includes recurrent mutations or amplification in genes associated with epigenetic regulation, cell signaling pathways, cell cycle regulation, and DNA repair mechanisms, often co-occurring in mutually exclusive combinations. Here, we will discuss the key alterations and their potential to be therapeutically targeted.

### 3.1 Epigenetic Dysregulation

The most significant advance in our understanding of DIPG biology has come from the discovery of unique recurrent somatic heterozygous mutations in genes encoding for two different histone H3 variants, H3.1 (*HIST1H3B, HIST1H3C*) and H3.3 (*H3F3A*) that are essential for epigenetic control of gene expression [32]. These mutually exclusive mutations in the histone H3 genes similarly result in a distinct single nucleotide variant leading to an amino acid substitution in the highly conserved N-terminal histone tail: lysine-to-methionine at position 27 (K27M). Collectively, H3K27M mutations occur in 74-93% of DIPG tumors [32-37]. The H3F3A mutation (H3.3K27M) is found in approximately two-thirds (56%-80%) of DIPGs and is associated with a later age of disease onset (7-10 years) and poorer overall survival (9 months) [32, 33, 35, 38]. Interestingly, H3.3K27M mutations also exclusively occur in diffuse midline gliomas effecting the thalamus and spinal cord. In contrast, H3.1K27M mutations are restricted to approximately one-fifth (17-31%) of DIPGs, correlated with an earlier age of onset (4-6 years), distinct clinicopathological and radiological features, and slightly longer survival (15 months) [32, 38].

Whilst the precise functions of H3K27M are still to be determined, evidence suggests that the mutation imparts a gain-of-function activity inhibiting the Polycomb repressive complex 2 (PRC2) via direct sequestration of the histone methyltransferase Enhancer of zeste homolog 2 (EZH2) resulting in a global reduction in H3K27me3 [39, 40]. Despite a global hypomethylation phenotype in H3K27M DIPGs, selected genomic regions of histone hypermethylation (gain of H3K27me3) are also observed, particularly in intergenic regions. As expected, genes with aberrant gain or loss of H3K27me3 demonstrate decreased or increased expression, respectively, strongly implicating epigenetic control of gene expression as an underlying mechanism driving DIPG pathogenesis [40]. Since epigenetic modifications are reversible, many studies have focused on the therapeutic potential of epigenetic modifiers. These include the use of inhibitors of H2K27 demethylases (JMJD3 and GSKJ4), histone deacetylase inhibitors (panobinostat and sodium valproate), catalytic EZH2 inhibitors (EPZ6438) and BET inhibitors, all which have shown promise in preclinical models (Table 2, Table 3).

Interestingly, H3.1K27M and H3.3K27M mutations co-occur with exclusive genetic alterations, likely accounting for the distinct clinical features described above. *TP53* mutations, *PDGRFA* mutations/amplifications, amplification at 17p11.2 targeting *TOP3A*, amplification of *CCND2, ATRX, ATM* and *ASXL1* mutations largely co-segregate with H3.3K27M. In contrast, H3.1K27M exclusively co-occurs with *ACVR1* and *BCOR* mutations and an enrichment of downstream PI3K pathway mutations (*PIK3CA* and *PIK3R1*) [32, 35, 36, 41]. Interestingly, *MYCN/ID2* mutations are enriched in H3 wildtype DIPG [35]. The specific functional importance of these co-segregating genomic alterations is now an area of intense investigation and preclinical results strongly suggest that combination therapy targeting both H3K27M and specific partner mutations will be necessary in the clinic.
<table>
<thead>
<tr>
<th>Genomic abnormality</th>
<th>Incidence (%)</th>
<th>Cellular changes</th>
<th>Clinical outcome</th>
<th>Targeted therapies tested</th>
<th>Therapeutic limitations</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MUTATIONS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histone H3</td>
<td>80</td>
<td>Hypomethylation of histone H3 proteins, initiated by the conversion of a lysine to methionine residue, resulting in aberrant cell-cycle function that initiates oncogenesis</td>
<td>Worse prognosis compared to non-histone mutated tumors</td>
<td>HDAC inhibitors such as panobinostat have yielded promising results in preclinical studies, but clinical trials published so far have shown intolerable toxicity at the effective dose. Other potentially promising HDAC inhibitors with promising preclinical results include JMJ3 and GSKJ4.</td>
<td>Commonly occurs in combination with other mutations; further investigation into combination therapies is necessary.</td>
<td>[42-44]</td>
</tr>
<tr>
<td>1) H3.3 (H3F3A)</td>
<td>60-71</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2) H3.1 (HIST1 H3B)</td>
<td>12-18</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDGFRA*</td>
<td>≈32</td>
<td>The most commonly mutated/amplified tyrosine kinase receptor in DIPG. Phosphorylation of these receptors triggers downstream activation of the PI3K and MAPK pathways.</td>
<td>Co-segregate with histone H3 mutations Enriched pro-neural expression Clinically aggressive</td>
<td>PDGFRA inhibitors tested Dasatinib/vandetanib/cabozanitinib (moderate potency) Crenolanib (potent)</td>
<td>Predominant cytostatic effect Poor CNS penetration (limited by drug efflux transporters, e.g. P-glycoprotein)</td>
<td>[42, 45-49]</td>
</tr>
<tr>
<td>PPM1D/TP53</td>
<td>22-40</td>
<td>PPM1D and TP53 are cell cycle regulatory mutations which are mutually exclusive in DIPG. PPM1D mutation/amplification and its consequent overexpression leads to suppression of multiple targets, e.g. p53. Mutated TP53 with histone H3.3 mutations occur in 30% of DIPGs allowing for the evasion of cellular death.</td>
<td>Co-segregate with histone H3 mutations ↑ metastases</td>
<td>Small molecule PPM1D inhibitors currently investigated by preclinical trials.</td>
<td>NA</td>
<td>[50-52]</td>
</tr>
<tr>
<td>ACVR1</td>
<td>20-30</td>
<td>Gene encodes the activin A type tyrosine kinase receptor (ALK2) Mutation activates the BMP pathway causing phosphorylation of downstream proteins, upregulating the signaling targets ID1/2, subsequently promoting cell progression via interaction with the Rb1 and p21 pathways.</td>
<td>Co-segregate with histone H3.1 mutations ↑ median OS</td>
<td>No DIPG trials performed on ALK2 inhibitors K02288 (highly selective ALK2 inhibitor) has been shown to inhibit the BMP-induced SMAD pathway; further preclinical models necessary.</td>
<td>NA</td>
<td>[33, 34, 53-55]</td>
</tr>
</tbody>
</table>
### Accessory drivers

| PIK3R1/PIK3CA | 15 | Can also be resultant from upstream activating changes in receptor kinases. Oncogenes within the PI3K pathway are an obligate partner of histone H3.3 present in clonal populations | ↑ angiogenesis  
• Stem cell formation | No DIPG trials performed on PI3K inhibitors  
• PI3K/mTOR inhibitor NVP- BEZ235 has demonstrated increased sensitization to RT and temozolomide with increased survival in non-brainstem glioblastoma murine models  
• AKT inhibitors  
• Rapamycin | Agents tested were non-specific to DIPG | [42, 47, 55-57] |
| BCOR/BCORL1, NF1, PTEN | <1 | NA | NA | NA | [33] |

### COPY NUMBER ABNORMALITIES

| PDGFRA ampl | 15-30 | As above, see * |
| CDK4/6 ampl | 7-18 | Amplifications involve the phosphorylation and inactivation of the Rb1 pathway, resulting in release of transcription factors required for cell cycle progression.  
• ↑ cell proliferation | CDK4/6 inhibitor tested  
• Palbociclib | Predominant cytostatic effect | [42, 55, 57] |
| CCND1/2/3 ampl | ≈15 | Concomitant mutation/amplification of the tyrosine kinase receptor pathways occur in approx. 20% of DIPGs  
NA | NA | [45, 66-69] |
| 1q gain, MET, IGF1R, MYCN/MYC | <1 | Although commonly found in adult gliomas, are found only in a minority of DIPGs | NA | Agents tested  
• Monoclonal antibodies (e.g. nimotuzumab)  
• Small molecule inhibitors (e.g. erlotinib)  
• EGFRvIII-targeting peptide vaccines  
• Therapies were based on effective agents used in adult regimes and weren't DIPG-specific. | [33, 42, 45, 58-65] |

### CELL CYCLE REGULATION

| PARP1/2 overexpression | N/A | Nuclear proteins involved in sensing DNA damage and initiating repair mechanisms to escape apoptosis.  
• ↓ response to radiotherapy | PARP1/2 inhibitor tested  
• Veliparib/olaparib/niraparib (synergistic effect with RT and temozolomide)  
• Clinical trials currently underway (e.g. ABT-888) | NA | [45, 66-69] |
| WEE1 overexpression | N/A | A regulatory kinase at the G2 checkpoint which facilitates DNA repair and cell survival.  
• ↓ cellular sensitivity to DNA-damaging agents | WEE1 inhibitor tested  
• MK-1775 (synergistic effect with RT in murine models)  
• Clinical trials currently underway | NA | [70-72] |
### Table 3: Selected preclinical DIPG targeted drug studies (published up to June 2018)

<table>
<thead>
<tr>
<th>Drug/agent class</th>
<th>Agent/s investigated</th>
<th>Media</th>
<th>Effect observed (Yes/No)</th>
<th>Conclusion</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAR T-cells</td>
<td>Anti-GD2 CAR T-cells</td>
<td>In vitro and in vivo (murine models)</td>
<td>Y</td>
<td>Anti-GD2 CAR T cells incorporating 4-1BBz costimulatory domains demonstrate potent cytotoxic effects towards H3K27M+ cells and engrafted tumors through antigen-dependent cytokine generation</td>
<td>[73]</td>
</tr>
<tr>
<td>EZH2 inhibitor and BET inhibitor</td>
<td>EPZ6438 and JQ-1</td>
<td>In vitro and in vivo (murine models)</td>
<td>Y</td>
<td>Synergistic effects in inhibiting tumor growth by blocking cell proliferation and promoting cell apoptosis</td>
<td>[74]</td>
</tr>
<tr>
<td>HDAC inhibitor</td>
<td>Panobinostat</td>
<td>In vitro and in vivo (murine models)</td>
<td>Y</td>
<td>Potent cytotoxic effects</td>
<td>[75, 76]</td>
</tr>
<tr>
<td></td>
<td>Sodium valproate</td>
<td>In vitro</td>
<td>Y</td>
<td>Cytotoxic effects as a monotherapy, with minimal toxicity to murine neural cells</td>
<td>[77]</td>
</tr>
<tr>
<td>Lytic infection agent</td>
<td>Parvovirus H-1</td>
<td>In vitro</td>
<td>Y</td>
<td>Mild cytostatic effects observed after infection at sub-lethal doses</td>
<td>[78]</td>
</tr>
<tr>
<td>mTORC1/2 kinase inhibitor</td>
<td>TAK228</td>
<td>In vitro and in vivo (murine models)</td>
<td>Y</td>
<td>Cytotoxic effects through inhibition of the mTOR/akt pathway induced a significant survival benefit in some DIPG murine models.</td>
<td>[79]</td>
</tr>
<tr>
<td></td>
<td>AZD2014 (mTORC1/2 inhibitor) compared to Everolimus (mTORC1 inhibitor)</td>
<td>In vitro</td>
<td>Y</td>
<td>Combined mTORC1/2 inhibition has greater efficacy compared to MTORC1 inhibition alone, which has little to no effect.</td>
<td>[80]</td>
</tr>
</tbody>
</table>
| PARP1/2 inhibitors                | Veliparib/Olaparib/Niraparib                                                         | In vitro and in vivo (murine models) | Y                        | Cytostatic effects, most pronounced with niraparib  
  ↑ response to radiotherapy                                                                                                                                             | [81]  |
| PDFGRA inhibitors                 | Dasatinib and Cabozatinib                                                           | In vitro                     | Y                        | Cytostatic effects with synergistic effects between dasatinib and cabozatinib                                                                                                                                            | [82]  |
| PI3K inhibitor and MEK inhibitor   | Perifosine and trametinib                                                           | In vitro                     | Y                        | Cytotoxic effects through synergistic inhibition of the PI3K/akt and MEK/ERK pathways                                                                                                                                    | [83]  |
| PLK1 selective inhibitor (new generation) | BI 6727                                                                           | In vitro                     | Y                        | Significant cytostatic effects with mild cytotoxic effects  
  ↑ response to radiotherapy                                                                                                                                         | [84]  |
| VGEF inhibitor                    | Bevacizumab                                                                          | In vivo (murine models)       | N                        | Inconclusive results due to insufficient drug biodistribution                                                                                                         | [85]  |
| Systematic drug screen            | Screen of 83 agents                                                                  | In vitro and in vivo (murine models) | Y                        | 10 of the most potent agents were listed  
  BMS-754807, listed as the most potent agent, significantly inhibited cell proliferation in vivo regardless of the histone H3.3 mutation status                                                                 | [86]  |
3.2 Cell Signaling Pathway Mutations

Recurrent mutations and gene amplification involved in key mitogenic and developmental signaling pathways have been described in a significant proportion of DIPGs and are commonly found to coexist with H3 mutations. The more commonly affected pathways include the PDGF, EGFR and BMP pathways.

The platelet-derived growth factor (PDGF) signaling pathway is involved in the regulation of cell growth and division, and angiogenesis. PDGF ligands bind to the cell membrane protein tyrosine kinase receptors PDGFRα and PDGFRβ leading to activation of other proteins by phosphorylation. PDGFRα is one of the commonly mutated PDGF receptors found in malignancies and is the most commonly mutated and/or amplified tyrosine kinase receptors in DIPG, occurring in 5% and 30% of tumors respectively [42, 45-47]. PDGFRα mutations/amplification most commonly co-occur with H3.3K27M. The PDGFRα inhibitors, dasatinib and cabozantinib, have been investigated in preclinical DIPG drug studies. These compounds have demonstrated cytostatic modes of action and are synergistic when administered together. However, PDGFRα inhibitors are known to have poor CNS penetration, limited primarily by drug efflux transporters. Further animal studies are necessary to investigate whether the co-administration of an efflux transporter inhibitor may improve CNS drug concentrations and subsequent drug efficacy. Inhibition of vascular endothelial growth factor (VEGF) is known to reduce downstream activation of PDGRA. VEGF inhibitors have been investigated in DIPG cell lines, so far with inconclusive results. Despite the preclinical data to date, the development of a genetic mouse model driven by PDGF-B overexpression, strongly supports a role for this pathway in DIPG tumorigenesis [87]. Recent effort has also focused on targeting its downstream effector pathway, PI3K/AKT/mTOR, that is also strongly implicated in DIPG.

EGFR mutations and amplification are among the most common genetic aberrations found in adult glioblastomas but are scarcely found in DIPGs. It is therefore not surprising that despite the large number of clinical trials investigating EGFR-targeting agents, none have shown efficacy so far. Similarly, there are varying reports on the prevalence of other mutations in kinase pathways, including MET mutations, which are seen in a minority of DIPGs. Trials conducted so far have failed to prove their potential as a DIPG therapeutic target [45, 59-61].

The bone morphogenetic protein (BMP) signaling pathway is affected in 20-30% of DIPGs, predominantly in the form of activating mutations in ACVR1, a gene encoding type I BMP receptor (ALK2), that exclusively co-occur with H3.1K27M. To date, 7 different ACVR1 mutations have been described in DIPG, resulting in ligand-independent constitutive activation of the pathway, upregulation of phosphorylated SMAD1/5 transcriptional effectors and upregulation of downstream BMP targets, leading to promotion of cell cycle progression via the RB and p21 pathways [33, 37, 88, 89]. Similar gain-of-function mutations have been described in the rare inherited bone disease, fibro-dysplasia ossificans progressiva (FOP) [90]. Despite significant biological differences between FOP and DIPG, including the inherited nature of FOP, the study of ACVR1 functions in FOP may be useful in the overall DIPG analysis. Limited ALK2 inhibitor drug studies have been performed on either tumor types and interpretation of results has been inconclusive. The role of ACVR1 in DIPG is yet to be determined [91]. With the availability of highly-specific ALK2 inhibitors, further preclinical studies are necessary to investigate their effects on potentially susceptible DIPG cell lines.
3.3 Cell Cycle Dysregulation

Genetic abnormalities involving essential cellular pathways are also found in a third of DIPGs, commonly coexisting with oncogenic mutations in the histone genes or kinase pathways as accessory drivers. The more commonly affected pathways include the RB and PI3K-Akt pathways.

Genetic abnormalities in the RB pathway that are described in DIPG include focal deletion of RB itself, amplifications of CCND1/2/3 (cyclin D1/D2/D3), and downstream CDK4/6 (cyclin-dependent kinase 4/6), collectively occurring in approximately 30% of tumors [42, 55, 57]. These cellular proteins contribute to phosphorylation of RB and the coordination of G1/S cell cycle progression. The increased activity of these proteins contributes to tumor growth. The CDK4/6 inhibitor, palbociclib, has been studied in DIPG murine models demonstrating a cytostatic mechanism of action but with limited efficacy [57]. As these abnormalities are often coexistent with other genetic aberrations, combination therapy may potentially prove to have greater effectiveness.

The PI3K/AKT/mTOR pathway is commonly implicated in glioblastoma as well as in other tumor types. Mutations in this pathway affect cellular signaling associated with control of the cell cycle and include alterations in tyrosine kinase receptors, growth factors, and interacting pathways including Ras/Raf/MEK/ERK. These mutations affect approximately 15% of DIPGs and include recurrent mutation/amplification of PIK3CA, mutations of PIK3R1, and mutation/loss of PTEN. Recently, there has been substantial preclinical activity in determining the efficacy of PI3K/mTOR inhibitors in DIPG, often in combination with other targeted agents. Inhibition of PI3K/AKT pathway with perifosine in combination with the MEK inhibitor, trametinib, synergistically reduced DIPG cell viability, while use of the dual HDAC/PI3K inhibitor, CUDC-907, as a radiosensitizer looks promising, acting by inhibiting radiation-induced DNA repair pathways [83, 92]. However, more preclinical work is needed to further investigate the potential role of inhibiting these pathways, with particular consideration directed to ensuring adequate agent tumor penetration.

The Hedgehog pathway has been investigated as another potential therapeutic target owing to earlier studies demonstrating its upregulation in DIPG [93]. However, these molecular abnormalities are uncommon, and found to be insufficient to cause DIPG formation in murine models. Although the role of the Hedgehog pathway in tumor formation remains unclear, studies into the efficacy of Hedgehog pathway inhibitors, including Smoothened inhibitors, are still currently being undertaken in clinical trials [2, 93-95].

3.4 Inactivation of DNA Repair Mechanisms

Mutations in the TP53 pathway are the second most common genetic aberration in DIPG with mutations or copy number alterations observed in 42-71% of tumors and can co-occur in the presence or absence of H3 mutations. Interestingly, PPM1D, encoding a phosphatase (WIP1) that negatively regulates p38 MAPK and is induced by TP53, is mutated in approximately 10% of DIPG. Functionally, PPM1D mutations are equivalent to TP53 mutations, and they are largely mutually exclusive with PPM1D mutations observed in 50% of DIPGs that are wildtype for TP53 [50-52, 96]. These mutations appear to function primarily as accessory drivers in the oncogenic process and research into their therapeutic potential is scarce. However, they may have a role in therapeutic synergy with other agents in combination therapy.

Other DNA repair proteins found to be affected in DIPG include the PARP1/2 and WEE1 proteins. Their prevalence in DIPG cohorts has not yet been formally studied. Nonetheless, their
potential as molecular targets have been studied in preclinical models with both PARP1 inhibitors and WEE1 inhibitors demonstrating radio-sensitization effects. Further studies are needed to investigate their clinical efficacy [66-68, 71, 72, 97, 98].

4. Translation of DIPG Genomics

With the recent surge in our understanding of DIPG genomics, attention has now turned to translating this knowledge into meaningful therapeutic strategies using preclinical models. Several drug classes are currently being trialed in preclinical studies both in vitro, and in vivo using murine models (Table 3). Studies published to date have mainly tested agents targeting epigenetic dysregulation and aberrant kinase pathway activity, using targeted therapies either FDA-approved or in late-stage clinical trials for other tumor types. Many studies have shown therapeutic potential of targeted therapies in vitro. However, the cytostatic or cytotoxic effects observed in vitro only partially translate into in vivo murine models due to inability to efficiently cross the blood brain barrier, high levels of systemic toxicity owing to their wide-ranging effects on different pathways and cell types, and only modest improvements in survival. These findings, in part, highlight the critical importance of relevant and reliable in vitro and in vivo models that has until recently been lacking for DIPG.

4.1 Patient-Derived Cell Models

Increased frequency of early post-mortem, stereotactic biopsies and refinement of DIPG culture conditions and supplementation has enabled the development of cohorts of patient-derived DIPG cell models. Slowly but surely these models are now becoming sufficient in number to accurately represent the heterogeneity of the disease and are often extensively characterized at a genomic level with accompanying clinical annotations [99, 100]. These models have provided an invaluable resource to the research community with which to explore underlying biology and identify new therapeutic avenues. The accompanying genomic and molecular annotations of these models is now enabling the next wave of phenotype-genotype analyses to uncover primary genetic drivers of disease, relevance of co-occurring alterations and functional importance of targetable pathways, in a cohort and even patient-specific context [99, 100]. However, the normal limitations of in vitro cell models also apply to DIPG. These include potential of the cells to genomically or molecularly “drift”, and responses to be different in the absence of supporting tissues and other signals, including immunomodulation. As a result, in vitro studies are often subsequently validated using in vivo disease models.

4.2 Orthotopic Xenograft Models

The availability of increased patient-derived cell models has also permitted the development of orthotopic cell-derived DIPG xenograft models that replicate the invasive nature of the disease and maintain key genomic and molecular characteristics. Direct injection of human DIPG cells into the fourth ventricle/pons of immune-deficient mice has not only enabled the interrogation of gene function and tumor biology but enabled evaluation of potential therapies on DIPG in situ, including an intact blood brain barrier, and potential systemic toxicities. Moreover, the labelling of tumor cells prior to injection, most commonly using luciferase constructs, to permit imaging,
allows researchers to monitor tumor growth and response to therapy using longitudinal imaging techniques [93]. Patient-derived orthotopic xenograft models, whereby patient tumor and stromal cells are injected without culture, also represents a valuable in vivo preclinical system. Typically, these patient-derived xenografts are maintained by serial passaging in immune-deficient mice to avoid any potential culture artefact. The limitation is related to absence of expression of reporters for imaging. Whilst these models remain the gold standard for in vivo investigation for DIPG preclinical studies, the use of immune-deficient mouse strains largely precludes the investigation of immunotherapeutic agents.

4.3 Genetic Mouse Models

The first genetic model to closely resemble DIPG preceded more recent genomic and molecular tumor characterization and was driven by germline Ink4a-ARF deletion and PDGFB overexpression in the Nestin-expressing neural stem cells of the fourth ventricle and aqueduct in neonatal mice [87]. This method utilized the replication-competent avian sarcoma-leukosis virus long-terminal repeat with splice acceptor/tumor virus A (RCAS/Tva) system whereby RCAS-PDGFB-expressing DF1 chicken fibroblast cells were injected into Nestin tv-a;Ink4a-ARF/ mouse pups at postnatal day 3. Despite very closely representing human DIPG histopathology, tumors do not exclusively arise in the pons. Subsequent variations of this model have since been developed to more closely replicate the genomics of human DIPG. This includes RCAS-PDGFB, RCAS-Cre, RCAS-H3.3K27M expressing DF1 cells intracranially injected into Neastin tv-a;p53lox/lox neonatal mice and RCAS-PDGFB, RCAS-Cre, RCAS-H3.3K27M expressing DF1 cells intracranially injected into Pax3 tv-a;p53lox/lox neonatal mice [39, 101]. Most recently, in utero electroporation of the pontine nuclei-containing lower rhombic lip of embryonic day 12.5 mouse embryos with a piggyBac transposon vector to overexpress H3.3K27M in combination with a non-transposable gRNA/Cas9 targeting Trp53 (H3.3K27M;p53 KD) resulted in neoplastic transformation with 100% penetrance 6 months following surgery [102]. Moreover, electroporation of a transposable shRNA targeting ATRX into this same model (H3.3K27M;ATRX/p53 KD) resulted in focally restricted lesions and reduced tumor latency to 4 months whilst the additional overexpression of wildtype PDGFRA cDNA using the piggyBac transposon (H3.3K27M;PDGFRA;ATRX/p53 KD) led to increased invasion and further reduced tumor latency to 21 days [102]. The different histopathological features of these models provide some insight into the importance of co-occurring genomic alterations and aberrant protein interactions on tumorigenesis. Whilst genetic mouse models of DIPG provide the benefit of studying tumors that arise in situ, in immunocompetent animals, they are currently restricted to narrow or broad cells of origin harboring limited and specific genetic alterations and are therefore only representative of a small cohort of the disease. As our understanding of tumor biology and the genetic and molecular interactions underlying DIPG continue to increase, so too will our ability to generate additional models that accurately represent the known heterogeneity of disease.

5. Clinical Application

Comprehensive reviews of DIPG-specific clinical trials have previously been published, analyzing a total of 55 DIPG-specific trials covering the period from 1984 to March 2011 [10, 11]. Despite the large number of clinical trials, no clear improvement in either the quality of life or length of survival has been demonstrated, and most studies are, in hindsight, complicated by the wide
variance in the selection criteria and absence of accompanying genomic and molecular data. Additionally, a systematic clinical review from the Netherlands has emphasized the necessity of cross-national cooperation to prevent potential epidemiological bias [5]. The pharmacologic agents investigated to date are largely based on therapies proven to convey survival benefit in the treatment of adult gliomas. The only agents with activity against DIPG genomic targets which have been investigated are anti-EGFR tyrosine kinase inhibitors, and results to date are inconclusive, possibly owing to varied selection criteria. One study requiring tissue biopsy on diagnosis, investigated the effects of erlotinib, and achieved an improved median survival of 12 months. However, the outcome was reported as inconclusive, and further Phase III studies were deemed necessary to validate the results [58].

In this review, we have also tabulated clinical trials published between March 2011 to June 2018 (Table 4). The agents investigated are still primarily common chemotherapeutic agents with anti-EGFR agents continuing to be one of the few drug classes being tested targeting known genetic abnormalities in DIPG tumors. Short-term stable disease was reported in an 8-patient clinical trial using a PI3K pathway-specific AKT inhibitor and an mTOR inhibitor, but it failed to improve overall survival. Generic alkylating agents such as temozolomide have been extensively studied in several trials, with no proven survival benefit. Other studies included agents such as VEGF inhibitors and mTOR inhibitors also resulted in negative or inconclusive results (Table 4).

Low drug bioavailability around the tumor due to its anatomical position within the blood brain barrier and its encapsulated morphology has also been proposed as a reason for lack of conclusive and positive outcomes in DIPG clinical trials. As a result, studies are now being undertaken to maximize drug delivery to the site. Besides the conventional chemotherapy delivery through intravenous therapy, other novel routes of drug delivery are being investigated. Of these methods, convection enhanced delivery (CED) has been the most extensively studied with in vivo murine and primate studies proving efficacy of drug delivery [103, 104]. CED utilizes a site-directed catheter which delivers a high concentration of the therapeutic agent directly to the tumor bed. This method was developed with the purpose of achieving reliable drug levels of agents with low bioavailability across the blood brain barrier. Although a number of trials have been conducted investigating the application of CED with conventional anticancer treatments, only recently has the delivery of targeted or radioimmunotherapy agents been described for DIPG patients suggesting that it is a safe and rational therapeutic option for these patients [105-108]. Despite potential barriers to the successful application of CED, including unequal distribution of the drug to variegated tumor tissue and necessity of neurosurgery expertise for its insertion, this is an avenue with promise that warrants further attention.

In addition to CED, several other innovative methods are also on the horizon. These include intranasal chemotherapy administration, drug delivery via continuous interstitial infusions, and through the medium of human neural and mesenchymal stem cells [109]. These ideas are still in the preclinical stages of investigation but have already showed promise in murine experimental models [110-112].

In addition to the most recent clinical trials outlined in Table 4, there are also a number of ongoing trials yet to be published (Table 5). Many of these ongoing trials are testing more promising agents targeting a range of different genomic abnormalities specific to DIPG tumors. Their results are much anticipated and will provide a platform on which directed therapeutic regimes can be formed through more comprehensive genomic understanding on a biopsy-oriented, case-specific level.
<table>
<thead>
<tr>
<th>Drug class</th>
<th>Agent/s investigated</th>
<th>Study type</th>
<th>Study population (DIPG)</th>
<th>Inclusion criteria</th>
<th>Effect observed</th>
<th>Conclusion</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>AKT inhibitor and mTOR inhibitor</td>
<td>Perifosine and Temsirolimus</td>
<td>Prospective</td>
<td>I</td>
<td>MRI</td>
<td>Y/INC</td>
<td>Stable disease achieved for a median of 3 months in 6 children, survival benefit not clarified, no dose-limiting toxicity</td>
<td>[113]</td>
</tr>
<tr>
<td>Alkylating agent - with radiosensitizers</td>
<td>Temozolomide - Vincristine or - Gs- platinum</td>
<td>Prospective</td>
<td>N/A</td>
<td>MRI + Biopsy</td>
<td>N</td>
<td>No clinically significant benefit observed, reported median survival of 15.6 months</td>
<td>[114]</td>
</tr>
<tr>
<td>Anti-angiogenic protocol (Angiocomb protocol)</td>
<td>Thalidomide and Etoposide and Celecoxib</td>
<td>Prospective</td>
<td>N/A</td>
<td>MRI</td>
<td>N</td>
<td>No significant survival benefit, reported median survival of 11.7 months observed with greater levels of toxicity</td>
<td>[115]</td>
</tr>
<tr>
<td>Autologous dendritic cell vaccine</td>
<td>AEMPS PET 15-215</td>
<td>Prospective</td>
<td>I</td>
<td>MRI</td>
<td>INC</td>
<td>Improved survival with median survival of 13 months</td>
<td>[116]</td>
</tr>
<tr>
<td>Kinase inhibitor (anti-EGFR)</td>
<td>Nimotuzumab</td>
<td>Prospective</td>
<td>II</td>
<td>MRI</td>
<td>INC</td>
<td>Median survival 3.2 months, a few demonstrated prolonged survival</td>
<td>[117]</td>
</tr>
<tr>
<td>Kinase inhibitors</td>
<td>Vandetanib vs Vandetanib and Dasatinib</td>
<td>Prospective</td>
<td>I</td>
<td>MRI</td>
<td>INC</td>
<td>Difference in radiation field was another variable studied between the two trial groups. No significant survival benefit was identified as a difference between the two trial groups. A few reported patients appeared to have demonstrated prolonged survival, but this was not the primary outcome and was not thoroughly explored.</td>
<td>[118]</td>
</tr>
<tr>
<td>Monoclonal antibody (anti-VEGF)</td>
<td>Bevacizumab</td>
<td>Prospective</td>
<td>I</td>
<td>MRI</td>
<td>N</td>
<td>No survival benefit proven (median survival 10.4 months), several discontinued therapies due to substantial toxicity (grade 3 hepatotoxicity and grade 4 thrombocytopenia)</td>
<td>[119]</td>
</tr>
<tr>
<td>Recombinant interferon</td>
<td>Pegylated Interferon α-2b (PEG-Intron)</td>
<td>Prospective</td>
<td>II</td>
<td>MRI</td>
<td>INC</td>
<td>No significant increase in progression-free survival, treatment may have potentially caused a higher incidence of distant diffuse disease</td>
<td>[120]</td>
</tr>
<tr>
<td>Telomerase inhibitor</td>
<td>Imetelstat (GRN163L)</td>
<td>Prospective</td>
<td>II</td>
<td>MRI</td>
<td>N</td>
<td>Premature study closure due to toxicity and CNS bleeding from resultant intracranial bleeding</td>
<td>[121]</td>
</tr>
</tbody>
</table>

N/A = not applicable, Y = benefit observed, N = no benefit observed, INC = inconclusive results (e.g. not statistically significant)
<table>
<thead>
<tr>
<th>Drug class</th>
<th>Agent/s investigated</th>
<th>Trial ref no.</th>
<th>Current status</th>
<th>Registration date</th>
</tr>
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<tbody>
<tr>
<td><strong>Single agents</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Alkylating agent</td>
<td>Melphalanhydrochloride</td>
<td>NCT01688401</td>
<td>Suspended</td>
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<td></td>
<td>Temozolomide</td>
<td>UECTR2007-00128-42-DE</td>
<td>C/NP</td>
<td>02/06/2008</td>
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<tr>
<td>Anticancerine</td>
<td>Doxorubicin</td>
<td>NCT02758366</td>
<td>R</td>
<td>27/04/2016</td>
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<td></td>
<td>Gemcitabine</td>
<td>NCT02992015</td>
<td>R</td>
<td>29/11/2016</td>
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<tr>
<td>Anthracyclines</td>
<td>Pidilizumab</td>
<td>NCT01952769</td>
<td>NR/NP</td>
<td>15/09/2013</td>
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<td></td>
<td>Pembrolizumab</td>
<td>NCT02359565</td>
<td>R</td>
<td>09/02/2015</td>
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<tr>
<td>Anti-VEGF agents</td>
<td>Bevacizumab</td>
<td>NTR3518</td>
<td>R</td>
<td>09/07/2012</td>
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<td>CDK4/6 inhibitor</td>
<td>Abemaciclib</td>
<td>NCT02644460</td>
<td>R</td>
<td>15/12/2015</td>
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<td></td>
<td>Ribociclib</td>
<td>NCT02607124</td>
<td>R</td>
<td>16/11/2015</td>
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<td>HDAC inhibitor</td>
<td>Panobinostat</td>
<td>NCT02899715</td>
<td>R</td>
<td>13/09/2016</td>
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<td></td>
<td>MTX-110 (panobinostat nanoparticle preparation)</td>
<td>NCT03566199</td>
<td>R</td>
<td>12/06/2018</td>
</tr>
<tr>
<td>Integron inhibitor</td>
<td>Valproic acid</td>
<td>RBR-7yspsd</td>
<td>Withdrawn</td>
<td>30/05/2016</td>
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<td>Vorinostat</td>
<td>NCT01189266</td>
<td>NR/NP</td>
<td>25/08/2010</td>
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<td>Immunomodifier</td>
<td>Lenalidomide</td>
<td>NCT01222754</td>
<td>C/P</td>
<td>15/10/2010</td>
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<td>Integron inhibitor</td>
<td>Cilengitide</td>
<td>NCT01165333/ EUCTR2009-016870-33-FR</td>
<td>C/NP</td>
<td>16/03/2010</td>
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<td>Kinase inhibitor</td>
<td>AZD1775</td>
<td>NCT01922076</td>
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<td></td>
<td>ONC201</td>
<td>NCT03416530</td>
<td>R</td>
<td>23/01/2018</td>
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<td>Monoclonal antibody (anti-EGFR)</td>
<td>Nimotuzumab</td>
<td>NCT00600584</td>
<td>C/P</td>
<td>11/04/2008</td>
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<tr>
<td>Oncolytic adenovirus</td>
<td>DNX-2401</td>
<td>NCT03178032/ EUCTR2016-001577-33-35</td>
<td>R</td>
<td>18/08/2016</td>
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<td>PDGFIR inhibitor</td>
<td>Crenolanib</td>
<td>NCT01393912</td>
<td>NR/NP</td>
<td>11/07/2011</td>
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<tr>
<td>Peptide vaccine</td>
<td>H3.3K27M Peptide Vaccine</td>
<td>NCT02960230</td>
<td>R</td>
<td>02/11/2016</td>
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<tr>
<td></td>
<td>WT1 peptide-based vaccine</td>
<td>JPRN-UMIN000013257</td>
<td>C/P</td>
<td>26/02/2014</td>
</tr>
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<td>Recombinant interferon</td>
<td>Pegylated interferon α-2b (PEG-Intron)</td>
<td>NCT00036569</td>
<td>C/P</td>
<td>10/05/2002</td>
</tr>
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<td>Topoisomerase I inhibitor</td>
<td>Irinotecan</td>
<td>NCT03086616</td>
<td>R</td>
<td>16/03/2017</td>
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<tr>
<td><strong>Multiple agent regime</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Adenoviral vector encoding cytokine IL-12 and Small-molecule activator ligand</td>
<td>Ad-RTS-hIL-12 and Veledimex</td>
<td>NCT03330197</td>
<td>R</td>
<td>16/10/2017</td>
</tr>
<tr>
<td>ALK inhibitor and kinase inhibitor</td>
<td>Crizotinib and Dasatinib</td>
<td>NCT01644773</td>
<td>NR/NP</td>
<td>17/07/2012</td>
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<td>Antineoplastons</td>
<td>Atengenal and Astugenal</td>
<td>NCT02742883</td>
<td>NR/NP</td>
<td>12/04/2016</td>
</tr>
<tr>
<td>Antimetabolite (pyrimidine analogue), Topoisomerase I inhibitor and Monoclonal antibody (anti-VEGF) and Kinase inhibitors (anti-EGFR)</td>
<td>Gemcitabine, Irinotecan and Bevacizumab and Erlotinib and Everolimus</td>
<td>NTR2391</td>
<td>R</td>
<td>24/06/2010</td>
</tr>
<tr>
<td>Cellular preparation</td>
<td>Autologous dendritic cells pulsed with lysated allogenic tumor lines</td>
<td>NCT02840123/ EUCTR2015-003362-84-E5</td>
<td>C/P</td>
<td>31/05/2016</td>
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<td>Cyclin D1/CDK4/CDK6 inhibitor and mTOR inhibitor</td>
<td>Ribociclib and Everolimus</td>
<td>NCT03355794</td>
<td>R</td>
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<td>HDAC inhibitor and Monoclonal antibody (anti-VEGF)</td>
<td>Valproic acid and Bevacizumab</td>
<td>NCT00879437</td>
<td>C/P</td>
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<td>HDAC inhibitor and Kinase inhibitor</td>
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<td>NCT02233049</td>
<td>R</td>
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<td>Vandetanib and Dasatinib</td>
<td>NCT00996723</td>
<td>C/P</td>
<td>15/10/2009</td>
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<td>Monoclonal antibody (anti-VEGF) and Kinase inhibitor (anti-EGFR) and Alkylating agent</td>
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<td>NCT01182350</td>
<td>C/P</td>
<td>12/08/2010</td>
</tr>
</tbody>
</table>

# All clinical trials up to June 2018 listed in the WHO trial registry involving a chemotherapeutic intervention for DIPG patients n=5 have been included
R = recruiting; NR = not recruiting
C = completed; P = published; NP = no publications found
It should be noted that the rarity of DIPG has a significant impact on the ability to accelerate clinical research. The difficulty in recruiting sufficient patient numbers to demonstrate a potential survival advantage necessitates multi-institutional international trials and therefore can restrict the number of simultaneous trials. Accordingly, the weight of preclinical data will play an important role in prioritizing the next wave of clinical investigation.

6. The Future

Given the lack of success in achieving improved patient outcomes for DIPG to date, future focus is on tumor genomic and molecularly-informed targeted therapies and improving drug accessibility to the site of disease. To achieve this, better understanding of the genomic landscape of DIPGs can yet be achieved through improving the protocolization of tissue and liquid biopsies for tumor subtyping, and cross-national collaboration of DIPG research with coordination of DIPG databases, such as the International DIPG Registry and SIOPE DIPG network, to achieve a comprehensive epidemiologic profile for the malignancy.

A greater number of promising preclinical studies focused on DIPG targeted therapies is anticipated in the coming years. This will lay the groundwork on which further translational clinical trials should be conducted. Owing to the complex facets of DIPG, recent preclinical research has expanded to cover a broader range of treatment options as well as adjunct supportive therapies, utilizing both intrinsic and extrinsic pathways to target this tumor.

Extrinsically, recent identification of potential DIPG targeted therapies have put a greater emphasis on targeting the tumor’s unique genomic makeup (Table 2). Historically, DIPG clinical trials have investigated chosen agents based on their effectiveness against anatomically and histologically similar adult counterparts, which have unfortunately failed to demonstrate any clinical success (Table 4). In recent years, a number of promising DIPG genomic-targeting therapeutic agents have been reported with positive outcomes in both in vitro and in vivo murine models (Table 3). Improvements in the numbers and heterogenous representation of preclinical in vitro and in vivo models will significantly enhance the transferability of preclinical results to the clinical setting. Several clinical trials extending from these studies are currently in the recruitment phase. A rationalized approach matching therapies to patient-specific tumor gene and pathway dependencies provides clinical potential for improving quality of life and/or survival outcomes for children with DIPG.

Intrinsically, recent developments in immunotherapy have demonstrated promising results in the utilization and augmentation of the children’s own immune system to target and destroy tumor cells. Recent preclinical investigations of DIPG genomically targeted CAR-T cells have achieved promising in vitro and in vivo results, but are yet to be translated to clinical practice [73]. This may be challenged by the microenvironment of DIPG tumors, which are neither highly immunosuppressive nor inflammatory [125]. Therefore, concurrent research into methods of the delivery, activation, and retention of these cells in the tumor environment will be necessary. However, this area remains one of enormous potential and intense interest.

Owing to the inaccessibility of DIPG’s anatomical location, further development in CNS drug delivery methods and cellular engineering is necessary to improve the potential clinical efficacy of both molecular and cellular agents. In addition to further research into invasive approaches such as CED and infusions, recent developments in non-invasive ultrasound-guided delivery of
therapeutic agents through microbubbles could also provide means of accurately targeting tumor cells to maximize therapeutic effect whilst minimizing systemic toxicity. It is also likely that the modification of already established targeted therapies with proven preclinical activity, to increase blood brain barrier permeability, could significantly strengthen the therapeutic arsenal required to treat this disease.

Further research into concurrent treatments such as radiotherapy can also be anticipated in the coming years. Some positive results have been seen in the re-irradiation trials published, and trials of larger numbers will be necessary to investigate the efficacy, dosage and toxicity of additional radiotherapy.

7. Conclusion

To improve the morbidity and mortality of children with DIPG, it is imperative to develop cross-national/international collaboration in protocolled biopsying and genomic analysis, and the consequent identification and development of genomically informed targeted therapies. Improvements in in vivo models and greater insight into effective methods of drug and cellular delivery will aid in improving efficacy in clinical translation. Together with concurrent radiotherapy and supportive treatments, increasing clinical trials of preclinically tested DIPG targeted therapies has the potential to improve the quality of life and survival outcomes in children with DIPG.

Author Contributions

TZ and JEC contributed to the writing of the manuscript. Review and editing was performed by SK, RF and PD.

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Competing Interests

The authors have declared that no competing interests exist.

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Sporadic and Hereditary Hemangioblastoma: The Role of Endothelial Cells

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Special Issue: Tumors of the Central Nervous System

Abstract

Hemangioblastomas (HBs) are benign, highly vascularized tumors of the central nervous system. Approximately 75% of HBs are sporadic, while 25% are associated with von Hippel–Lindau (VHL) disease. HBs consist of two main components: a rich capillary network composed of vascular endothelia and pericytes, within large vacuolated stromal cells, which
harbor the genetic defect. The mechanism by which the VHL gene product (pVHL) causes HB is not completely clear. Wild-type pVHL is involved in the response to hypoxia, targeting HIF-α for ubiquitination and degradation in the presence of oxygen, but not under hypoxic conditions. Thus, it is postulated that mutated pVHL stabilizes HIF-1α even under normoxic conditions, resulting in upregulation of cellular proliferative and angiogenic genes that promote tumorigenesis. In addition to VHL mutations, a variety of genes and microRNAs that promote angiogenesis and cell proliferation have been implicated in the pathogenesis of HB. To date, no biomarkers for the prediction of HB onset, recurrence, or progression have been identified. We recently proposed the quantification of circulating endothelial cells (CECs) and their progenitors, circulating endothelial progenitors (CEPs), as potential biomarkers for monitoring the presence of HB and its recurrence after surgical resection of the tumor. In this review, we discuss the possible role of these cells in the onset of HB and the technical challenges for their accurate identification and quantification.

1. Introduction

Hemangioblastomas (HBs) are biologically benign, highly vascularized tumors that account for 1%–2.5% of all intracranial tumors and 7%–12% of posterior fossa neoplasms in adults [1]. The majority of HBs evolve as solitary sporadic lesions. However, in 25% of cases they are associated with von Hippel–Lindau (VHL) disease (OMIM 193300), an autosomal dominant multi-organ neoplastic syndrome, with complete penetrance, and variable expression, caused by mutations in the tumor suppressor VHL gene [2, 3]. Affected patients may develop visceral tumors, such as clear cell renal carcinomas, pheochromocytomas, renal and pancreatic cysts, neuroendocrine tumors, epididymal cystadenomas, and ovarian cysts, and central nervous system (CNS) malignancies, such as cerebellar, brainstem and spinal hemangioblastomas, retinal angiomatosis, and endolymphatic sac tumors. The disease has a prevalence of 2–3 per 100 000 and an estimated incidence of between 1 in 36 000 and 1 in 52 000 live births [4, 5]. All mutation carriers usually develop clinical features by 65 years of age [6]. Although VHL disease was recognized as a specific syndrome in the early 1900s [7-9], it was not until 1993 that Latif identified the VHL gene on the short arm of chromosome 3 (3p25-26) [10]. The VHL gene encodes the VHL protein, which is mainly responsible for hypoxia-inducible factor-1 alpha (HIF-1α) degradation under normoxic conditions.

The symptoms of HBs depend on the location of the tumor. Cerebellar HBs more commonly present with headache, ataxia, nausea, vomit, dysmetria, and hydrocephalus. Brainstem HBs cause dysarthria, dysphagia, bradycardia, dyspnea, anorexia, and hiccups. Hyposthenia, hypo- or paresthesia, and pain are typical symptoms of spinal cord HBs [11]. Magnetic resonance imaging is the best diagnostic technique, through which HBs are visualized as a contrast-enhancing mass, eventually associated with a cyst or syrinx. In VHL disease, surgery is reserved to those patients who suffer symptoms, or in cases of HBs showing progressive growth in a potentially dangerous location. If the tumor is completely removed, there are usually no recurrences. However, new lesions may occur in VHL patients due to the natural history of the disease.
Although many studies have elucidated the details of the molecular pathways involved in the development of HBs in patients affected by VHL, the genetic bases of sporadic HBs are still largely unclear, and new hypotheses have also been proposed for VHL-related HBs.

2. Hemangioblastomas and Their Genetic Basis

The genetic basis of HBs has been partially clarified. VHL-related HBs are thought to be caused by the combination of a first, inherited mutation in the tumor suppressor VHL gene and a second, acquired mutation, according to Knudson’s two-hit hypothesis. The VHL tumor suppressor gene is located on the short arm of chromosome 3 (3p25-26), and is expressed in several tissues both inside and outside of the CNS [12]. The VHL gene coding sequence, consisting of approximately 14500 base pairs, is formed by three exons, each comprising 712 nucleotides [13]. Two forms of VHL mRNA exist, differing in the presence or absence of exon 2, thus encoding two distinct VHL proteins, both of which function as active oncosuppressors [14]. Germ-line and sporadic mutations of the VHL gene involve all three exons, and encompass missense mutations along with non-sense mutations, micro-deletions/insertions, splice site mutations and large deletions [12]. Some studies also suggest that hypermethylation of the promoter region of VHL is a possible epigenetic mechanism of protein inactivation [15, 16]. Hypermethylation can occur in normally unmethylated sites that are rich in 5’-CG-3’ sequences known as CpG islands.

The role of the VHL gene in the development of sporadic HBs has been debated, with several studies showing that VHL mutation does not cause sporadic HBs, while others indicate the involvement of the VHL gene [17, 18]. However, recent observations have confirmed that sporadic HBs may also be associated with cryptic VHL inactivation [19].

In addition to the VHL gene, other genetic alterations have also been implicated in the tumorigenesis of HBs. Comparative genomic hybridization studies showed that DNA losses at 6q are frequent alterations in HBs [20]. Other studies confirmed that loss of heterozygosity (LOH) occurs concurrently at 6q and 3p in almost 70% of cases, suggesting that a tumor suppressor gene, namely ZAC1, located at 6q is involved in the development of HB [21, 22]. The ZAC1 gene, which encodes an inducer of cell cycle arrest and apoptosis, is characterized by LOH in a significant number of sporadic HBs [20]. Moreover, in 90% of analyzed sporadic HBs cases, the ZAC1 gene promoter is hypermethylated.

3. Molecular and Cellular Features of Hemangioblastomas

As stated previously, HBs are benign CNS tumors occurring either sporadically or associated with VHL syndrome. It has been shown that mutations in the VHL gene leading to loss of its functions are involved in the pathogenesis of HBs in familial [23] as well as most sporadic cases [19].

Although genetic defects in VHL predispose individuals to HBs, the mechanisms by which the VHL gene product (pVHL) causes neoplastic transformation have not been fully elucidated. In the presence of oxygen, the wild-type pVHL degrades HIF-α by ubiquitination. In contrast, under hypoxic conditions, the wild-type pVHL does not recognize and bind to HIF-1α, thus preventing its degradation. Similarly, it is postulated that mutated pVHL stabilizes HIF-α, resulting in downstream upregulation of cellular proliferative and angiogenic genes, such as vascular endothelial growth factor and platelet-derived growth factor, which promote tumorigenesis [24]. Moreover, in
addition to VHL mutations, a variety of genes and microRNAs (miRNAs) have been implicated in the pathogenesis of HBs, all of them being functionally involved in cell proliferation and angiogenesis promoting pathways [25].

Histologically, HBs consist of two main components: a rich capillary network (composed of vascular endothelia and pericytes) within large vacuolated stromal cells, which harbor the genetic defect [12]. However, their cytological origin is uncertain and controversial. It has been hypothesized that the stromal cells are derived from embryonic, developmentally arrested hemangioblasts [26], which are multipotent common precursors of hematopoietic and endothelial cells [27]. More recently, a subpopulation of stage-specific embryonic antigen-1 positive cells has been detected. These cells are characterized by the capability of differentiating into stromal-like cells and vascular cells in the presence of the specific HB niche, and deemed to be the tumor-initiating cells in both sporadic and familial HBs [28]. Concerning vascular cells, it has been suggested that they undergo intensive reactive angiogenesis within the pseudo-hypoxic (pVHL-deficient) environment of the tumor [29]. However, de novo tumor-derived vasculogenesis has been reported, corroborating the hypothesis that hemangioblasts represent the neoplastic cells from which HBs originate [29].

4. Circulating Endothelial Cells and Their Progenitors

Circulating endothelial cells (CECs) and their progenitors, circulating endothelial progenitors (CEPs), are non-hematopoietic cells present in the blood. They are characterized by different origins: indeed, CECs arise from the mature endothelium of the vessel wall in response to a damaging stimulus, whereas CEPs are derived from the bone marrow and participate in vascular repair and homeostasis [30].

Phenotypically, there is a lack of consensus about the surface markers that unambiguously distinguish CECs from CEPs, mainly because they cannot be characterized by a single marker [31]. Thus, a combination of markers is required. Several markers occur on both cell types, including CD34, CD31, CD146 and CD309, while CD133 is the sole antigen that seems to be expressed on CEPs alone and is subsequently lost in mature CECs [32]. Accordingly, CECs and CEPs are usually defined by the expression of endothelial markers (such as CD34, CD31 and CD146) together with the absence of the pan-hematopoietic marker CD45 within viable, nucleated blood cells [33]; the addition of the progenitor marker CD133 allows discrimination between the two populations. Other properties that differentiate CECs from CEPs are the ability of CEPs to form colonies in vitro with high proliferative potential, and the uptake of acetylated low-density lipoproteins [30].

In healthy individuals, detection of CECs and CEPs in the peripheral blood is a rare event because renewal of the endothelial layer takes place continuously, but at a low replication rate of 0.6–1% per day [34]. Thus, one of the major challenges to the quantification of these cells is represented by their low frequency in the blood. The recent, extraordinary advances and standardization of the technologies available for identifying, quantifying and characterizing rare populations of circulating cells [35, 36] have made the detection of CECs and CEPs possible. In particular, immunomagnetic isolation and fluorescence-activated cell sorting have been successfully employed for cell isolation, while flow cytometry has allowed multiparametric analysis [34].
Although rarely found in healthy individuals, increased levels of CECs and CEPs have been detected in patients affected by several pathological conditions, encompassing cardiovascular, autoimmune and neoplastic disorders, and correlate with disease severity [37-41]. These observations suggest that CEC and CEP numbers could be used in clinical practice as non-invasive, blood-based markers for detecting a variety of diseases and monitoring their clinical course, although the achievement of a consensus on the value of the identification and enumeration of CECs and CEPs for this purpose remains to be established.

5. Endothelial Cells in Hemangioblastomas: A Possible Predictive Role?

Although benign, HBs can be a significant source of neurological morbidity or even mortality following intratumoral hemorrhage or cystic expansion of the tumor. Surgical resection of symptomatic or progressing lesions is the preferred treatment modality and can be curative, although observation is reasonable for asymptomatic lesions with minimal growth [24]. Unfortunately, no biomarkers have yet been identified to predict the onset, recurrence, or progression of HB.

Although the studies supporting CEC and CEP enumeration and monitoring as candidate biomarkers are limited, the data obtained so far are promising. Bhatt et al. investigated mature CECs and CEPs, along with their ratio, in renal cell carcinoma (RCC), a condition often associated with VHL disease other than HBs [42]. A significant increase in CEPs was observed either in patients with VHL disease and RCC or in those with sporadic RCC compared to patients with VHL disease without RCC and healthy. Moreover, CEPs decreased after surgery in patients with non-metastatic sporadic RCC. Overall, these observations paved the way for the use of CEP counts as a monitoring strategy for patients with VHL syndrome [42].

In addition, more recently, we implemented a flow cytometric method for accurately quantifying CECs and CEPs in the peripheral blood of a cohort of nine patients with HB (8 VHL-affected, 1 with sporadic HB), before and after surgery, in order to assess their use as direct markers of the presence of the tumor [43]. We took advantage of a high-speed, acoustic focusing flow cytometry technique to detect staining of a panel of markers encompassing CD276, a tumor endothelial marker that characterizes a subset of CECs arising specifically from the tumor endothelium [44-46]. A higher percentage of CEPs was detected in HB patients before surgical resection of the tumor in comparison to that in healthy individuals, and the level decreased in HB patients one month after surgery, corroborating the hypothesis that baseline CEP percentage and kinetics could be a valid tool for monitoring HB onset and recurrence [43].

It is well-established that co-treatment with antiangiogenic drugs prevents the rapid mobilization of pro-angiogenic bone marrow-derived CEPs, and their subsequent tumor colonization [47, 48]. Thus, the aforementioned findings could have important clinical implications with the potential to be translated from the bench to clinical practice. However, treatment with antiangiogenic agents could importantly limit the use of CEPs as a reliable marker in surgically non-resectable HBs.

6. Concluding Remarks and Future Perspectives

Most of the studies aimed at clarifying the role of genetics in the tumorigenesis of HBs have focused on the VHL gene. Its biallelic inactivation is the cause of the development of the tumor in
patients affected by VHL disease. Although it is now accepted that the VHL gene also has a role in sporadic cases, and recent studies indicate crucial contributions of other genes in the tumorigenesis of sporadic HBs. These alterations affect several pathways downstream of the VHL protein and are involved in neovascular formation.

Moreover, recent studies showed a correlation between the presence of HBs and CEP levels, indicating a possible involvement of CEPs in the formation of HBs and a potential new predictive marker. We propose that these cells can be used as non-invasive, blood-based predictors by employing high-sensitivity flow cytometric techniques, which will overcome challenge presented by the rarity of CEPs in the peripheral blood and the need for multiparametric analysis to unambiguously identify these cells. Confirmation of the predictive role of CEPs for monitoring HBs could pave the way to the establishment of a diagnostic tool useful for neurosurgeons involved in the treatment and cure of HB.

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Author Contributions

E.B. and A.F. conducted on line search and critical analysis of the current literature on the topic; A.D.G., L.G. and S.D.B. contributed to the writing of sections 1, 4 and 5; G.P. contributed to the writing of section 1 and 2; E.B., A.F., A.V.M., M.N., A.C. and M.P. wrote and revised the manuscript.

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Magnetic Resonance Imaging Approaches for Predicting the Response to Hyperoxic Radiotherapy in Glioma-Bearing Rats

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Abstract

Background: Despite important advances in multimodal therapeutic options, glioblastoma (GBM), the most frequent and aggressive form of all astrocytomas, remains with a median overall survival period of 15 months. A direct correlation between GBM hypoxia and higher aggressiveness, poor prognosis and greater resistance to different treatments has been established. However, because of intratumoral and interindividual heterogeneity, it has not been possible to assess accurately the hypoxia degree from physiopathological parameters or neuroimaging methods. This study aims to develop and evaluate a magnetic resonance imaging (MRI) approach to identify more precisely those tumors that could improve the outcome through an oxygen targeted therapy.

Methods: To assess the efficacy of radiotherapy in animals irradiated under air and oxygen breathing, we implemented a GBM animal model obtained by intracranial injection of glioma C6 cells in rats. MRI studies, based on the oxygen-induced contrast in blood (BOLD) and
tissues (TOLD), were carried out to evaluate the effect of the modulation in oxygen breathing conditions on the tumors in vivo. The efficacy of the oxygen breathing therapies was determined by the relative tumor volume at the end of the experiment, compared to its size on the day before the treatment.

**Results:** Our results categorized the tumors in responding, non-responding and intermediate behaviors. While BOLD analysis did not show any statistical difference between animals, either breathing air or oxygen, TOLD parameters allowed for the identification of the tumors with higher responses to hyperoxygenic radiotherapy.

**Conclusions:** The non-invasive oxygen enhanced MRI acquisitions proposed here show promising potential to identify those tumors that would generally improve their response to a hypoxia targeted treatment.

**Keywords**
Glioblastoma multiforme; magnetic resonance imaging; TOLD contrast; BOLD contrast; hypoxia; radiotherapy; oxygen modulation; animal model

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**1. Introduction**

Despite decades of intensive research in different fields, high-grade gliomas (WHO grade III and IV) are still considered incurable, with very unfavorable prognosis [1]. With current medical managements, these malignant brain tumors show mean survival times after diagnosis of 2 to 3 years for anaplastic astrocytoma, and around 15 months for glioblastoma multiforme (GBM) [2]. GBM is, in fact, the most aggressive and lethal intracranial tumor, depicting the highest morbidity and mortality outcomes, notwithstanding its relatively low incidence. Typical treatments of GBM involve surgery followed by chemotherapy and radiation therapy [3], but the 5 years survival prediction remains less than 3% [4]. One of the possible explanations for this lack of treatment success relies in the low availability of oxygen in certain tumor regions [5].

Hypoxia, or low oxygen tension (pO$_2$), is an important characteristic of advanced solid tumors. It results from an imbalance between oxygen consumption and supply, and has been long recognized as an important determinant of tumor aggressiveness, patient outcome and resistance to different therapeutic interventions including, chemotherapy, radiotherapy and surgical tumor resection [6]. Whereas hypoxia is deleterious to most normal cells causing their death, neoplastic cells can develop adaptive mechanisms to survive under conditions of oxygen limitation [7]. In GBM patients, the hypoxic core, or local hypoxic foci, are highly resistant to different treatments and will induce, most likely, tumor relapse [8]. Low oxygen tension is also linked to treatment resistance at different levels [7]. As a result, several strategies have been previously proposed to alleviate tumor hypoxia and potentiate treatment, including hyperoxic/hyperbaric gas breathing, increasing the ability of blood to carry oxygen (transfusions, administration of erythropoietin), the use of artificial oxygen carriers (perfluorocarbons), hypoxic cell-selective cytotoxins, intensity modulated radiation therapy and gene therapies [7, 9-11]. However, despite promising perspectives in preclinical studies, the new therapeutic interventions delivered only marginal benefits at the clinical level. In particular, the Overgaard meta-analysis [12] concluded that this
lack of efficacy was most likely due to the current inabilities to accurately measure tumor hypoxia in vivo, and stratifying adequately those patients that would, most likely, benefit from hypoxia targeted interventions.

Consequently, considerable efforts have been devoted to develop in vivo oxygen measurement techniques [9, 13, 14]. But despite the different approaches investigated, there is not currently a technique that fulfills all the desired requirements, and consequently, none of them has been widely adopted in practice [15]. Besides, numerous studies reported high variability of intra- and inter-tumor oxygen tension even within patients with the same tumor type [9, 16, 17], stressing the importance of imaging tumor hypoxia for each patient individually. In the past few years, oxygen enhanced MRI (OE-MRI) has been proposed as an interesting alternative to evaluate the tumoral pO\textsubscript{2} in vivo, since it is completely non-invasive, makes use of an endogenous contrast, has adequate temporal and spatial resolution and is easy to implement in the current clinical MRI routines [18]. As a main drawback, it does not provide quantitative pO\textsubscript{2} values, although several studies reported a direct link between OE-MRI derived parameters and changes in pO\textsubscript{2} [19, 20]. OE-MRI takes advantage of two contrast mechanisms directly influenced by oxygenation status: Blood Oxygen Level Dependent (BOLD) and Tissue Oxygen Level Dependent (TOLD). Both BOLD and TOLD measurements, based on changes in signal intensity (SI), coupled with hyperoxic or hyperbaric gas challenge have been tested in preclinical settings [21-23] and in volunteer human patients [24, 25]. Here, we hypothesized that these techniques could provide additionally valuable information to assess tumor response to therapeutic interventions modulating hypoxia levels.

On these grounds, the present study reports on the influence of oxygenation in tumor response to radiotherapy using an orthotopic model of glioblastoma multiforme (GBM) in rats. We explore the use of OE-MRI (BOLD and TOLD) to identify those specific tumors that will benefit from a hypoxia targeted therapy (breathing pure oxygen), designed generally to improve treatment responses.

2. Materials and Methods

The experimental procedures were approved by the highest institutional ethical committee (Community of Madrid) and met the national (R.D. 53/2013) and the European Community guidelines (2010/62/UE) for care and management of experimental animals. Animals were housed in the animal premises of our institution (Reg. No. ES280790000188) and cared by specialized personnel.

2.1 Animal Handling

Male Wistar rats, of body weight (b.w.) 230 ± 20 g, were used. Animals were kept in cages in a controlled room with a 12-h cycle of light and darkness, temperature of 22°C ± 2°C and water and food access ad libitum. The study comprised 19 C6 glioma-bearing animals, divided into two groups treated with radiotherapy: 9 rats irradiated under normoxic conditions (air breathing, group 1) and 10 rats irradiated in hyperoxia (100% oxygen breathing, group 2).
2.2 Tumor Implantation

Authenticated rat glioma C6 cells were obtained from the American Type Culture Collection (ATCC number CCL-107, Manassas, VA, USA). Cells were grown in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with HEPES, 10% fetal bovine serum (FBS) and antibiotics. Briefly, animals were anaesthetized intraperitoneally (i.p.) with a mixture of ketamine/mendetomidine (75/0.5 mg/kg) and placed in a stereotaxic holder. Then, $10^5$ C6 cells in 10 μl of medium were injected in the right caudate nucleus, through a small craniotomy. After surgery, rats were induced to recover with atipamezole (5 mg/kg) subcutaneously (s.c.) administrated. They also received s.c. meloxicam (0.5 mg/kg) as analgesia during the following 3 days [26].

2.3 MRI Studies

2.3.1 General

In vivo MRI experiments were performed on a Bruker AVANCE III system (Bruker Medical GmbH®, Ettlingen, Germany) using a 7.0-T horizontal superconducting magnet, equipped with a gradient insert (60 mm inner diameter) with a maximum intensity of 360 mT/m and a $^1$H selective birdcage resonator (38 mm inner diameter). Anesthesia was initiated in an induction box through the inhalation of a mixture containing 3%-4% of isoflurane in air (1 L/min), and maintained during the experiment through a nose mask delivering 1%-1.5% Isoflurane in air (or oxygen). Animals were then placed in a heated probe, which maintained the core body temperature at approximately 37°C. The physiological status of the animals was monitored by a gating system designed for small animals (SA Instruments, Inc., Stony Brook, NY, USA) providing respiratory rate and body temperature through the imaging experiment.

2.3.2 Anatomical Imaging

For assessing the tumor growth, T2-weighted (T2W) spin-echo (SE) images were acquired with a rapid acquisition with relaxation enhancement (RARE) sequence and the following parameters: repetition time (TR) = 3000 ms, echo time (TE) = 60 ms, averages (Av) = 3, RARE factor = 8, acquisition matrix (Mtx) = 256 × 256, in-plane resolution of 136 × 136 μm$^2$, slice thickness = 1.5 mm and 10 slices in coronal orientation (total acquisition time of 3.36 min). Besides, T2W images were acquired as reference at the end of the complete MRI study.

2.3.3 BOLD Experiments

T2*-weighted (T2*W) images were acquired using a FLASH sequence and the following parameters: TR = 300 ms, TE = 20 ms, Av = 1, Mtx = 128 × 128, in-plane resolution of 234 × 234 μm$^2$, slice thickness = 1.0 mm and 3 slices in axial orientation (total acquisition time of 38.4 s).

2.3.4 TOLD Experiments

T1-weighted (T1W) FLASH images were acquired using TR = 30.5 ms, TE = 5 ms, Av = 1, and the same geometrical parameters as T2*W images for BOLD studies (total acquisition time of 3.9 s).
2.3.5 Contrast Enhanced (CE) Studies

CE-T1W images were acquired after i.p. administration of 0.2 M Gd-diethylenetriaminepentaacetic acid (Magnevist®, Bayer, Whippany NJ, USA) at a dose of 0.25 mmol/kg b.w. Images were obtained with a SE sequence using TR = 500 ms, TE = 10.6 ms, Av = 2, Mtx = 256 × 256, in-plane resolution of 136 × 136 μm², slice thickness = 1.5 mm and 10 slices in coronal orientation (total acquisition time of 3.12 min).

2.3.6 Overall Description of the Experiment Design

Tumor growth follow-up was carried out by acquiring T2W and CE-T1W images, from the day 7-9 after the implantation to the day 38-40, or until the animal experiment reached the endpoint according to ethical procedures. OE-MRI experiments were performed the day before the irradiation as previously described [21]. Briefly, the animals were positioned and anatomical images were acquired in the coronal plane. These images were used to position the axial BOLD and TOLD images so the middle slice goes through the higher section of the tumor. Five alternated BOLD and TOLD studies were performed while the animal was breathing air and these were used as a baseline. Immediately after, the breathing gas was changed to 100% oxygen (group 2, n = 10 animals), or remained as air (group 1, n = 9 animals), and fifteen alternated BOLD and TOLD sequences were acquired. At the end of the experiment, T2W anatomical images with the same geometry as BOLD and TOLD were obtained.

2.4 Radiotherapy

At day 19-21 after C6 cells implantation, animals were irradiated during 11 minutes -either under normoxic (group 1) or hyperoxic conditions (group 2)- by selecting a dose of 10 Gy in a biological irradiator (Shepherd Mark J-30 800 Ci 137Cs). Briefly, the glioma-bearing rats were anesthetized by injecting a mixture of ketamine/medetomidine (75/0.5 mg/kg, i.p. injected) prior to the irradiation. When rats showed proper anesthesia level, they were covered with a homemade lead tube, leaving the head uncovered, thus allowing the radiation reach the tumor. The animals were then placed in the irradiator system over the spinning platform. In order to assure hyperoxia for group 2, once the rat was in the irradiation chamber, the air was displaced by flowing pure oxygen at 5 bar for 15 minutes prior to the irradiation, and this oxygen level was maintained during the whole irradiation procedure.

2.5 Data Analysis

2.5.1 Tumor Volume Analysis

Images were analyzed with Image J software (NIH, USA) [27] and the data obtained treated with Excel software (Microsoft, USA). The volume of the tumors was calculated from anatomical CE-T1W images. In order to normalize tumor response to the therapy, the tumor volume measured the day before the irradiation was chosen as the basal size (value of 0) from which we determined the GBM evolution. Any positive change reflects tumor growth whereas negative change depicts tumor shrink. The relative tumor volume at the end of the experiment (RTVf) was selected for the purposes of analysis.
2.5.2 OE-MRI Analysis

To assess the GBM response to oxygen modulation, we evaluated in each animal the relative changes of TOLD and BOLD signals in the tumor related to the contralateral hemisphere, considered as healthy tissue. These two regions of interest (ROIs), of approximately the same size, were manually selected and delineated in three adjacent slices. Briefly, the signal intensity (SI) of the 5 first images (while the animal was breathing air) from T1W (TOLD contrast) or T2*W acquisitions (BOLD contrast), were averaged in order to obtain the intensity baseline. Normalized changes in SI for each ROI were calculated according to Equation 1:

$$\Delta SI(\%)_i = \frac{SI_i}{\left(\sum_{i=1}^{5} S_{i}/5\right)} \times 100 - 100$$  \hspace{1cm} [Eq. 1]

where $i$ is the image number, and $S_{i}$ the averaged signal intensity of the ROI in that image.

The TOLD signal in the tumor (T) or in the contralateral hemisphere (C) was calculated as the mean changes of $\Delta SI$ during oxygen breathing according to the Equation 2:

$$TOLD_{T or C}(\%) = \frac{\sum_{i=1}^{15} \Delta SI(\%)_i}{15}$$  \hspace{1cm} [Eq. 2]

Finally, the relative changes of TOLD signal in the tumor, related to the changes in the contralateral hemisphere, were obtained using Equation 3:

$$\Delta TOLD(\%) = \frac{TOLD_T - TOLD_C}{TOLD_T} \times 100$$ \hspace{1cm} [Eq. 3]

Equivalent equations 2 and 3 were applied for assessing the BOLD studies.

2.5.3 Statistical Analysis

Statistical analyses were performed using GraphPad Prism 4 software (GraphPad Software, Inc., San Diego, CA, USA). The differences between values were determined by unpaired two tailed Student’s t-tests analysis with Welch's correction. Correlation between parameters was assessed using two tailed Pearson correlation coefficients ($R$). In all cases, P values lower than 0.05 were considered to be statistically significant.

3. Results

3.1 Oxygen-Enhanced Magnetic Resonance Imaging

The response to the hyperoxic gas challenge was heterogeneous, but TOLD and BOLD changes within each animal studied were highly consistent. Figure 1 illustrates the obtained results with a representative tumor. The $\Delta SI$ maps, measuring signal intensity change in T1W or T2*W images, depict regions with positive (orange) and negative (blue) changes for BOLD (upper panels) and TOLD (lower panels) images during air (Figure 1A & D) and oxygen (Figure 1B & E) breathing, whereas regions of no change are shown in black.
Figure 1 Oxygen-Enhanced MRI. Response maps, as ΔSI, in normalized T2*W (A, B) and T1W (D, E) OE-MRI studies, are overlaid on T2W-SE images of a representative GBM animal. Brain images were acquired while breathing air (A, D) and oxygen (B, E). Tumor is enclosed in a red line. Color-based scale bar shows percentage change in ΔSI. Graphics C and F show the mean BOLD (C) and TOLD (D) responses measured in three adjacent image slices on tumor (filled circles) and contralateral healthy tissue (empty circles). Data are represented as the mean value ± standard error of the mean (SEM).

Mean values were stable during air breathing (Figure 1C for BOLD, and 1F for TOLD), with standard errors ranging from 1 to 4% for BOLD and 1 to 5% for TOLD. When the breathing gas was changed to oxygen, the maps showed an overall increase of the ΔSI values, although areas of negative change become also detectable (Figure 1B and 1E for BOLD and TOLD respectively). BOLD signal in the tumor increased very quickly after oxygen insult (10%), reaching a plateau (15%) at 1.5 min and remaining constant until the end of the experiment. The contralateral healthy hemisphere showed higher variability with time, depicting a cyclic response to oxygen that is attenuated with time until reaching a stable value of around a 2% increase with respect to the baseline. TOLD responses followed a similar pattern but depicted smaller change in ΔSI (7%), as was expected.

In order to evaluate the potential influence of the GBM size in the OE-MRI derived parameters, we investigated whether BOLD and/or TOLD signals in the tumor were driven by its volume the day before the irradiation, when the OE-MRI studies were carried out. Although both BOLD (Figure 2A) and TOLD (Figure 2B) signals showed a tendency to increase with the tumor size, this is not statistically significant (R = 0.336, P = 0.159 for BOLD and R = 0.078, P = 0.75 for TOLD). The same
analysis but using the ΔBOLD (Figure 2C) or ΔTOLD (Figure 2D) instead, yielded similar patterns, not showing significant relationship with tumor size either ($R = 0.241$, $P = 0.321$ for ΔBOLD, and $R = 0.38$, $P = 0.11$ for ΔTOLD).

![Graph A](https://via.placeholder.com/150)

![Graph B](https://via.placeholder.com/150)

![Graph C](https://via.placeholder.com/150)

![Graph D](https://via.placeholder.com/150)

**Figure 2** Relationship between OE-MRI data and the tumor volume. Graphics show the correlation analysis of OE-MRI parameters versus the tumor size the day before the irradiation. Panels A and B show the results for BOLD and TOLD measurements in the tumor; C and D present the ΔBOLD and ΔTOLD data, respectively.

We also assessed the possible correlation between BOLD and TOLD changes. As shown in Figure 3A, tumor BOLD and TOLD responses are significantly matched, with a trend of higher TOLD being associated with larger BOLD ($R = 0.614$, $P = 0.005$). Nevertheless, this relationship is lost when the changes are tested relative to the contralateral hemisphere (Figure 3B): ΔBOLD vs ΔTOLD ($R = -0.035$, $P = 0.886$).
Figure 3 Relationship between BOLD and TOLD responses. Correlation analysis between BOLD and TOLD signals measured in the tumor from OE-MRI studies (A), and between ΔBOLD and ΔTOLD changes from the same experiment (B).

3.2 Radiation Response

Table 1 presents some of the relevant data of the study, including the tumor volume the day before the irradiation, the relative tumor volume at the end of the experiment, and the parameters obtained from the OE-MRI acquisitions.

Taking RTVf as a measure of therapy efficacy, we categorized the tumors in three different groups: animals with a clear positive response in which the glioma volume notably decreased (RTVf < -50%), rats that did not respond to the radiation whose tumor size at least doubled in size (RTVf > 100%), and subjects with an intermediate behavior that could not be clearly included, at least at the end time of the study, in either of the two previous groups (-50% < RTVf < 100%). Under this assumption, in the cohort of animals irradiated in hyperoxic condition, 4 of them were responding (11, 12, 14 and 18), 4 did not respond (10, 13, 16 and 17) and 2 presented an intermediate behavior (15 and 19); while in the group treated under normoxia, only 2 responded (3 and 4), 4 were non-responding (1, 5, 6 and 7) and 3 were identified with the intermediate behavior (2, 8 and 9). Analyzing the data, there were no statistical differences between group 1 (rats 1-9) and 2 (rats 10-19) before irradiation, either in tumor volume or any of the OE-MRI measured parameters. Nevertheless, we found a statistically significant difference in the ΔTOLD values between responding and non-responding animals that were irradiated under pure oxygen breathing (P = 0.004). Interestingly, this did not happen for animals irradiated in normoxic conditions, or when BOLD related parameters were tested.
Table 1 GBM tumor characteristics and OE-MRI parameters.

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<th>RTVf\textsuperscript{b}</th>
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BC: Breathing condition
TV-pre: Tumor volume the day before the irradiation (pre-treatment)
a: Data are presented as mm\textsuperscript{3}
RTVf: Relative tumor volume at the end of the experiment
b: Data are presented as percentage (%)
T: Tumor

Figure 4 shows anatomical CE-T1W acquisitions of two representative animals at several time points before and after the irradiation. Images A-D correspond to a non-responding animal treated under normoxic conditions whereas images E-H represent a responding animal irradiated under oxygen breathing.
Figure 4 Representative CW-T1W MRI of tumor evolution, before (12 and 3 days) and after (9 and 18 days) the irradiation. Top images correspond to a GBM bearing animal that did not respond to irradiation under normoxic conditions (A-D); while the bottom ones (E-H), correspond to an animal that did respond to irradiation in hyperoxia.

Finally, we pursued to evaluate the correlations between tumor response to treatment, as measured by the RTVf, and OE-MRI parameters. When ΔBOLD response was tested (Figure 5A), no correlation was found for any of the two groups ($R = -0.044$, $P = 0.91$ for air and $R = 0.24$, $P = 0.5$ for oxygen breathing animals respectively). This was also the case for ΔTOLD response in animals irradiated during air breathing (Figure 5B, $R = 0.401$, $P = 0.28$).

Nevertheless, a strong negative correlation was found between ΔTOLD and RTVf for animals breathing oxygen during irradiation, with $R = -0.846$ and $P = 0.002$. Within the cohort of tumors irradiated during oxygen breathing, all the responding animals present a ΔTOLD > 40%, whereas all the non-responding in the same group showed a ΔTOLD < 40%. Those tumors with an intermediated behavior showed mixed values for ΔTOLD (48 and 8%) despite showing similar responses to irradiation (RTVf = 41 and 55 % respectively, corresponding to tumors 15 and 19 in Table 1).

As we found animals responding to irradiation in both normoxic and hyperoxic groups, we evaluated whether OE-MRI could be a good predictor of the tumor response to radiotherapy irrespective of the breathing conditions during the treatment. If we put all the tumors together, we find no correlation between any of the OE-MRI parameters (BOLD, TOLD, ΔBOLD, ΔTOLD) and the relative volume at the end of the study (data not shown).
Figure 5 Correlations between OE-MRI parameters and the relative tumor volume at the end of the experiment. A: No correlation was found when ΔBOLD was tested, either for tumors irradiated under normoxia (blue points) or hyperoxia (red points). B: A strong correlation was observed for ΔTOLD (%) in animals that inhaled pure oxygen during irradiation ($R = -0.846$, $P = 0.002$), but not in those treated under air breathing ($R = 0.401$, $P = 0.28$).

4. Discussion

It is widely accepted that tumor hypoxia plays a central role in therapy failure and poor patient outcome [15, 28, 29]. This is especially important in radiotherapy, with several studies linking its success to tumor oxygenation [30, 31]. Consequently, numerous strategies have been developed over the years to modulate tumor pO$_2$ to improve therapy efficacy. Among them, hyperoxic or hyperbaric gas breathing [7] have been a very popular choice due to its easy application [22], low cost and minimal impact in patient health [32]. Several studies have proved the suitability of this strategy with different tumor types, including prostate [33], liver [34], breast [35], cervix [36, 37] and brain [25, 38]. However, despite the success achieved in pre-clinical settings, the expectations became much attenuated when translated to the clinic [7, 39]. One of the possible reasons for this
circumstance, is the lack of a proper method to identify those patients that would benefit from a hypoxia directed intervention [12, 13, 15], as neither, the tumor oxygenation nor the response to hypoxia directed therapies could be inferred from histopathological or conventional imaging studies. This situation emphasized the need to measure tumor pO₂ and/or response to hypoxia directed therapies on a patient by patient personalized manner.

Oxygen enhanced MRI provides a convenient methodology to measure tumor response to hypoxia targeted interventions, as it is sensitive to changes in oxygenation, does not require an external contrast agent nor ionizing radiation, it is non-invasive and uses currently available MRI technology. All these circumstances, promise to facilitate translation to the clinical environment and its combination with additional MRI techniques yielding multiparametric information. OE-MRI is based on the different magnetic properties of hemoglobin in vessels, and molecular oxygen in tissues. Hemoglobin is diamagnetic in its oxygenated form whereas paramagnetic when unbound to molecular oxygen. The ratio oxy- to deoxy-hemoglobin influences the apparent transverse relaxation rate, which is the basis for the BOLD contrast. BOLD has been used in many functional MRI studies as a surrogated marker of neuronal activation [9] and more recently, to investigate the response of tumors to hyperoxic gas challenges [19, 40]. Several studies have recently taken advantage of the weak paramagnetic properties of molecular oxygen in aqueous solvents, because of its two unpaired electrons. Due to this circumstance, dissolved molecular oxygen will decrease the longitudinal relaxation time of tissue water. Unlike with hemoglobin, this relationship is nearly linear at physiological conditions [41], and this is the basis of TOLD contrast [21]. Several studies have shown the feasibility of this approach to assess changes in tumor oxygenation coupled with the modulation breathing atmospheres [21, 42].

On these grounds, the present study aimed to assess the potential prognostic utility of OE-MRI as an in vivo and non-invasive imaging strategy to predict the tumor radiotherapy response in hyperoxic conditions. A GBM model generated by C6 glioma cell intracranial injection was selected due to its favorable characteristics: it is hypoxic (pO₂ ≈ 12-14 mm Hg) but still responsive to pO₂ modulation by hyperoxic gas breathing [43]; reacts to radiation; presents a high grade of heterogeneity [44], which more accurately reflects the clinical situation; and grows orthotopically in a non-homogenous pattern between individuals, increasing malignancy as it develops [45]. We found that our model presents a high-grade of variability in response to radiotherapy, irrespective of the tumor size or the conditions used during treatment (Table 1, Figure 4 & 5). To improve tumor oxygenation, we employed pure oxygen breathing. Finally, we selected radiotherapy since its outcome can be improved by increasing tumor oxygenation [20, 46].

The goal of any applied therapy is to achieve the maximum treatment response in the affected tissue while minimizing damage to the surrounding normal tissue. In order to monitor this, we focused here on those parameters that best depict the difference in oxygenation status between tumor and contralateral brain, instead of relying merely on the glioma response. On these grounds, we chose ΔBOLD or ΔTOLD as our reference parameters, since they reflect the above-mentioned difference between cancerous and healthy tissues. ΔSI maps during air breathing (Figure 1 A & D) showed fluctuation in SI during baseline conditions both in healthy and in tumor tissue. This fact reflects the normal tissue fluctuation in oxygenation status, and it is the basis for resting state functional MRI [47]. Besides, it is well established that tumor volume is not a good predictor of either tumor oxygenation or treatment outcome [48], and it has little or no influence on OE-MRI studies [41]. Since, to the best of our knowledge, no previous information was available on OE-
MRI in orthotopic C6 GBM model, we investigated the influence of tumor size on BOLD and TOLD responses (Figure 2). Although there is a tendency towards higher BOLD and TOLD responses with higher tumor volume, these are not statistically significant. We also confirmed that tumor size before the treatment is not a good predictor of the therapy outcome, as measured by the RTVf. It does not matter whether we consider all the tumors together, we separated them based on the irradiation conditions, or in its responsiveness, there was not any statistically significant correlation between these two parameters. Nevertheless, we found that $\Delta$TOLD is a parameter that is highly correlated ($R = -0.85, P = 0.002$) with the RTVf in the cohort of animals irradiated under hyperoxic conditions (Figure 5B). Furthermore, $\Delta$TOLD was also significantly different for responding vs non-responding tumors treated under oxygen breathing ($P = 0.004$). No correlation, or significant difference, was found in the analysis of tumors that were irradiated while animals were breathing air.

These results are consistent with those obtained in previous studies using a prostate tumor model (AT1) implanted in the flank. Tumor TOLD response was closely related to tumor growth delay following single [20] or hypofractionated [22] radiotherapy. Sheng Li et al found that T2* has a prognostic value in detecting those patients with cervical squamous carcinoma who have a higher probability of responding positively to chemotherapy [49]. Similar results were reported when assessing BOLD as a predictor of the therapeutic response to chemoradiotherapy in cervical cancer [50]. Jiang et al. [51] also found that large BOLD response correlated with better chemotherapy outcome in patients with locally advanced breast cancer.

There are several limitations to our study. First, due to the high grade of heterogeneity in the tumor response to treatment, the number of animals irradiated in oxygen breathing conditions showing improvement (four) or no responsiveness (four) is too small to establish a strong predictive value. Furthermore, we found that animals depicting intermediate behavior (two) presented mixed responses in $\Delta$TOLD. Thus, more subjects should be added to further assess the predictive potential of this parameter. Second, the therapy was applied at a single time point. It would be also interesting to investigate whether OE-MRI has predictive value at different time points during GBM development. Future studies will consider these and other factors, such as using other parameters to measure therapy success or combining the information provided by OE-MRI with multiparametric MRI studies that can be also be used as surrogate markers of the tumor status.

5. Conclusions

In summary, OE-MRI studies show a promising potential to identify those tumors that would benefit from oxygen breathing during irradiation and, more generally, from any hypoxia targeted therapy. It also reveals profound consequences of the modulation of oxygen content in the breathing gas mixture, for the outcome of therapy. It also reveals that the tumor response cannot be readily predicted from physiopathological parameters or convectional imaging studies.

Author Contributions

All authors contributed equally to the conception and design of the work, data collection, data analysis and interpretation, drafting the article, critical revision of the article and final approval of the version to be published.
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Competing Interests

The authors have declared that no competing interests exist.

References


Correlation of CT and MR Perfusion and Permeability Parameters for Intracranial Tumors

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Abstract

Background: Perfusion imaging, mainly MR perfusion (MRP), is performed frequently for brain tumor imaging. CT perfusion (CTP) is less studied as a method for characterizing brain tumors. The purpose of this study was to demonstrate the usefulness of CTP perfusion parameters in evaluating brain tumors and to compare it with MRP measures in the same patient population.

Methods: Patients underwent CTP and MRP imaging prospectively. Images were processed with vendor-provided and vendor-neutral software. Four regions of interests were placed in whole tumor, solid portion of the tumor, region of maximum perfusion and contralateral normal white matter. Absolute and normalized values of cerebral blood flow (CBF), cerebral blood volume (CBV), and permeability were obtained for both CTP and MRP and compared using correlation and linear regression.

Results: We compared CTP and MRP in 20 patients with intracranial tumors. With vendor-provided software, we found significant correlation for absolute CBV in the region of maximum perfusion ($r^2 = 0.26$, $p = 0.031$) and for normalized CBV ($r^2 = 0.29$, $p = 0.020$) and...
normalized CBF ($r^2 = 0.34$, $p=0.011$) in the whole tumor. With vendor-neutral software, we found significant correlation for normalized CBF in whole tumor ($r^2 = 0.38$, $p=0.008$) and in solid component ($r^2 = 0.47$, $p = 0.002$). There were no significant correlations for the permeability parameters.

**Conclusions:** In comparing CTP and MRP methods, several statistically significant positive correlations were seen for CBF and CBV values. CTP may potentially be used interchangeably with MRP, for imaging of brain tumors, especially when MRP is contraindicated.

**Keywords**
CT perfusion; MR perfusion; intracranial tumours

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1. **Introduction**

Advanced imaging is becoming standard of care for characterization and preoperative planning of brain tumors, predominately with MRI. MR perfusion (MRP) has been shown to be an effective technique for predicting tumor angiogenesis and tumor grade; verifying tumor involvement that may not demonstrate enhancement; and differentiating tumor recurrence from post radiation pseudoprogression [1, 2].

Both CTP and MRP use the same basic principles to estimate tissue perfusion; by use of dynamic whole brain imaging during bolus contrast injection. Contrast is used to establish a density change in CTP and T2* shortening in MRP [3]. Cerebral blood volume (CBV) measures the total volume of blood in the intravascular space in a selected region of interest (ROI) and is measured in milliliters of blood per 100 g of brain tissue. Cerebral blood flow (CBF) measures the volume of blood moving through a given volume of 100 g of brain tissue per minute. MRP values are reported as relative (depicted as rCBV and rCBF) because MRP perfusion values are determined in comparison to that in the contralateral normal white matter. Permeability surface area product (PS) and the transfer constant (Ktrans) estimate the leakiness of the blood brain barrier, by measuring diffusion of contrast from the intravascular to the interstitial space [4]. Both parameters should be close to zero in normal brain. Ktrans can also be measured with MRP, however, there is inherent error in permeability measurements with MRI due to extravasation of gadolinium, altering the T1 and T2* signal dynamics [3]. K2 permeability values are corrected using a statistical model which account for T1 and T2* leakage effects.

CTP is often used in patients with contraindications to MRP. However, little is known about the comparability of perfusion parameters between the two modalities for the evaluation of brain tumors. A direct comparison of CTP with MRP has not been well studied in the same subset of patients with intracranial tumors. The purpose of our study was to directly compare CTP and MRP perfusion parameters in patients with intracranial tumors. It was hypothesized that perfusion parameters obtained from CTP would correlate with those obtained from MRP.
2. Methods

The study was approved by our institutional research ethics board (study number 1018631). CTP and MRP performed in patients with intracranial tumors in a prospective study were retrospectively analysed.

Patient selection- Consecutive patients presenting to our institution with a diagnosed brain tumor were approached to participate in our study. Individuals who provided informed consent and had both CTP and MRP performed within 7 days of each other were eligible for the study. In all cases, MRP was obtained first followed by CTP. The acceptable timeframe between the two imaging modalities was limited to 7 days to reduce the chances of tumor progression. Patients with a contraindication to imaging by MR or CT were excluded.

Imaging Technique- CT Perfusion - All patients underwent a 9.6-cm-coverage brain CTP protocol (80 kV, 100mAs, 128 x 0.6 mm collimation, 9.6-cm scan volume in the z-axis by using an adaptive spiral scanning technique ["shuttle mode"], CT dose index of 122.64 mGy), with 18 scans every 1.67 seconds, 5 scans every 3 seconds and 4 scans every 15 seconds, resulting in a total scanning time of 100.06 seconds on the 128-section dual-energy CT scanner (Sensation Definition; Siemens Healthcare, Erlangen, Germany). A total of 40 mL of nonionic iiodinated contrast media (iopamidol, Isovue-370; Bracco Diagnostic, Vaughan, Ontario, Canada) was injected at a rate of 5 ml/s, followed by a saline flush of 40 mL sodium chloride at 5 ml/s and a start delay of 5 seconds. Axial images with a section thickness of 5 mm were reconstructed without overlap and sent to the Picture Archiving and Communication System (PACS). CTP was done as a part of the prospective research study.

MR Perfusion- Axial T2*-weighted imaging gradient echo (GE) EPI sequence with TR- 2000, TE-26, flip angle-5, Matrix-96x128, Nex-1, FOV-22, were acquired with a temporal resolution of 2 seconds during and after injection of 0.1 mmol/kg body weight or 0.2 ml/kg body weight of MultiHance (gadobenate dimeglumine; Bracco Imaging, Canada, Montreal, QC) at a rate of 5 ml/s. Imaging was performed on a 1.5T MRI system (Signa HDxt, GE Healthcare). The total time of acquisition was 1 minute and 20 seconds. The acquisition covered the whole head with 20, 5 mm thick slices and inter-slice spacing of 1.5 mm. MRP was performed as part of standard of care.

Image Analysis and Post Processing- Vendor-provided software- CTP analysis was performed first with the vendor-provided Neuro-VPCT software (Siemens Healthcare) based on the semiautomatic deconvolution “Tumor” algorithm. Motion correction and bone segmentation was performed automatically; automatic arterial and venous vessel identification, vessel segmentation threshold, and depiction of a healthy hemisphere for normalization were done. The ROIs were determined by a radiology resident (KG) under the supervision of a fellowship trained neuroradiologist (JS). All ROIs were kept a consistent size, which was approximately 5 mm². CBF and CBV value was obtained in four different ROIs: (1) whole tumor, (2) solid portion, (3) region of maximum perfusion (ROMP), and (4) contralateral normal white matter (Figure 1). MRP data were analyzed first using the vendor provided software package (Functool, GE Healthcare). The rCBV and rCBF values were obtained using four ROIs in approximately the same locations as the CTP analysis (whole tumor, solid portion, ROMP, and contralateral normal white matter) (Figure 1). Care was taken to avoid inclusion of large cerebrospinal fluid (CSF) space or large blood vessels in the ROIs.
Vendor-neutral software- Both CTP and MRP were also processed with an automated vendor-neutral software Oleasphere (Olea Medical Solutions Inc). CBF, CBV, and permeability values were obtained using the same four ROIs in approximately the same locations. Gray-scale and color-coded perfusion parameter maps were stored in a Digital Imaging and Communications in Medicine (DICOM) format.

CT and MR perfusion values were recorded as both absolute and normalized values (compared to the contralateral normal appearing white matter ROI). The MR vendor-provided software did not have the function to process permeability, therefore, comparison of permeability parameters were only performed from vendor-neutral software.

Statistics- Linear regression was used to compare the CTP and MRP parameters from both vendor-provided and vendor-neutral software and Pearson’s correlation coefficients were calculated. A p-value of < 0.05 was considered significant. STATA 13.0 software was used for statistical calculations.

![Figure 1](image_url)

**Figure 1** Region of interest (ROI) placement on a cerebral blood flow (CBV) map in a patient with a right temporal lobe brain tumor. A- whole tumor; B- solid portion; C- region of maximum perfusion (ROMP) and D- contralateral normal white matter.

3. Results

From March 2014 to March 2015, 21 patients (13 males; mean age: 65 years, range: 51-76 years) were newly diagnosed with brain tumors and underwent both CTP and MRP. The most common diagnosis was glioblastoma multiforme (17 patients), followed by metastasis (3) and grade 3 astrocytoma (1). Mean time interval between CTP and MRP was 6.8 hours (range: 1 - 49 hours).
MRP of one patient was excluded due to excessive motion artefact. Data from only 18 patients were analyzed on the vendor-provided software as 2 patients were excluded due to file corruption that prohibited post-processing. Overall, the absolute values obtained from MRP were smaller compared to that from CTP with vendor-provided software (Table 1). There was a strong positive correlation between absolute CBV values in the ROMP ($r^2 = 0.26$, $p = 0.031$) and normalized CBF in the whole tumor ($r^2 = 0.34$, $p = 0.011$). There was only a weak but significant positive correlation for normalized CBV ($r^2 = 0.29$, $p = 0.020$). The ROMP absolute CBV value performed with CTP could be predicted from MRP value by the following equation, $CT = 5.39 + 0.49(MR)$. There was no significant linear relationship between CTP and MRP for the remainder of the perfusion parameters.

### Table 1
Mean absolute and normalized (“n”) cerebral blood volume (CBV) and cerebral blood flow (CBF) CT and MR perfusion values with vendor-provided software, and correlation.

<table>
<thead>
<tr>
<th></th>
<th>CT</th>
<th>MR</th>
<th>Correlation (r-squared)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBV Max</td>
<td>8.5</td>
<td>6.2</td>
<td>0.26</td>
<td>0.031*</td>
</tr>
<tr>
<td>CBV WT</td>
<td>4.9</td>
<td>3.3</td>
<td>0.16</td>
<td>0.102</td>
</tr>
<tr>
<td>CBV Solid</td>
<td>6.1</td>
<td>4.1</td>
<td>0.09</td>
<td>0.226</td>
</tr>
<tr>
<td>CBV CW</td>
<td>1.7</td>
<td>0.7</td>
<td>0.12</td>
<td>0.165</td>
</tr>
<tr>
<td>nCBV Max</td>
<td>5.1</td>
<td>10.3</td>
<td>0.08</td>
<td>0.264</td>
</tr>
<tr>
<td>nCBV WT</td>
<td>2.9</td>
<td>5.4</td>
<td>0.29</td>
<td>0.020*</td>
</tr>
<tr>
<td>nCBV Solid</td>
<td>3.7</td>
<td>6.7</td>
<td>0.16</td>
<td>0.094</td>
</tr>
<tr>
<td>CBF Max</td>
<td>72.7</td>
<td>38.8</td>
<td>0.01</td>
<td>0.771</td>
</tr>
<tr>
<td>CBF WT</td>
<td>48.1</td>
<td>17.9</td>
<td>0.01</td>
<td>0.627</td>
</tr>
<tr>
<td>CBF Solid</td>
<td>58.3</td>
<td>22.6</td>
<td>0.003</td>
<td>0.841</td>
</tr>
<tr>
<td>CBF CW</td>
<td>24.1</td>
<td>5.1</td>
<td>0.11</td>
<td>0.188</td>
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<tr>
<td>nCBF Max</td>
<td>3.4</td>
<td>8.5</td>
<td>0.07</td>
<td>0.304</td>
</tr>
<tr>
<td>nCBF WT</td>
<td>2.3</td>
<td>3.8</td>
<td>0.34</td>
<td>0.011*</td>
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<tr>
<td>nCBF Solid</td>
<td>2.7</td>
<td>4.8</td>
<td>0.11</td>
<td>0.177</td>
</tr>
</tbody>
</table>

CT: computed tomography; MR: magnetic resonance; CI: confidence interval; WT: whole tumor; Max: region of maximum perfusion (ROMP); Solid: solid portion of tumor; CW: contralateral white matter. CBV units = ml/100 ml; CBF units = ml/100g/min. *Denotes significant difference (P<0.05).

Twenty patients were analyzed using the vendor-neutral software. The CBV and CBF values were similar between CTP and MRP, except MRP CBF values were overall larger than CTP (Table 2). There was a weak but significant positive correlation between normalized CBF values of the whole tumor ($r^2 = 0.38$, $p = 0.008$) and solid component ($r^2 = 0.47$, $p = 0.002$). There was no significant correlation between absolute CBF and CBV values. There was also no significant correlation between the permeability parameters from CT (Ktrans and PS) and MR (K2) (Table 3). Table 4 shows the correlation between the CBF and CBV computed from vendor provided and vendor
neutral CT and MR perfusion. This showed significant correlation between CBV and CBF of whole tumor and CBV of solid component of the tumor on CT perfusion.

**Table 2** Mean absolute and normalized ("n") cerebral blood volume (CBV) and cerebral blood flow (CBF) CT and MR perfusion values with *vender-neutral software*, and correlation.

<table>
<thead>
<tr>
<th></th>
<th>CT</th>
<th>MR</th>
<th>Correlation (r-squared)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBV Max</td>
<td>8.4</td>
<td>8.2</td>
<td>0.02</td>
<td>0.634</td>
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<tr>
<td>CBV WT</td>
<td>4.2</td>
<td>4.0</td>
<td>0.07</td>
<td>0.318</td>
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<tr>
<td>CBV Solid</td>
<td>4.9</td>
<td>4.9</td>
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<td>0.679</td>
</tr>
<tr>
<td>CBV CW</td>
<td>0.9</td>
<td>0.8</td>
<td>0.06</td>
<td>0.341</td>
</tr>
<tr>
<td>nCBV Max</td>
<td>9.5</td>
<td>10.6</td>
<td>0.08</td>
<td>0.285</td>
</tr>
<tr>
<td>nCBV WT</td>
<td>4.5</td>
<td>5.2</td>
<td>0.06</td>
<td>0.328</td>
</tr>
<tr>
<td>nCBV Solid</td>
<td>5.5</td>
<td>6.4</td>
<td>0.07</td>
<td>0.300</td>
</tr>
<tr>
<td>CBF Max</td>
<td>31.7</td>
<td>70.7</td>
<td>0.01</td>
<td>0.750</td>
</tr>
<tr>
<td>CBF WT</td>
<td>21.9</td>
<td>40.0</td>
<td>0.18</td>
<td>0.090</td>
</tr>
<tr>
<td>CBF Solid</td>
<td>24.1</td>
<td>49.3</td>
<td>0.16</td>
<td>0.112</td>
</tr>
<tr>
<td>CBF CW</td>
<td>12.4</td>
<td>8.9</td>
<td>0.21</td>
<td>0.066</td>
</tr>
<tr>
<td>nCBF Max</td>
<td>2.6</td>
<td>9.1</td>
<td>0.17</td>
<td>0.098</td>
</tr>
<tr>
<td>nCBF WT</td>
<td>1.8</td>
<td>5.4</td>
<td>0.38</td>
<td>0.008*</td>
</tr>
<tr>
<td>nCBF Solid</td>
<td>2.0</td>
<td>6.7</td>
<td>0.47</td>
<td>0.002*</td>
</tr>
</tbody>
</table>

CT: computed tomography; MR: magnetic resonance; CI: confidence interval; WT: whole tumor; Max: region of maximum perfusion (ROMP); Solid: solid portion of tumor; CW: contralateral white matter. CBV units = ml/100 ml; CBF units = ml/100g/min. *Denotes significant difference (P <0.05).

**Table 3** Correlation of CT (Ktrans and PS) and MR (K2) permeability values obtained with *vender-neutral software*.

<table>
<thead>
<tr>
<th></th>
<th>Correlation (r-squared)</th>
<th>p-value</th>
<th>Correlation (r-squared)</th>
<th>p-value</th>
</tr>
</thead>
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<tr>
<td></td>
<td>CT Ktrans and MR K2</td>
<td></td>
<td>CT PS and MR K2</td>
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<td>Permeability Max</td>
<td>0.05</td>
<td>0.349</td>
<td>0.10</td>
<td>0.180</td>
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<tr>
<td>Permeability WT</td>
<td>0.02</td>
<td>0.507</td>
<td>0.01</td>
<td>0.840</td>
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<tr>
<td>Permeability Solid</td>
<td>0.04</td>
<td>0.389</td>
<td>0.01</td>
<td>0.795</td>
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</table>

CT: computed tomography; MR: magnetic resonance; CI: confidence interval; PS: permeability surface area product; Ktrans: transfer constant; Max: region of maximum perfusion (ROMP); WT: whole tumor; Solid: solid portion of tumor. *Denotes significant difference (P <0.05).
Table 4 Mean absolute and normalized ("n") cerebral blood volume (CBV) and cerebral blood flow (CBF) computed from vendor provided and vendor neutral CT and MR perfusion values and correlation.

<table>
<thead>
<tr>
<th></th>
<th>Vendor-provided</th>
<th>Vendor neutral</th>
<th>Correlation (r-squared)</th>
<th>p-value</th>
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<td><strong>CT</strong></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>CBV Max</td>
<td>8.59</td>
<td>8.42</td>
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<td>0.150</td>
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<tr>
<td>CBV WT</td>
<td>5.04</td>
<td>4.22</td>
<td>0.37</td>
<td>0.010*</td>
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<tr>
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<td>6.27</td>
<td>4.97</td>
<td>0.25</td>
<td>0.042*</td>
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<td>CBV Contra</td>
<td>1.71</td>
<td>0.94</td>
<td>0.01</td>
<td>0.960</td>
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<td>CBF Max</td>
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<td>31.75</td>
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<td>0.816</td>
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<tr>
<td>CBF WT</td>
<td>49.93</td>
<td>21.87</td>
<td>0.25</td>
<td>0.041*</td>
</tr>
<tr>
<td>CBF Solid</td>
<td>59.76</td>
<td>24.06</td>
<td>0.02</td>
<td>0.555</td>
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<tr>
<td>CBF Contra</td>
<td>24.27</td>
<td>12.44</td>
<td>0.10</td>
<td>0.217</td>
</tr>
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<td><strong>MRI</strong></td>
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<tr>
<td>CBV Max</td>
<td>6.39</td>
<td>8.21</td>
<td>0.07</td>
<td>0.299</td>
</tr>
<tr>
<td>CBV WT</td>
<td>3.33</td>
<td>4.03</td>
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<td>4.13</td>
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<td>0.81</td>
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<td>0.17</td>
<td>0.095</td>
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<td>CBF WT</td>
<td>18.51</td>
<td>40.03</td>
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<td>0.068</td>
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<td>CBF Solid</td>
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<td>49.28</td>
<td>0.08</td>
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</tr>
<tr>
<td>CBF Contra</td>
<td>5.28</td>
<td>8.88</td>
<td>0.21</td>
<td>0.067</td>
</tr>
</tbody>
</table>

CT: computed tomography; MR: magnetic resonance; CI: confidence interval; PS: permeability surface area product; Ktrans: transfer constant; Max: region of maximum perfusion (ROMP); WT: whole tumor; Solid: solid portion of tumor. *Denotes significant difference (P < 0.05).

4. Discussion

Our study showed some significant correlations between CT and MR perfusion parameters for both vendor-provided as well as vendor-neutral software, although these were not consistent between different ROIs. A positive linear relationship was shown with absolute CBV values using a ROI in the ROMP and normalized CBF in the whole tumor. Permeability parameters did not show any correlation as these parameters (CT Ktrans and PS and MR K2) actually measure different phenomenon and cannot and in fact should not have been compared directly [5, 6]. Only one other study has assessed the relationship between CTP and MRP perfusion values, in a small selected group of patients with high grade gliomas [7]. This study showed that normalized CBV in ROMP on both CTP and MRP had linear correlation. The MRP technique used in that study was a T2 weighted spin echo EPI sequence. This was different from the technique used in our study,
which was a T2* gradient echo EPI sequence. No study has compared CTP to MRP values using a T2* gradient echo EPI technique.

While the use of MRI as an imaging modality has grown progressively in the medical management of brain tumors, its implementation remains contraindicated in several patient populations, such as individuals with cardiac pacemakers, implanted hearing aids, or neurostimulators [8]. In comparison to MRP, CTP also offers the advantages of lower costs, faster scans, and more accessibility [9]. CTP has been used extensively for the characterization of acute stroke and possesses promising application in tumor imaging [1, 10-13], despite the risk of a small dose of ionizing radiation (2-3 mSv). The correlations shown in this study, although somewhat limited, could potentially increase the use of CTP in the investigation of brain tumors. However, more research is required to determine if modifications in imaging acquisition and post-processing techniques may lead to further equivalency between CTP and MRP methods. Despite being significant (p < 0.05) the correlation coefficient has remained weak to strong (r < 0.50). A possible explanation for the relatively poor correlation could be the differences in patient angulation, slice selection and spatial resolution between CT and MRI. A T2* gradient echo EPI sequence was used for MRP acquisition, rather than a spin echo EPI sequence as in the previous study. T2* gradient echo EPI is more sensitive to the T2* susceptibility effects from gadolinium and less sensitive to the masking effects of T1 [13, 14], therefore, this technique may lead to changes in perfusion values compared to a spin echo EPI sequence.

Limitations- ROIs of the whole tumor or solid portion were difficult to reproduce with CT and MRI, especially with vendor-provided software, due to slight differences in slice selection, angulation and spatial resolution. The ROMP identified on MRP studies often showed no density (black) on CT perfusion maps with no perfusion value, possibly due to the threshold set up. Due to this, we had to put ROIs in the region adjacent to the ROMP and this might have resulted in less than optimal correlation between the values on CTP and MRP. Perfusion and permeability values for each region of interest were recorded only once rather than taking an average of multiple measurements. Multiple ROIs in the same region may more accurately reflect the true perfusion value, especially for the ROMP. Despite including consecutive presenting brain tumors, the study remains limited by a small sample size. A study with larger sample size may demonstrate better comparability between CTP and MRP for brain tumors.

In conclusion, significant positive correlations were seen for CBF and CBV values between CTP and MRP methods. CTP may potentially be used interchangeably with MRP, for imaging of brain tumors, especially when MRP is contraindicated.

Author Contributions

Kirsten Greenlaw-Collected the data, wrote the first draft and edited the manuscript. Jai Shankar- Original idea, data analysis, final manuscript editing and submission.

Competing Interests

The authors have declared that no competing interests exist.
References


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Case Report

Cerebellopontine Angle Glioblastoma with Concurrent Spinal Cord Involvement: A Case Report and Review of Literature

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Abstract
Objective: To report a unique case of cerebellopontine angle glioblastoma with concurrent spinal cord involvement.

Background: Glioblastoma (GBM) is the most common primary malignancy of the central nervous system (CNS), comprising 46.6% of all CNS malignancies. By anatomic location, cerebellopontine angle (CPA) GBMs are exceedingly rare. To our knowledge, the following case represents the tenth reported case of CPA GBM and the first with a corresponding spinal cord tumor on presentation.

Methods: Retrospective chart review was conducted for a patient with CPA GBM. The patient consented to the publication of her diagnostic studies.

Result: A 43-year-old female presented with a 3-month history of right ear hearing loss. Brain magnetic resonance imaging (MRI) demonstrated a right CPA mass as well as a cervical spinal cord mass of unknown histology. Pathology showed GBM, IDH wildtype and negative for the H3 K27M mutation, while the cervical spinal cord lesion was not amenable to biopsy. She received proton beam therapy with 60 Gy to the GBM and 50.4 Gy to the spinal cord...
tumor in 30 fractions each with concurrent Temozolomide. She received 2 cycles of adjuvant Temozolomide prior to her demise due to progressive disease.

**Conclusion:** There are four possible origins of CPA glioblastoma: the cerebellum, brainstem, root entry zone of cranial nerve VIII, and heterotopic glial cells of the leptomeninges. Prognosis does not appear to depend on tumor origin. Outcomes are likely optimized by maximal safe resection followed by radiation and concurrent Temozolomide. Spinal cord involvement of malignant tumors can significantly adversely affect survival outcomes in such patients.

1. Introduction

Glioblastoma (GBM) is the most common primary malignant tumor of the central nervous system (CNS), comprising 46.6% of all CNS malignancies [1]. By anatomic location, the posterior fossa is a relatively uncommon location of GBM. A study of the Los Angeles County Cancer Surveillance Program shows the highest incidence for GBMs was in the frontal lobe, while the lowest incidence for GBM occurred in the posterior fossa (defined as tumors of the ventricular system, brainstem, cerebellum, and CPA (cerebellopontine angle)) [2]. CPA GBMs are exceedingly rare such that to our knowledge, the following case represents the tenth reported case of CPA GBM and the first with a corresponding spinal cord tumor on presentation.

2. Case

A 43-year-old female presented with a 3-month history of hearing loss in the right ear. A brain MRI in December 2015 showed a right CPA mass deemed to be acoustic neuroma and an expansile cervical spinal cord mass of unknown origin. She did not receive contrast at the time due to her pregnancy. She was lost to follow up and presented in December 2016 with dizziness and bilateral hand numbness that led to a repeat brain MRI with contrast, which showed progression of the brain tumor and areas of enhancement at the C3-C4 levels. (Figure 1). She underwent subtotal resection of the right CPA mass in January 2017 as a gross total resection was deemed unsafe by her surgeon. (Figure 2). Pathology demonstrated the lesion to be a GBM (IDH wild-type, MGMT promoter unmethylated, and absent H3 K27M mutation). The cervical spinal cord lesion was not amenable to biopsy due to the location. After resection, she had further neurological worsening. Neurologic exam showed dysarthria, decreased right hearing, hypotonia of the right arm, bilateral hip flexor weakness, right appendicular dysmetria, upper motor neuron signs on the right, and a wide-based ataxic gait. She received proton beam therapy to both the CP angle mass and the cervical spine mass, as the cervical spine mass was also treated as if it were GBM. She received 60 Gy to the brain tumor in 30 fractions and 50.4 Gy to the spinal cord tumor in 30 fractions with proton beam therapy. She received concurrent Temozolomide at 75 mg/m² during the 30 fractions of proton beam XRT. Post radiation MRI of the brain and spine showed decreased size of the enhancing right CPA mass; however, there was increased size of the enhancing, expansile mass in the cervical cord extending from C2 to C7 (Figure 3). She initially deferred adjuvant monthly Temozolomide, opting for a holistic approach instead.
**Figure 1** (A) Axial gadolinium enhanced brain MRI demonstrating a right cerebellopontine angle mass in December 2016 prior to surgical resection. (B) Sagittal MRI of the Cervical spine with gadolinium in December 2016 demonstrating areas of enhancement at the C3 C4 levels. (C) Axial T2/FLAIR demonstrating extensive edema in the right cerebellopontine angle extending into the brainstem and the cerebellum.

**Figure 2** Post-operative axial T1 post contrast brain MRI demonstrating subtotal resection of the right CPA mass.
In August 2017, follow-up MRI of the brain and spine showed increased size of both the brain and cervical cord lesions. She started adjuvant Temozolomide at a dose of 200 mg/m2. Following two cycles of adjuvant Temozolomide, she developed new complete right lower motor neuron type facial palsy as well as worsening of her gait ataxia, sensory loss, and appendicular dysmetria. MRI of the brain and cervical spine showed further progression (Figure 4). Bevacizumab was then recommended. However, the patient had rapid clinical decline and died prior to initiation of Bevacizumab.

**Figure 3 (A)** Axial gadolinium enhanced brain MRI after completion of proton beam radiation. **(B)** Sagittal gadolinium enhanced cervical spine MRI after completion of proton beam radiation.

**Figure 4 (A)** Axial gadolinium enhanced MRI of the brain showing increase in the size of the right cerebellopontine angle mass after completion of 2 cycles of adjuvant Temozolomide. **(B)** Sagittal gadolinium enhanced MRI of the cervical spine demonstrating expansile mass in the cervical spine after completion of 2 cycles of adjuvant Temozolomide.
3. Discussion

This is the tenth reported case of CPA GBM, and the first with a corresponding spinal cord tumor on presentation. Of the ten cases, this is the second case to undergo administration of Temozolomide, and the first to undergo off label use of proton beam therapy. This case is also unusual due to the longer survival of the patient despite being untreated for a year. A summary of the clinical characteristics of these cases are listed in Table 1. Nearly all cases presented within three months of symptom onset. The most common symptoms were headache, nausea, vomiting, hearing loss, and gait disturbance. The most common signs were ataxia and cranial nerve dysfunction.

A tumor can occupy the CPA in one of two ways. First, it can extend from heterotopic glial nests of the meninges in a process known as primary leptomeningeal gliomatosis [3]. Second, it can grow exophytically from the brain parenchyma and gain access to the CPA by passing through the foramen of Luschka [4]. Of the ten cases, one originated from primary leptomeningeal gliomatosis, four tumors arose from the cerebellum, and the remainder arose from either the brainstem parenchyma or the root entry zone of cranial nerve VIII. The root entry zone of a cranial nerve is the extension of glial tissue proximal to Schwann cell insulation. The root entry zone of cranial nerve VIII is by far the longest of the cranial nerves measuring roughly 10 mm in length [4]. This would suggest that cranial nerve VIII has the highest risk for tumorigenesis amongst the cranial nerve root entry zones. Ultimately, there does not appear to be a difference in prognosis based on tumor origin in this small sample.

Histologically, four of the ten cases were made up by an uncommon variant of glioblastoma known as giant cell GBM, which is seen in only one percent of all GBM cases [5]. Giant cell GBMs are characterized by multinucleated giant cells with abundant cytoplasm, and tend to occur in younger patients. Of the four cases of giant cell GBM, all occurred in patients younger than 30, but this did not render improved outcome. It is unclear why the giant cell GBM variant would have a predilection for the CPA [5].
Table 1: Summary of reported cases of cerebellopontine angle glioblastoma.

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Age (years), Sex</th>
<th>Symptoms</th>
<th>Signs</th>
<th>Symptom Duration (months)</th>
<th>Involved Side</th>
<th>Tumor Origin</th>
<th>Histopathology</th>
<th>Surgery</th>
<th>Radiotherapy</th>
<th>Chemotherapy</th>
<th>Post-op follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ahn et al (1997)</td>
<td>79, M</td>
<td>GD, HL, NV</td>
<td>A, N, CN</td>
<td>3</td>
<td>Left</td>
<td>Cerebellum</td>
<td>GBM</td>
<td>STR</td>
<td>No</td>
<td>No</td>
<td>Died, 3 weeks</td>
</tr>
<tr>
<td>Swaroop et al (1997)</td>
<td>22, M</td>
<td>GD, HL, FP</td>
<td>A, N, CN</td>
<td>12</td>
<td>Right</td>
<td>Brainstem</td>
<td>GCGBM</td>
<td>Biopsy</td>
<td>Yes</td>
<td>No</td>
<td>NA</td>
</tr>
<tr>
<td>Kasliwal et al (2006)</td>
<td>11, NA</td>
<td>GD, HA, NV, AP, VL, FW</td>
<td>A, CN, PL, HP</td>
<td>0.5</td>
<td>Right</td>
<td>Cerebellum</td>
<td>GCGBM</td>
<td>Biopsy</td>
<td>No</td>
<td>No</td>
<td>Died, 2 months</td>
</tr>
<tr>
<td>Rasalingam et al (2008)</td>
<td>9, M</td>
<td>GD, HA, DV, FV, ED</td>
<td>A, N, CN, PL</td>
<td>0.5</td>
<td>Right</td>
<td>Brainstem</td>
<td>GBM</td>
<td>STR</td>
<td>No</td>
<td>No</td>
<td>Died, 2 months</td>
</tr>
<tr>
<td>Wu et al (2011)</td>
<td>60, M</td>
<td>HL, DA, DP</td>
<td>CN</td>
<td>2</td>
<td>Left</td>
<td>CN VIII</td>
<td>GBM</td>
<td>STR</td>
<td>No</td>
<td>No</td>
<td>Died, 2 months</td>
</tr>
<tr>
<td>Salunke et al (2012)</td>
<td>59, M</td>
<td>GD, HL, HA, NV, FW</td>
<td>A, CN, PL, HP</td>
<td>3</td>
<td>Right</td>
<td>Brainstem or CN VIII GBM</td>
<td>GBM</td>
<td>STR</td>
<td>Yes</td>
<td>No</td>
<td>NA</td>
</tr>
<tr>
<td>Taraszewska et al (2013)</td>
<td>29, F (NF1 patient)</td>
<td>HA, NV</td>
<td>A, N, HP</td>
<td>NA</td>
<td>Bilateral</td>
<td>PLG</td>
<td>GCGBM</td>
<td>STR</td>
<td>No</td>
<td>No</td>
<td>Died, 7 days</td>
</tr>
<tr>
<td>Matsuda et al (2014)</td>
<td>69, M</td>
<td>FP</td>
<td>Normal exam</td>
<td>NA</td>
<td>Right</td>
<td>Cerebellum</td>
<td>GBM</td>
<td>STR</td>
<td>60 Gy</td>
<td>Temozolomide</td>
<td>Stable, 24 months</td>
</tr>
<tr>
<td>Present Case</td>
<td>42, F</td>
<td>HL, NV, PA, DZ</td>
<td>A, CN, BP, HR</td>
<td>3</td>
<td>Right</td>
<td>Brainstem or CN VIII GBM</td>
<td>GBM</td>
<td>STR</td>
<td>60 Gy to brain, 50.4 Gy to spinal cord</td>
<td>Temozolomide</td>
<td>Progression, 8 months</td>
</tr>
</tbody>
</table>

NA = not available
HL = hearing loss, GD = gait disturbance, NV = nausea, vomiting, HA = headache, FP = facial pain, AP = abdominal pain, VL = vision loss, FW = focal weakness, DA = dysarthria, DP = dysphagia, PA = paresthesia, DZ = dizziness, DV = double vision, FV = fever, ED = ear discharge
A = ataxia, N = nystagmus, CN = cranial nerve palsy, PL = papilledema, HR = hyperreflexia, HP = hemiparesis, BP = bilateral paresis. GBM = glioblastoma, GCGBM = giant cell glioblastoma, PLG = primary leptomeningeal gliomatosis.
Primary GBMs arise de novo and render a relatively poor prognosis when compared to secondary GBMs, which develop from lower grade gliomas. Hallmark features of primary GBMs include absence of mutations in IDH1 and TP53. Mutations of IDH1 and TP53 are more often associated with secondary GBMs. The molecular characteristics of the ten cases are listed in Table 2. Testing for IDH1 and TP53 mutations was scant overall. Of the two cases that were tested for IDH1 mutation, both showed absence of this mutation. Of the four cases that were tested for TP53 mutation, two showed absence of this mutation. Overall, further investigation is required to determine the prevalence of primary versus secondary GBMs at the CPA. Methylation of the promoter region of the DNA repair gene, MGMT, renders improved response to Temozolomide. The present case had an unmethylated promoter region. No other cases reported MGMT status. This patient’s tumor was negative for NF1 and NF2 mutations.

Table 2 Molecular characteristics of reported cases of cerebellopontine angle glioblastoma.

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Age (years), Sex</th>
<th>Histopathology</th>
<th>GFAP</th>
<th>TP53 mutation</th>
<th>IDH1 R132H</th>
<th>MGMT</th>
<th>H3 K27M</th>
<th>ATRX mutation</th>
<th>TERT mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ahn et al (1997)</td>
<td>79, M</td>
<td>GBM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Swaroop et al (1997)</td>
<td>22, M</td>
<td>GCGBM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rasalingam et al (2008)</td>
<td>9, M</td>
<td>GBM</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wu et al (2011)</td>
<td>60, M</td>
<td>GBM</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salunke et al (2012)</td>
<td>59, M</td>
<td>GBM</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Taraszewski et al (2013)</td>
<td>29, F (NF1 patient)</td>
<td>GCGBM, PLG</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Matsuda et al (2014)</td>
<td>69, M</td>
<td>GBM</td>
<td>+</td>
<td>-</td>
<td>wt</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present Case</td>
<td>42, F</td>
<td>GBM</td>
<td>+</td>
<td>-</td>
<td>wt</td>
<td>UM</td>
<td></td>
<td></td>
<td>+</td>
</tr>
</tbody>
</table>

Legend

UM = unmethylated, wt = wild type

The most common tumor of the CPA is vestibular schwannoma, comprising 80% of CPA tumors [6]. Since treatment for vestibular schwannomas and GBMs differ greatly, it is important to distinguish the two radiographically and histologically. Posterior fossa GBMs are characterized by ill-defined tumor margins, peritumoral edema, and moderate to marked ring-like enhancement with gadolinium contrast [7], which was noted in this patient. Vestibular schwannomas are homogenously enhancing rather than ring-enhancing in 67% of one case series [8], cystic schwannomas, however can show ring enhancement. Thus, it is important that gadolinium contrast be administered whenever possible to
help distinguish between these two tumor types. Diffusion restriction, when present may suggest central nervous system involvement of lymphoma [9]. These radiographic distinctions may be critical in determining the urgency of obtaining a histologic diagnosis as well as planning future treatments and determining prognosis.

Regarding outcome, three of the ten cases reported survival at three months post-surgery (see Table 1). The first case was of a 15-year-old girl who presented two months after symptom onset with left cerebellar giant cell GBM. She underwent gross total resection and radiotherapy [10]. No other case underwent gross total resection. The second case was of a 69-year-old man with a right cerebellar GBM who underwent subtotal resection, radiotherapy, and chemotherapy with Temozolomide [11]. The third case (the present one) had similar intracranial tumor management to the second case except for the proton beam therapy in our case compared to standard XRT in the second case. Also, the presumed cervical spinal cord GBM complicated management. Proton beam therapy for intracranial GBMs have shown relative sparing of healthy brain tissue, while its safety and efficacy in spinal cord GBM have not been studied [12]. In the present case, post radiation MRI showed decreased size of the intracranial mass but increased size in the cervical cord mass.

The utility of Temozolomide in spinal cord GBMs are not well studied. In a systematic review of patients with primary spinal cord GBMs, there was no significant difference between patients treated with Temozolomide compared to patients who received other treatment modalities, though there was a trend toward improved median overall survival (16 months to 10 months) in the Temozolomide group [13]. In this small sample of patients with CPA GBMs, resection followed by radiation and Temozolomide rendered the best outcome.

4. Conclusion

We report the tenth case of GBM of the CPA and the first with a corresponding spinal cord tumor on presentation. There are four possible origins of CPA GBM: the cerebellum, brainstem, root entry zone of cranial nerve VIII, and heterotopic glial cells of the leptomeninges. Prognosis does not appear to depend on tumor origin. We recommend maximal safe resection followed by radiation and Temozolomide, although the efficacy of standard XRT compared to proton beam radiotherapy has not been definitively determined. The added component of this patient’s spinal cord tumor likely significantly contributed to her poor outcome.

Author Contributions

All authors have contributed equally to the preparation of this manuscript.

Competing Interests

The authors have declared that no competing interests exist.

References


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Review

Sleep Disorders in Children with Central Nervous System Tumors

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Abstract:
Sleep complaints are common in pediatric patients with central nervous system (CNS) tumors. These problems may result from disruption of normal homeostatic, circadian, neuroendocrine, and cardiorespiratory pathways and vary by tumor location and treatment received. Children with tumors within the hypothalamus and surrounding regions are prone to excessive daytime sleepiness. Sleep-related breathing disorders, especially those involving abnormal control of breathing, may occur in patients with tumors of the brainstem and posterior fossa. Maintaining a high index of suspicion for sleep disorders in children with CNS tumors is essential for early recognition and treatment. In this article, we will review the various sleep problems reported in pediatric brain tumor survivors, explore underlying neurobiological mechanisms, and discuss approaches to screening and diagnosis.
Keywords
CNS tumors; craniopharyngioma; posterior fossa tumors; hypersomnolence; narcolepsy; circadian rhythm disorder; sleep disordered breathing; sleep apnea; sleep-related hypoventilation

1. Background

Sleep is an essential physiologic function for growth, cognition, and physical and emotional well-being. It is particularly important in childhood, when the brain and body are undergoing rapid development. Normal sleep occurs through a complex interplay between homeostatic, circadian, neuroendocrine, and cardiorespiratory pathways. Cancer and cancer treatment may cause disruptions and imbalances in these pathways, resulting in abnormal sleep. Compared to the general population, pediatric cancer survivors are more likely to experience sleep problems. Patients with a history of brain tumors are particularly prone to sleep issues. In this article, we will review the physiology of normal sleep, describe the sleep problems and pathophysiologic mechanisms behind disrupted sleep in children with central nervous system (CNS) tumors, and discuss strategies for screening, diagnosis, and management.

1.1 Sleep Mechanisms and Physiology

Sleep and wakefulness. Sleep is fundamentally important in promoting growth, repair, and neurocognitive functioning. The timing, duration, and quality of sleep depend on complex, balanced interactions between sleep-promoting and wake-promoting pathways. The majority of these pathways are housed in the basal forebrain, diencephalon, and brainstem. The balance between sleep and wakefulness is governed by two distinct biologic processes: sleep-wake homeostasis (Process S) and circadian rhythm (Process C)[1]. Homeostatic drive to sleep intensifies with continued wakefulness over time and subsides as an individual sleeps. During the day, the sleep-dependent homeostatic drive is counteracted by sleep-independent circadian arousal mechanisms. Circadian rhythms are coordinated by an internal pacemaker housed in the bilateral suprachiasmatic nuclei (SCN) of the anterior hypothalamus [1]. The near-24 hour circadian rhythm is externally entrained by the light-dark cycle on Earth through direct input received by the SCN from retinal ganglion cells via the retinohypothalamic tract, which runs through the optic chiasm [2, 3]. Additional input from the intergeniculate leaflet and median raphe nucleus modulate circadian phase shifts [3]. The SCN sends afferents to other regions of the hypothalamus and not only regulates sleep and wakefulness but also body temperature, hormone release, metabolism, and feeding behaviors through complex feedback loops. The primary pathway for circadian regulation of sleep flows from the SCN to the nearby subparaventricular zone, which in turn projects to the dorsomedial hypothalamic nucleus and connects subsequently to the wake-promoting lateral hypothalamus and the sleep-promoting ventrolateral preoptic area [3–5]. The SCN also exerts influence through output to the paraventricular hypothalamic nucleus [3–5]. The paraventricular hypothalamic nucleus in turn sends projections that affect hypothalamic and pituitary hormone release and via a separate pathway, melatonin release from the pineal gland [5].
The diurnal rhythm of melatonin release is essential in maintaining a normal 24-hour sleep-wake cycle [1, 4].

The ascending reticular activating system (ARAS) promotes wakefulness and cortical arousal and is inhibited by input from the sleep-promoting ventrolateral preoptic area. The ARAS originates near the junction between the midbrain and pons then splits into two branches which send projections to nuclei in the thalamus, hypothalamus, basal forebrain, and cerebral cortex [4, 5]. These branches comprise cholinergic and monoaminergic cell populations, as well as neurons containing the excitatory neuropeptide orexin, which is synthesized in the lateral hypothalamus [4, 5]. Orexin-containing neurons in the lateral hypothalamus project to many of the major nuclei involved in sleep and wakefulness and are postulated to play a crucial role in maintaining cortical arousal and stabilizing the sleep-wake balance [4, 5]. Individuals with narcolepsy often have reduced cerebrospinal fluid (CSF) orexin levels and animal models with dysregulated orexin transmission demonstrate sleep-wake and REM sleep derangement [5].

These various pathways work together in a coordinated pattern of excitation and inhibition to maintain wakefulness and cortical arousal during the day and promote normal restorative sleep at night. Disruption of any one component may throw this intricate system off balance, leading to abnormal or inappropriate sleep. In patients with brain tumors, these disruptions may occur as sequelae of the primary neoplasm or as a consequence of cancer treatment.

Control of breathing and maintenance of ventilation. The primary goal of respiratory control during sleep is maintenance of homeostasis, with regulation of blood gases, to preserve normal function. Central chemoreceptors located along the ventral medulla respond to subtle fluctuations in PaCO₂ through changes in pH [3]. Detection of hypercapnia leads to stimulation of breathing, coordinated by the respiratory central pattern generator, which consists of neuronal aggregates housed within the brainstem [3]. When activated, this neuronal circuitry generates rhythmic motor patterns that affect the depth and frequency of ventilatory breaths [3, 6]. Central chemoreceptor activation and response is the primary driver of the ventilatory response to hypercapnia, augmented by smaller contributions from peripheral chemoreceptors [3]. The peripheral chemoreceptors are located at bifurcation of the carotid arteries and are most sensitive to changes in PaO₂. The peripheral chemoreceptors send afferents via the glossopharyngeal nerve to the nucleus of the solitary tract (NTS) in the dorsal medulla, where they converge with afferents from peripheral baroreceptors and pulmonary stretch receptors [7]. The NTS integrates this information to produce coordinated sympathetic, parasympathetic, and respiratory reflex responses via projections to other nuclei within the medulla and pons [7]. The retrotrapezoid nucleus of the ventral medullary group is one of the receiving nuclei and also sends afferents back to the NTS and plays an important role in coordinating respiratory drive [7]. Pontine nuclei receive input from the NTS and send modulatory feedback [7]. Peripheral sensory input is received through stimulation of juxtacapillary (J) receptors of the alveoli by irritation, interstitial fluid, and capillary distention [3]. Signals are then sent to the brainstem via the vagus nerve. These signals elicit rapid shallow breathing or in extreme cases, apnea.

While the majority of the major neurologic processes that coordinate respiratory drive are housed in the brainstem, the cerebellum plays a supporting role. The cerebellum aids in rhythmic pattern generation and motor coordination, supporting the cyclical act of breathing [8–11]. It helps synchronize contraction of the muscles that maintain upper airway patency with diaphragmatic contraction, supporting optimal inspiratory flow [9]. The deep nuclei of the
cerebellum, particularly the fastigial and medial nuclei, may help coordinate motor responses to the respiratory stimuli [10, 11].

In normal individuals, central respiratory drive and ventilatory muscle power work in conjunction to sufficiently overcome the respiratory load and maintain adequate ventilation. During sleep the body is vulnerable to respiratory insufficiency as a result of normal sleep-related physiologic changes in breathing mechanisms. During normal sleep minute ventilation decreases, resulting in an approximately 20% decline in alveolar ventilation [3]. The ventilatory responses to hypoxia and hypercapnia are blunted, requiring larger deviations from baseline to evoke a ventilatory response [3]. Upon initiation of sleep and withdrawal of the wakefulness stimulus, upper airway tone decreases and airway resistance increases, leading to decreased air flow and lower minute ventilation [3, 12]. Despite these physiologic variables which decrease ventilation during sleep, normal individuals continue to maintain adequate ventilation in the absence of additional impairment. However, tumors of the brainstem, cerebellum, and nearby regions may disrupt the neuromotor pathways that maintain respiratory drive and response to chemosensory input. This additional insult may provoke abnormal breathing and respiratory insufficiency during the already-vulnerable sleep state. Specific sleep related breathing disorders and their anatomic tumor associations will be discussed later in this review.

1.2 Assessment Tools for Pediatric Sleep Complaints

Clinicians have a variety of tools available to aid in assessing sleep complaints, characterizing sleep patterns, and evaluating for sleep-related breathing disorders. Validated questionnaires use patient or parent-reported information to identify specific symptoms of concern and determine their severity. Patients may be asked to keep a sleep diary to record sleep quality and duration from day to day. When sleep problems are identified, further objective testing may be indicated in order to determine symptom etiology. An individual’s baseline sleep-wake pattern can be assessed through the use of an actigraph, a wearable device that continuously detects level of activity and uses an algorithm to determine sleep duration and quality from day to day [13]. Overnight polysomnography provides a comprehensive assessment of a single night’s sleep, including information on sleep architecture, respiratory function, and abnormal movements or behaviors [14]. Multiple sleep latency testing (MSLT) in conjunction with polysomnography is used to evaluate for narcolepsy in individuals complaining of excessive daytime sleepiness. The MSLT consists of four to five 20-minute nap opportunities set apart in two-hour intervals, with the goal of defining time to onset of sleep (sleep latency) and sleep-onset rapid-eye movement sleep (SOREM)[15]. Maintenance of wakefulness testing is similar to MSLT but instead measures the ability to stay awake in a sleep-promoting environment [15]. The application of these tests in assessment and diagnosis of specific sleep disorders will be covered later in this review.

1.3 Pediatric CNS Tumor Prevalence, Morbidity, and Mortality

Childhood cancer encompasses a wide variety of malignancies that vary in prevalence by age and sex. CNS tumors represent 26% of all childhood cancers, second only to leukemia [16]. The most common pediatric CNS tumors are astrocytomas, which may arise throughout the CNS and vary in grade and medulloblastomas, which develop in the posterior fossa and are always malignant [17]. While cancer remains the second leading cause of death in children,
improvements in detection and treatment have led to marked improvement in survivorship [16, 17]. However, as cancer survivors, pediatric brain tumor patients have the highest prevalence of long-term morbidities [18]. CNS tumor management is guided by location, size, histology, and grade of the neoplasm. Resection, chemotherapy, and/or cranial radiation therapy (CRT) are the cornerstones of treatment. These treatments, in addition to damage from the tumor itself, can lead to long-term physical sequelae, including motor, sensory, and cognitive deficits, hydrocephalus, seizures, and endocrinopathies [19, 20]. Complications vary by primary tumor location and type of treatment received. Cranial radiation exposure independently increases risk of subsequent neoplasms, seizures, neurologic deficits, and neurocognitive impairment [19]. Patients with tumors within or near the hypothalamus, especially those who have undergone radiation therapy, are prone to neuroendocrine dysfunction and obesity due to disruption of the hypothalamic-pituitary axis. In addition to physical sequelae, pediatric CNS tumor survivors are at higher risk for psychological distress, depression, and lower quality of life than their peers [19, 20]. While data in children are lacking, adult childhood cancer survivors are more likely than their healthy siblings to report fatigue, disrupted sleep, and daytime sleepiness [21]. The presence of sleep issues in cancer survivors is associated with lower health-related quality of life [21, 22]. Thus, early recognition and treatment of sleep problems in pediatric cancer survivors is important not only for physical health and healing but also for preserving mental health and emotional well-being.

1.4 Pediatric Brain Tumors and Sleep

From its onset through survivorship, cancer has the ability to negatively impact sleep through disruption of normal circadian, homeostatic, cardiorespiratory, hormonal, and behavioral influences. Cancer-related psychological distress may also elicit or augment sleep disturbances. There is a greater prevalence of sleep complaints in children with a history of CNS tumors compared to those with other childhood cancers [23–25]. Children with tumors near the hypothalamus, thalamus, and brainstem are the most likely to experience sleep disturbances [23–27]. In 2011, Rosen et al reported that out of 70 pediatric cancer patients referred for sleep problems, 68% had a history of CNS malignancies [24]. Out of all referred patients with CNS neoplasms 73% had hypothalamic, thalamic, or brainstem tumors. In another report of pediatric CNS tumor survivors referred for sleep problems, Mandrell and colleagues found that 55% of referred patients had tumors of the hypothalamus or nearby regions [26]. As previously discussed, a multitude of neurochemical processes controlling sleep are housed within the basal forebrain, diencephalon, and brainstem. In brain cancer patients, the primary tumor, surgical resection, chemotherapy, and radiation may damage and disrupt these pathways, leading to inadequate, ineffective, or dysregulated sleep [23, 25, 27]. Exposure to cranial radiation therapy is associated in a dose-dependent manner with sleep-wake disturbances both during therapy and into adulthood [21, 28, 29]. Neuroendocrine dysfunction may result from disruption of the hypothalamic-pituitary axis and lead to obesity, snoring, and obstructive sleep apnea [23, 26]. Damage to structures in the brainstem and posterior fossa involved in control of breathing may lead to central sleep apnea and hypoventilation [8, 30, 31]. Sleep problems are a significant factor negatively impacting quality of life in cancer survivors but often go under recognized and under reported [21, 32]. In the following sections, we will review the prevalence and etiologies of various
sleep problems reported by children with CNS tumors and discuss our approach to screening, diagnosis, and management.

2. Sleep Problems in Children with CNS Tumors

2.1. Excessive Daytime Sleepiness and Narcolepsy

Excessive daytime sleepiness (EDS) is the most prevalent sleep complaint in pediatric cancer survivors and is more common after brain tumors than other pediatric malignancies [24, 31, 33]. It is the most common reason for referral to a sleep specialist in children with a history of CNS tumors [24, 26, 31]. Several retrospective studies of pediatric CNS tumor survivors have suggested a link between EDS prevalence and CNS tumor location, with a greater proportion of patients having possessed tumors within or near the hypothalamus [24, 26, 31, 34]. However, data remains mixed, as recent prospective surveys of pediatric brain tumor survivors found no association between tumor location and EDS [33, 35]. Other reported risk factors for development of EDS include obesity [26, 36], exposure to cranial radiation therapy [32, 34], and use of antiepileptic medications [34].

Excessive daytime sleepiness often represents the final common manifestation of an array of disorders affecting normal initiation and maintenance of sleep. EDS may be the result of a central neurologic process such as narcolepsy or occur secondary to sleep deprivation from insufficient sleep or sufficient but interrupted sleep. Inadequate sleep in cancer patients and cancer survivors can result from an innumerable host of physiologic, behavioral, and psychologic issues, including sleep-related breathing disorders, circadian rhythm disorders, insomnia, poor sleep hygiene, pain, anxiety, depression, and medication side effects. Subjective indicators of EDS include inappropriately falling asleep during routine daytime activities and new-onset daytime napping. It should be delineated from fatigue, which is characterized by lack of physical or mental energy [37]. EDS is objectively defined by the inability to maintain wakefulness and alertness during normal waking hours, with unintentional or inappropriate sleep occurring daily for three months or more [37].

When pediatric CNS tumor patients exhibit excessive daytime sleepiness in the absence of identifiable secondary contributors, narcolepsy and hypersomnia due to medical condition should be suspected. Narcolepsy is objectively defined by excessive daytime sleepiness plus sleep latency ≤ 8 minutes and two or more episodes of sleep-onset rapid-eye movement sleep (SOREM) on a multiple sleep latency test (MSLT) [37]. Narcolepsy is further classified as type 1 or type 2, with diagnosis of type 1 narcolepsy requiring presence of cataplexy and/or a reduced concentration of orexin in the CSF [37]. Hypersomnia is also defined by presence of excessive daytime sleepiness and MSLT demonstrating sleep latency ≤ 8 minutes. Multiple episodes of SOREMS are not present [37].

Secondary narcolepsy and hypersomnia due to medical condition are not uncommon in children with CNS tumors and may emerge near time of tumor diagnosis or during treatment and survivorship [26, 31, 38–40]. Patients with tumors of the sellar/parasellar and suprasellar/hypothalamic regions, particularly craniopharyngiomas, are especially vulnerable to developing these disorders [26, 38–41]. Mandrell and colleagues reported 31 pediatric patients with a history of brain tumors referred for sleep complaints [26]. Seventeen underwent MSLT and seven were subsequently diagnosed with hypersomnia or narcolepsy. All seven patients had a
history of sellar/parasellar, hypothalamic, or thalamic tumors. Weil et al recently reviewed 26 cases of narcolepsy associated with brain tumors within or near the hypothalamus [40]. Tumor types included craniopharyngiomas, adenomas, gliomas, and germinomas. Ten patients had narcolepsy with cataplexy. Four of five patients who underwent measurement of CSF orexin had abnormally low orexin levels. Twelve of the 26 total patients were symptomatic at the time of tumor diagnosis, while 13 developed narcolepsy after surgery, and one developed narcolepsy after cranial radiation therapy. Most of the patients who developed narcolepsy after surgery had craniopharyngiomas, which are often difficult to resect with significant risk for hypothalamic injury. The patients who did not require extensive surgical resection of the tumor were more likely to have improvement in their narcolepsy symptoms during the treatment course.

In patients with brain tumors damage to orexin-mediated arousal pathways is likely the driving mechanism underlying the development of secondary narcolepsy and hypersomnolence. This is supported by these disorders’ association with tumors in the vicinity of the hypothalamus and the finding of low CSF orexin in some affected patients [26, 40, 41]. Damage to the orexin-containing neurons of the lateral hypothalamus may occur as a result of tumor invasion, during surgical resection, and/or after cranial radiation therapy. The particular association between narcolepsy and craniopharyngiomas is likely attributable to both the proximity of the mass to the hypothalamus and the propensity for hypothalamic injury to occur during resection [40]. We recommend that a high index of suspicion for narcolepsy be maintained in all children with brain tumors near the hypothalamus. In these patients the threshold for objective evaluation of EDS with polysomnography and MSLT should be low.

2.2 Circadian Rhythm Sleep Disorders

Circadian rhythm sleep disorders are characterized by misalignment between an individual’s sleep pattern and the sleep pattern that is considered normal or desired [37]. Circadian rhythm sleep disorders occur through alterations in the circadian timing system [37]. These disorders can be diagnosed through the use of sleep logs or actigraphy and are classified into subgroups based on sleep pattern. Patients with delayed sleep-wake phase disorder fall asleep and wake up two or more hours later than is considered normal, while patients with advanced sleep-wake phase disorder go to bed and wake up earlier [37]. Delayed sleep-wake phase disorder is common in healthy adolescents and young adults with an estimated prevalence of 7-16%, while advanced sleep-wake phase disorder more often occurs in the elderly [37]. Non-24-hour sleep-wake disorder occurs when an individual operates on a sleep-wake cycle greater than 24 hours, leading to progressive delay in sleep and wake from day to day [37]. This disorder is thought to represent failure of 24-hour circadian entrainment due to the absence of photic light-dark input. Most individuals with non-24-hour sleep-wake disorder are blind, though sighted individuals can be affected. Irregular sleep-wake rhythm disorder is characterized by intermittent bouts of sleep throughout the day and night with no discernible rhythm [37]. Periods of sleep typically last less than four hours and patients often complain of excessive sleepiness and/or insomnia depending on the time of day. Irregular sleep-wake rhythm disorder typically occurs in individuals with neurocognitive or neurodegenerative disease and rarely affects healthy children [37].

There is a paucity of data on circadian rhythm sleep disorders in childhood cancer and it is difficult to speculate on the prevalence in patients with CNS tumors. Delayed sleep-wake phase
disorder may presumably occur in adolescent CNS tumor patients given its prevalence in the healthy adolescent population. Children with brain tumors that compromise visual pathways are at risk for blindness and thus at risk for developing non-24 hour sleep-wake disorder [19, 42]. In Rosen and colleagues’ 2011 study, three of 70 pediatric cancer patients were diagnosed with a circadian rhythm disorder [24]. Two of the three had CNS tumors involving the brainstem or hypothalamus. One was an adolescent diagnosed with delayed sleep-wake phase disorder and the other was blind with a likely diagnosis of non-24 hour sleep-wake disorder. Irregular sleep-wake rhythm disorder has been reported in patients with tumors of the pituitary and hypothalamic regions and may be present at time of diagnosis or after tumor treatment [43–47]. These irregular sleep-wake patterns may be attributable to disruption in normal diurnal melatonin secretion. Altered patterns of melatonin secretion with shifted or absent nocturnal peak and/or inadequate daytime suppression have been demonstrated in some patients with tumors of the sellar/parasellar, suprassellar/hypothalamic, and pineal regions [46–52]. The specific melatonin-regulating circadian pathway affected varies with tumor location. Tumors affecting the optic chiasm may interrupt photoneural input to the SCN travelling via the retinohypothalamic tract [51]. Sellar and suprasellar tumors may directly damage the SCN or its melatonin-regulating afferents [47, 50]. Pineal tumors may disrupt the distal end of the melatonin pathway, impairing melatonin release from the pineal gland [52].

The therapeutic approach to circadian rhythm sleep disorders includes interventions to aid in entrainment to the normal 24-hour circadian cycle and exogenous melatonin administration to shift an individual’s current circadian phase in the desired direction [53]. Strategically timed oral melatonin administration is the cornerstone of therapy, with additional behavioral interventions such as prescribed sleep-wake scheduling and deliberate periods of light avoidance and exposure [53]. This approach may be successfully applied to affected patients with CNS tumors [49, 52]. In one study, melatonin substitution in pediatric patients with craniopharyngiomas not only resulted in better diurnal variation in salivary melatonin but also yielded improvements in daytime sleepiness and physical activity [49].

### 2.3 Insomnia and Interrupted Sleep

Insomnia is characterized by difficulty initiating and maintaining sleep or experiencing non-restorative sleep. This disruption occurs despite adequate opportunities for sleep and leads to impairment in daytime function [37]. Insomnia often co-occurs with excessive daytime sleepiness and fatigue and like EDS, may have a multitude of secondary causes. Insomnia is purported to be the most common sleep problem in adults with cancer, including those with primary brain tumors [32, 54]. Insomnia and interrupted sleep are common complaints in children still receiving cancer therapy but are less often reported than other sleep complaints in cancer survivors [24, 55]. In Rosen et al’s 2011 study 17 out of 70 pediatric cancer patients were referred for insomnia, most of whom were still receiving cancer therapy [24]. The prevalence of insomnia in patients with brain tumors was not significantly different from those with non-CNS malignancies. Identified etiologies included behavioral issues, pain, and high-dose corticosteroid therapy. Robertson and colleagues also identified ongoing corticosteroid therapy to be associated with insomnia in adult patients with CNS tumors but notably found a lack of association between insomnia and tumor location or use of other medications such as antidepressants, antipsychotics, and stimulants [56].
Insomnia during cancer treatment may occur as a result of numerous physiologic, environmental, and psychologic factors which may negatively impact sleep; however, knowledge of insomnia prevalence and associations among pediatric brain tumor survivors remains limited. Nolan and colleagues compared adult survivors of childhood CNS tumors to matched controls and found no significant difference in overall sleep quality between the two groups, although survivors were nearly three times more likely to take longer to fall asleep [57]. Zhou et al reported insomnia in 25 of 98 adult childhood CNS tumor survivors but identified no statistically significant associations, including cancer diagnosis, cancer recurrence, type of treatment received, or presence of depression/anxiety [58]. Thus while it seems pediatric brain tumor survivors may experience more symptoms of insomnia than the general population, the etiology remains unclear.

2.4 Parasomnias and Sleep Related Movement Disorders

Parasomnias represent a heterogeneous category of sleep disorders involving undesirable physical behaviors or experiences that occur during sleep onset, within sleep, or during arousal from sleep [37]. Consciousness consists of three distinct states: wake, rapid eye movement (REM) sleep, and non-REM sleep. Normal transitions between these states arise through the coordinated interplay of various sleep and wake pathways. Parasomnias are thought to occur as a result of dysfunctional state-to-state transitions which leave the individual in an unstable, dissociated state of consciousness. Disinhibition of physiologic functions that are normally suppressed during sleep leads to abnormal behaviors [37]. Examples of these disorders include somnambulism, sleep-related eating, sleep terrors, and dream-enacting behaviors [37]. Video polysomnography is helpful in diagnosis by allowing characterization of the abnormal event and determining the sleep stage in which it occurs [37].

There is insufficient data to determine the prevalence of parasomnias in children with brain cancer compared to healthy children or children with other malignancies. There are isolated case reports of patients experiencing parasomnias as a presenting symptom leading to tumor diagnosis [59–62]. Pilotto and colleagues conducted a retrospective study of comparing 29 pediatric CNS tumor survivors to healthy controls and identified an increased frequency of parasomnias in patients [63]. However, the study was inadequately powered to detect associations with tumor location or treatment type. Which specific disorders were detected within the category of parasomnias was not reported by the authors.

Sleep related movement disorders are a group of conditions characterized by stereotypic movements that occur during sleep [37]. Sleep related movement disorders are distinguished from parasomnias by the simplicity of the abnormal movement. Disorders that fall into this category include bruxism, restless legs syndrome, periodic limb movement disorder, and sleep-related rhythmic movement disorder [37]. There is a paucity of data on prevalence of these disorders in cancer patients and even less in pediatric patients with CNS tumors. Some reports suggest that high-dose chemotherapy in cancer patients may be associated with restless leg syndrome and increased periodic limb movements during sleep [64, 65]. Ostacoli and colleagues reported a restless leg syndrome prevalence of 18.3% in adult patients undergoing therapy [64]. Restless leg syndrome was associated with anxiety, depression, and lower quality of life. Further research is needed to determine the prevalence of restless leg syndrome and other sleep related
movements disorders in children with cancer, including those with CNS tumors, to promote early
detection and treatment.

2.5 Sleep Related Breathing Disorders

Sleep related breathing disorders encompass a wide variety of issues affecting ventilation
during sleep, including obstructive sleep apnea (OSA), central sleep apnea (CSA), sleep-related
hypoxemia, and central hypoventilation [37]. Children with sleep related breathing disorders may
present with labored breathing or witnessed apneas during sleep, snoring, excessive daytime
sleepiness, non-restorative sleep, daytime hyperactivity, and behavioral issues. Polysomnography
is required for diagnosis and should be scored using the most contemporary American Academy of
Sleep Medicine (AASM) criteria [37, 66]. Children with CNS tumors may experience damage to the
neurochemical and neuromotor pathways that coordinate breathing or develop conditions which
lead to increased airway resistance, predisposing them to abnormal breathing during sleep [24, 26,
30, 31, 67]. It is difficult to estimate the overall prevalence of all sleep related breathing disorders
in pediatric patients with CNS tumors. In Rosen’s 2011 study, sleep related breathing disorders
were diagnosed in 28 out of 70 pediatric cancer patients, 20 of whom had CNS tumors [24].
Diagnoses included OSA, CSA, and sleep-related hypoxemia. Multiple sleep related breathing
disorders may co-occur in patients with tumors of the brainstem and posterior fossa [8, 9, 68].
Fujimoto et al recently reported a 12 year-old obese male presenting with OSA, CSA, and sleep-
related hypoventilation who was found to have a tumor within the medulla [68]. Lee and
colleagues described four pediatric patients with medulloblastomas involving the fourth ventricle
without brainstem invasion, all who developed symptoms of sleep-disordered breathing years
after resection, chemotherapy, and radiation [8]. All four patients demonstrated obstructive sleep
apnea, central sleep apnea, and hypoventilation on polysomnography. Thus it is important to
maintain a high suspicion for sleep related breathing disorders in patients with posterior fossa
tumors from diagnosis through survivorship, even when no brainstem involvement is suspected.

Obstructive sleep apnea. Obstructive sleep apnea (OSA) is the most common sleep related
breathing disorder in children, affecting up to 5% of the pediatric population with a peak incidence
from two to six years of age [69]. OSA occurs as a result of complete or partial airway obstruction
leading to intermittent episodes of reduced or absent airflow during sleep [37, 69]. In healthy
children, OSA is most often attributable to enlarged tonsils and adenoids. Other conditions that
contribute to airway obstruction include obesity and craniofacial abnormalities as well as
neurologic impairment, which may lead to hypotonia and weakness of airway and ventilatory
muscles [69–72].

Similar to healthy children, OSA in pediatric patients with CNS tumors may be multifactorial.
Obesity is a significant risk factor for OSA and may occur through unique mechanisms specific to
brain tumor patients. Children with brain tumors may develop obesity as a side effect of
medications such as corticosteroids, from reduced caloric expenditure in the setting of physical
morbidity, or as a result of neuroendocrine derangement. Examples of neuroendocrine
abnormalities that may cause weight gain include hypothyroidism, Cushing’s disease, and
hypothalamic obesity. Hypothalamic obesity results from damage to the hypothalamic pathways
regulating satiety and energy balance and is characterized by hyperphagia, decreased metabolic
rate, and rapid weight gain even after caloric restriction [73]. This damage may be the result of
tumor invasion, surgical injury, or radiation therapy [73]. Mandrell and colleagues retrospectively reviewed 31 pediatric CNS tumor survivors who underwent polysomnography and found that 14 had OSA, the majority of whom were obese [26]. Nine of the 14 patients had tumors of the sellar/parasellar and hypothalamic regions. Other tumor locations included the posterior fossa, pineal gland, and optic nerve. Independent of obesity, OSA may occur due to impaired contraction of the pharyngeal dilator muscles involved in maintaining upper airway patency [23, 67]. Normal function requires neuromotor input from the glossopharyngeal, vagus, and hypoglossal nerves, which exit the medulla just below the inferior cerebellar peduncles. These neural pathways may be disrupted by posterior fossa tumors, leading to discoordination or weakness of the affected muscle groups [67].

Treatment of OSA in healthy children and children with CNS tumors involves supporting airway patency through the use of positive airway pressure and interventions to alleviate the source of airway obstruction. Adenotonsillectomy should be considered in those with enlarged tonsils and adenoids, although in the presence of other tumor-related factors, OSA may persist despite surgery. Continuous positive airway pressure (CPAP) is an appropriate treatment for OSA in children without central sleep apnea or hypoventilation [74]. CPAP initiation and management should be overseen by a sleep physician or pulmonologist working in conjunction with a multidisciplinary team consisting of a respiratory therapist, nurse, and psychologist to aid in equipment fitting, habituation, and adherence [74]. Acclimatization techniques should be utilized to improve compliance and include trial of different mask types to determine best comfort and fit, use of mask during the day to accustom the child to the sensation, and use of ramp mode at the beginning of sleep to gradually increase the delivered pressure to goal. Weight loss should be encouraged in obese patients. Management of secondary obesity due to neuroendocrine dysfunction by an experienced endocrinologist is critical [75]. More research is needed to identify differences in treatment response and prognosis for improvement in children with CNS tumors and OSA compared to other children.

Central sleep apnea and sleep-related hypoventilation. Central sleep apnea (CSA) occurs when ventilatory control pathways fail to initiate respiratory effort, leading to pauses in breathing [37]. In patients with CNS tumors abnormal respiratory drive results from acquired injury to the pathways controlling ventilation, which may occur through direct tumor invasion or surgical resection [8, 9, 30, 31, 76]. While injury to the respiratory centers of the medulla is of particular concern, tumors in other regions of the brainstem, posterior fossa, and surrounding areas may also damage of key pathways and result in CSA [8, 9, 24, 26, 30, 31, 76]. Sleep related central hypoventilation represents a separate type of sleep related breathing disorder that often co-occurs with CSA and arises through similar mechanisms [8, 37, 77]. Like CSA, it is usually reported in relation to tumors of the brain stem and posterior fossa [8, 76, 78, 79].

Treatment of CSA and sleep-related hypoventilation is often challenging. Surgical tumor resection may lead to improvement in symptoms but does not guarantee complete resolution [78]. Patients may require long-term positive pressure ventilation during sleep, either non-invasively using bilevel positive pressure ventilation (BPAP) or invasively via tracheostomy and home mechanical ventilation. As with CPAP, initiation of BPAP should begin with mask fitting and acclimatization under the guidance of a multidisciplinary team. An age-appropriate backup rate should be used in all patients with central hypoventilation, bradypnea, or those that are unable to reliably trigger spontaneous breaths [80]. Patients should undergo titration of BPAP settings via
polysomnography to determine the optimal settings for adequate gas exchange per AASM guidelines [80]. Tracheostomy and mechanical ventilation should be considered in those with central apneas, bradypnea, or hypoventilation while awake. It should also be considered in those with intolerance to BPAP or poor treatment response. Treatment decisions should be made through bidirectional communication between the patient’s family and medical providers. These choices should be guided by the patient’s clinical status, presence of comorbid conditions, prognosis, and overall goals of care.

3. Screening and Diagnostic Approach

Given the prevalence of sleep complaints in children with CNS tumors, we recommend that the patient’s primary pediatrician or oncologist inquire about sleep problems during routine visits. The interview should elicit information on sleep-wake timing, sleep latency, excessive sleepiness, nighttime awakenings, perceived sleep quality, and daytime sleep habits. If significant sleep complaints are identified, referral to a pediatric sleep specialist should be considered. Preliminary evaluation of sleep-wake disturbances can begin with the use of validated questionnaires and patient sleep logs. Because of the age variation and the heterogeneous nature of sleep complaints, no single questionnaire is adequate for all patients. The Patient-Reported Outcomes Measurement Information System (PROMIS) questionnaires on Pediatric Sleep Disturbance and Sleep-Related Impairment may be used to obtain a subjective assessment of daytime sleepiness, impairment in daily function, and difficulties with sleep onset and maintenance [81,82]. The Pittsburgh Sleep Quality Index (PSQI) is a validated and widely used tool to evaluate sleep timing, sleep quality, and sleep efficiency [83]. Questionnaires that aid specifically in evaluating excessive daytime sleepiness in children include the Epworth Sleepiness Scale (ESS)[84] and the Pediatric Daytime Sleepiness Scale (PDSS)[85]. The ESS assesses severity of daytime sleepiness through self-reported propensity to fall asleep during various activities. The PDSS measures daytime sleepiness in relation to school performance to determine degree of impairment. Actigraphy should be considered in patients with symptoms of excessive daytime sleepiness, insomnia, or difficulty initiating and maintaining sleep [13]. Actigraphy is useful in assessing for abnormal sleep patterns caused by circadian rhythm sleep-wake disorders and central disorders of hypersomnolence. Patients with severe hypersomnolence or excessive daytime sleepiness, especially those with tumors of the hypothalamus and surrounding regions, should undergo polysomnography with a multiple sleep latency test to assess for narcolepsy. Evaluation for parasomnias with video polysomnography may be indicated in patients with frequent nighttime awakenings or abnormal sleep behaviors. Patient or parental reports of daytime sleepiness, hyperactivity, non-restorative sleep, snoring, irregular breathing, and/or witnessed apneas are concerning for sleep-disordered breathing and should prompt timely assessment with polysomnography.

4. Conclusions

Children with brain tumors are at increased risk for experiencing abnormal sleep from the time of tumor diagnosis, through treatment, and during survivorship. They may be affected by an array of pathologic sleep conditions including sleep related breathing disorders, narcolepsy, circadian rhythm sleep-wake disorders, insomnia, parasomnias, and sleep-related movement disorders. Recognizing associations between these disorders and tumor location or type of treatment
received may aid in prompt diagnosis and intervention. Due to their propensity to negatively impact overall health and quality of life, screening for sleep problems should be part of anticipatory care in children with CNS tumors.

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Author Contributions

Dr. Maloney performed the literature review. She conceptualized and wrote the majority of the manuscript. Dr. Lewinter assisted in reviewing the literature and writing the manuscript. Dr. Davidson Ward reviewed and edited the manuscript for concept, content, and clarity. Dr. Perez conceptualized the manuscript topic and scope. She guided the literature review and supervised the manuscript writing process. She reviewed and edited the final manuscript.

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TTF-1-Expressing Supratentorial Embryonal Tumors (PNET): A Clinicopathologic Study of Two Cases and Literature Review of TTF-1-Positive Primary Brain Tumors

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Abstract
Thyroid transcription factor 1 (TTF-1) is a nuclear polypeptide and a tissue-restricted member of the homeobox protein family that, when attached to DNA, plays a crucial role in regulating the expression of select genes during early embryonic development of the thyroid, lung, and brain.
As often happens, the initial enthusiasm for the diagnostic value of TTF-1 as a selective immunohistochemical marker of lung and thyroid tumors began to dwindle, due to its detection in tumors arising from other organs.

TTF-1-expressing primary brain tumors arising in the 3rd ventricle were first reported in 2004. More recently, tumors arising from the posterior hypophysis (pituicytomas, granular cell tumors of the sellar region, and spindle cell oncocytomas of the pituitary gland) as well as chordoid gliomas of the third ventricle and subependymal giant cell astrocytomas have been proven to be TTF-1 immunoreactive.

We present for the first time the expression of TTF-1 in two primary embryonal tumors (PNETs) of the anterior basal brain. One was in a 6½-year-old female and the other in a 36-year-old female.

General surgical pathologists and neuropathologists should be aware of the potential TTF-1 expression in tumors of the ventral forebrain.

Keywords
Thyroid transcription factor 1 (TTF-1); brain tumor; primitive neuroectodermal tumor (PNET); central nervous system (CNS); embryonal CNS tumor

1. Background and Aims

Human thyroid-specific transcription factor-1 (TTF-1), also known as thyroid-specific enhancer-binding protein (T/EBP or NKX2.1), is a 371 amino acid-long polypeptide (with a molecular weight of 38 to 40 kDa), and is a homeodomain-containing transcription factor of the NK-2 family [1]. Human TTF-1 is encoded by a single gene located on chromosome 14 and is organized into two or three exons and one or two introns [1, 2]. The known molecular targets of TTF-1 in the thyroid are thyroglobulin, thyroid peroxidase, and thyrotropin receptor genes [1, 2].

TTF-1 expression has also been found in the bronchioalveolar epithelium of the lung, where it similarly functions as a transcriptional activator of specific genes, such as alveolar type II cell surfactant protein genes and the bronchiolar Clara cell secretory protein gene [1, 3-7]. It has also been observed in the ventral forebrain where the responsive genes are mostly unknown. TTF-1 expression in the central nervous system (CNS) has been documented in the ventral neuroepithelium of the anterior wall of the third ventricle and hypothalamic areas, including the infundibulum, which is the site of derivation of the neurohypophysis [1, 3, 4, 8, 9].

TTF-1 plays a fundamental role in organogenesis by regulating the expression of additional genes that are directly involved in development and differentiation [6, 10-12]. Experimental studies have demonstrated that in T/EBP knock-out homozygous mice, the embryonic development of the thyroid, lung, hypothalamus, pituitary, and globus pallidus is dramatically impaired [1, 3, 10, 13-16]. Limited information exists on the regulation of TTF-1 gene expression by hormones, cytokines, and other biological agents [2].

For many years, TTF-1 has been extensively used for diagnostic purposes in surgical pathology as a lineage-specific immunohistochemical marker, most often for primary and secondary tumors of the lung and thyroid [17-21], and especially of neuroendocrine lineages [22].
Two monoclonal antibodies to TTF-1 are commercially available: the SPT24 clone (Novocastra Laboratories, Newcastle Upon Tyne, UK), which is currently known to be more sensitive than the other [23], and the 8G7G3/1 clone (Dakocytomation, Carpinteria, CA, USA), which conversely, is considered more specific [21, 24].

Over time, many reports soon documented its sporadic or frequent expression in tumors from other sites, such as the gastrointestinal tract, breast, urinary bladder, and prostate. The majority of these tumors are of neuroendocrine nature, mainly small-cell carcinomas [22, 25-29]. In a lesser proportion, others belong to a wide variety of cell and tissue types [23, 30-35], including nephroblastomas [29]. Subsequently, nuclear TTF-1 immunostaining was documented in primary brain tumors as well [36-46].

Therefore, the presence of TTF-1 immunoreactivity in a metastatic small cell (neuroendocrine) carcinoma cannot by itself be used as a sign of pulmonary or thyroidal origin, and in this situation, an extrapulmonary source cannot be excluded [21, 23, 29]. Likewise, even when dealing with metastatic non-small cell carcinoma, the possibility of a remote deposit from other sources (such as breast, uterus, gastrointestinal tract, and others) cannot be ruled out based on the sole TTF-1 immunopositivity in tumor cells [21, 23, 29]. This occurs also with the 8G7G3/1 clone, albeit in a lower percentage of cases, in comparison with the SPT24 clone [21].

In 2011, Galliani and Bisceglia analyzed TTF-1 expression with the 8G7G3/1 clone in a series of 117 varied embryonal tumors [47], excluding nephroblastomas, which represented the subject of a previous and separate study [29]. This large series of embryonal tumors, both in children and adults [47], considered 92 tumors from extra-CNS sites and 25 primitive CNS neuroectodermal tumors (cPNETs, which are currently referred to as CNS-embryonal tumors, according to 2016 WHO classification of tumors of the central nervous system [48]).

Of the extra-CNS embryonal tumors, 28 were peripheral PNETs (i.e., members of the Ewing’s sarcoma family), 14 were peripheral thoracoabdominal neuroblastomas, 18 were embryonal rhabdomyosarcomas, 16 were alveolar rhabdomyosarcoma, 5 were desmoplastic small round cell tumors, 4 were hepatoblastomas, 4 were pleuropulmonary blastomas, 1 was a paraganglioblastoma, 1 was a pancreatoblastoma, and 1 was an undifferentiated liver sarcoma. None of these expressed immunoreactivity for TTF-1 [47].

Moreover, the 25 central PNETs of our series included 14 infratentorial PNETs (medulloblastomas), 4 supratentorial PNETs, 1 inframedullary PNET, 3 supratentorial neuroblastomas (including 1 olfactory neuroblastoma), 2 pineoblastomas, and 1 retinoblastoma [47]. Of all these tumors, just one pediatric suprasellar (classic) PNET from 2008 expressed TTF-1, while all the rest failed to do so.

In the same year, another case of a TTF-1-expressing PNET in a 36-year-old woman was observed during routine work. This latter case was histologically diagnosed as PNET with anaplastic features, as previously reported by other authors [49, 50].

We report herein the findings as well as the clinical and morphological features of these two cases of TTF-1 immunoreactive suprasellar and parasellar PNETs, one in a female child and the other in a young lady, respectively, taking the opportunity to review the entire rubric of TTF-1 immunopositive primary brain tumors reported in the literature.
2. Case Reports

2.1 Case 1

Computed tomography (CT) scan and magnetic resonance imaging (MRI) of the head performed on a 6½-year-old girl, who was brought in semiconscious state to the emergency department of the Casa Sollievo della Sofferenza hospital in San Giovanni Rotondo (Italy) in 2008, disclosed an 8.0 cm midline, contrast-enhancing, suprasellar tumor that protruded into the 3rd ventricle and encased the intracranial carotid arteries (Fig. 1 [A-F]). The suprasellar tumor was partially resected.

Histopathologically, an intraoperative frozen section was interpreted as a small round cell tumor of unspecified origin (Fig. 2A). Permanent sections confirmed a small round blue cell neoplasm with perivascular pseudorosettes and Homer Wright rosettes, brisk mitotic and apoptotic activity, and foci of necrosis (Fig. 2 [B-G]).
Immunohistochemically, the tumor cells were focally positive for synaptophysin, neurofilaments, and glial fibrillary acidic protein (GFAP), and negative for S100 protein, desmin, myogenin, cytokeratins (wide spectrum), CD45, and CD99. INI-1 was normally expressed. Proliferation index, as assessed by Ki-67/MIB-1, was 50%.

Since a study of TTF-1 expression in small round cell tumors from any location was in progress, this case was included as well.

Fig. 2 (A-D) Histopathology. (A) Frozen section preparation, Hematoxylin & Eosin stain. Patternless sheets of monotonous neoplastic small-round-cells of indeterminate origin. (B) Paraffin embedded permanent section, H&E, low-power view. Embryonal tumor with early spotty necrosis in the center, and perivascular pseudorosettes narrow arrows. (C) H&E, higher magnification. Small, undifferentiated tumor cells with perivascular alignment (not indicated), and Homer Wright rosettes (broad arrow). (D) Neoplastic cells with cytoplasmic pseudoclearing, pericellular artifact.
Fig. 2 (E-G) Histopathology (cont’d). *Paraffin embedded permanent sections, Hematoxylin & Eosin stain.* Embryonal tumor exhibiting perivascular pseudorosettes (*narrow arrows*) and Homer Wright rosettes (*broad arrows*).

TTF-1 was detected according to the following procedure. Antigen retrieval was heat-induced on deparaffinized 4 μm sections for two cycles each of 15 minutes in 10-mM citrate buffer (pH=6) using a 360 W microwave oven. Immunostaining was performed with the labelled streptavidin-biotin peroxidase complex system (LSAB2), using the monoclonal 8G7G3/1 (Dako) antibody to TTF-1 diluted 1:30 in a Dako Autostainer (Dako). Endogenous peroxidase was blocked, diaminobenzidine was employed as a chromogen, and counter-stain was done with hematoxylin. The sections were evaluated by two observers and judged to be positive for TTF-1 in at least 50% of the nuclei of the tumor cells (Fig. 3 [A-B]).

Fig. 3 (A-B) TTF-1 immunohistochemistry (*monoclonal antibody 8G7G3/1*). Nuclear expression in at least 50% of the tumor cells.
Diagnosis: central PNET with early neuroblastic differentiation and TTF-1 expression.

Follow-up: The patient received craniospinal radiation and chemotherapy, including temozolamide and VP-16. Imaging performed one year after the partial resection revealed residual tumor. The patient died of her disease 20 months after the diagnosis.

2.2 Case 2

During CT and MRI investigation for intracranial hypertension, a 36-year-old female who was admitted in 2011 at the Santa Maria della Misericordia hospital in Udine (Italy) was found to have a huge ventral brain tumor involving the diencephalon (Fig. 4 [A-D]). Subtotal tumor resection was performed.

Histopathologically, the tumor was highly cellular and comprised of small round blue cells sprinkled with large, pleomorphic, hyperchromatic, and anaplastic-appearing nuclei; a high
mitotic–apoptotic index; and contained foci of necrosis. No rosettes or perivascular pseudorosettes were observed (Fig. 5 [A-H]).

![Image of histopathology](image)

**Fig. 5** (A-D) Histopathology. (A-B) Small round-blue cell tumor, showing microgeographical foci of necrosis in A (center towards the top) and B (top right and bottom left). (C-D) Higher power views. Several mitotic and apoptotic figures are seen. *(Hematoxylin & Eosin stain on paraffin embedded tissue sections).*

![Image of histopathology](image)

**Fig. 5** (E-H) Histopathology (cont’d). This tumor also showed throughout a variable range of anaplastic features with “bizarre giant cells”, apoptotic bodies, necrosis, and atypical mitoses, similar to the description made in the literature in two PNETs of adults *(the reader is advised to see panels 5 and 8 of Fig. 5 in ref. 50).*
Immunohistochemically, the tumor cells showed diffuse cytoplasmic immunoreactivity for synaptophysin and NSE. Focal immunopositivity in a certain percentage of cells was observed for nestin, Neu-N, and neurofilament proteins with mean values of 30%, 15%, and 30%, respectively (Fig. 6 [A-D]). Conversely, CD45, CD99, S100 protein, GFAP, chromogranin A, cytokeratin 7, cytokeratin 8 (CAM5.2), desmin, and myogenin were all negative. p53 was diffusely expressed in the nuclei of tumor cells. The Ki67 tumor proliferation fraction assessed with the MIB-1 antibody was very high (over 90%).

TTF-1 nuclear expression was detected with immunostaining using the monoclonal 8G7G3/1 antibody diluted 1:50 in the EnVision FLEX discovery system (Dako, Glostrup, Denmark) after heat-induced pretreatment of deparaffinized 4 µm tissue sections in citrate buffer (pH=6.1) for antigen retrieval. Endogenous peroxidase activity was blocked, dianobenzidene was used as a chromogen, and hematoxylin as a counterstain. Then, the immunoreactivity was evaluated by two observers, and judged to be positive in the nuclei of 50% of tumor cells (Fig. 6 [E-F]).

**Fig. 6 (A-F) Immunohistochemistry.**  
(A) [*Neu N*] Nuclear immunoreactivity in 50% of tumor cells. (B) [*Neurofilament proteins*] Cytoplasmic immunostaining with perinuclear crescentic positivity in 50% of tumor cells. (C) [*Synaptophysin*]. Diffuse cytoplasmic immunopositivity. (D) [*Nestin*] Cytoplasmic immunoreactivity in over 50% of tumor cells. (E) [*p53*] Variously strong nuclear expression. (F) [*TTF-1*] Intranuclear expression in over 50% of nuclei of tumor cells (*monoclonal antibody 8G7G3/1*). **Inset:** detail of immunoreactivity. **Note:** The estimated percentage of positive cells for panels A-D, and panel F, is displayed on the pictured tumor fields: the mean value for each marker is given in the text.
For the sake of completeness, Wilms' tumor 1 protein (WT1), which is a transcriptional activator or repressor factor that controls genes that mediate the epithelial-to-mesenchymal transition and shuttles between the nucleus and cytoplasm, was also included in this extensive immunohistochemical analysis, revealing focal cytoplasmic positivity (40% of tumor cells) with a partial crescentic perinuclear pattern.

**Diagnosis:** central PNET with early neuroblastic differentiation and TTF-1 expression.

**Follow-up:** follow-up was lost on the patient. It is presumed that she received radiation treatment in another institution. The patient died of tumor-related causes 2 years and 10 months after diagnosis.

3. Discussion

In the experimental rodent CNS model, TTF-1 expression was detected in the ependymal and subependymal cells of the ventral neuroepithelium of the 3rd ventricle, including neurons of select hypothalamic nuclei, astrocytes of the median eminence, pituicytes of the infundibular stalk and neurohypophysis, and in the adjacent extrahypothalamic (rat) suprachiasmatic nucleus and subfornical organ [3, 12, 15, 51, 52].

In 2004, Zamecnik et al. used monoclonal antibodies against TTF-1 (using the 8G7G3/1 clone) and documented TTF-1 expression in two cases of ependymomas (one grade II and one grade III, both localized in the 3rd cerebral ventricle in a 5-year old boy and in a 12-year old girl, respectively) out of 73 primary brain tumors investigated, including 33 astrocytic tumors of various grades, 27 classic ependymomas (11 grade II and 16 grade III), 7 medulloblastomas, and 3 gangliogliomas [36].

In 2006, Prok and Prayson used the same 8G7G3/1 clone and studied 50 cases of glioblastomas but did not prove immunopositivity in any [37]. In 2007, Galloway and Sim investigated TTF-1 expression in 28 cases of glioblastomas using both clones currently available (the 8G7G3/1 and SPT24): 14 glioblastomas proved positive when SPT24 clone was used, while no case was immunopositive with the 8G7G3/1 clone [38]. Galloway and Sim first questioned the specificity of TTF-1 immunopositivity in their 14 glioblastomas [38].

In 2009, Lee et al., using the 8G7G3/1 clone, studied a series of five pituicytomas and four granular cell tumors arising from the posterior lobe of the pituitary, all of which expressed TTF-1 [39].

Again in 2011, Kristensen et al. used the SPT24 clone and demonstrated TTF-1 immunoreactivity in approximately 18% of high-grade astrocytic and oligodendrocytic gliomas (10 immunopositive cases out of 56 grade III to grade IV tumors), and no immunoreactivity in all 47 low-grade astrocytic and oligodendrocytic tumors [40]. Additionally, they also found TTF-1 immunopositivity in one of three central neurocytomas, one of 18 ependymal tumors, and one of five choroid plexus tumors, but no positivity was observed in any of four pineal tumors, 11 meningiomas, eight PNETs, or four mixed glio-neuronal tumors [40]. However, Kristensen et al. could not document any immunoreactivity in any of the above brain tumors when the clone used was 8G7G3/1 [40].

In 2014, similar results to Kristensen’s study were experienced by Unal et al., who analyzed 45 primary brain tumors (one grade I, seven grade II, four grade III, 20 grade IV astrocytic tumors, 9 meningiomas, two oligodendrogliomas, one schwannoma, and one medulloblastoma) with both
monoclonal antibodies. These authors found four high-grade astrocytic tumors to be TTF-1 immunopositive when using the SPT24 clone, while no case was positive using the other clone [41].

All the above brain tumors investigated for TTF-1 expression were from several various areas and sites of the encephalon. Therefore, based on our own and others’ experiences [21, 24], it must be said that the SPT24 clone is more sensitive but less specific than the 8G7G3/1 clone, even in brain tumors pathology [36-41].

During the last decade, nuclear TTF-1 immunostaining has been specifically documented in most (if not all) sellar/suprasellar neurohypophyseal tumors studied.

In 2009, Lee et al. used the 8G7G3/1 clone and studied a series of five pituicytomas and four granular cell tumors arising from the posterior lobe of the pituitary gland: all of these neurohypophyseal tumors expressed TTF-1 [39].

In 2010, Bisceglia and Galliani also investigated seven differentiated brain tumors (two central neurocytomas, three pineocytomas, and four subependymomas located either in a lateral or the 4th ventricle), but did not observe any immunostaining in any of them [53].

In 2013, Mete et al. investigated seven spindle cell oncocytomas, four pituicytomas, and three granular cell tumors, all of which showed nuclear positivity for TTF-1 (using the SPT24 clone). On the basis of the same immunohistochemical profile, including several other markers, these authors also concluded that these three groups of tumors likely share a common histogenesis, and all are variants of pituicytoma [54]. This interpretation was already anticipated [55], concordant with reviews of the subject [56, 57, 58], which proposed nomenclature of spindle cell oncocytoma as oncocytic pituicytoma, granular cell tumor as granular cell pituicytoma, and sellar ependymoma as ependymal pituicytoma [56].

TTF-1 is considered to be an excellent marker of (both fetal and adult) pituicytes, the specialized glial cells of the neurohypophysis [39], although the matter of a common histogenesis for the entire group of TTF-1 immunopositive sellar/parasellar tumors (i.e., pituicytomas, granular cell tumors of the sellar region, spindle cell oncocytomas, and sellar ependymomas) is still not definitely settled [59-63].

Three separate cases of TTF-1 immunopositive mixed pituicytoma, one with classic pituicytoma, spindle cell oncocytoma, and an ependymal component [56], a second with epithelioid oncocytic, follicle formation, and ependymal differentiation [61], and a third with spindle cell oncocytoma and (pituicytic) ependymal differentiation [64], point to unify their derivation. Nuclear TTF-1 expression was documented in the ependymal component [64] (also called “follicle formation/ependymal differentiation” or “follicle-like organotypic differentiation”) in the classical pituicytomatous [56, 61] and in the spindle cell oncocytomatous component [64]. TTF-1 expression was detected using the monoclonal SPT24 antibody in two different cases [61, 64]. In a third case, the antibody used was not specified by the authors (presumably SPT24) [56].

In the 4th edition of the 2017 World Health Organization classification of pituitary tumors [65], all TTF-1 positive, non-neuroendocrine, low-grade (WHO grade I) sellar/suprasellar tumors of the posterior hypophysis (and infundibular stalk) are included and discussed, and subsequently reiterated and emphasized [66-68].

Parenthetically, on reviewing this subject, we found that approximately 70 cases of (classic) pituicytomas [46, 57], 25 cases of spindle cell oncocytomas [62], approximately 100 cases of sellar granular cell tumors [58], and 9 cases of intrasellar ependymomas [69, 70] have been recorded in literature thus far.
Furthermore, in 2014, Michotte et al. reported for the first time TTF-1 expression (with the 8G7G3/1 clone) in a chordoid glioma, a low-grade tumor (WHO grade I) with selective predilection for the 3rd ventricle, thus expanding the group of TTF-1 immunoreactive sellar/parasellar tumors [42].

Using both monoclonal antibodies, Bielle et al. reported in 2015 on 17 cases of such tumors assembled from a multi-institutional study. They found universal immunoreactivity in all of them (17 out of 17) using the SPT24 clone with a 60 to 100% positive index of tumor cells. When the 8G7G3/1 clone was employed, less intense and variable immunoreactivity was detected in 15 of 17 cases as follows: 5 to 20% positive cells were observed in 11 cases, 30 to 70% in 3 cases, and no significant reactivity in 1 [43]. These investigators also demonstrated TTF-1 expression in both the embryonal and adult lamina terminalis of the rat, giving support to the hypothesis of the origin of the chordoid glioma from ependymal cells or tanycytes of the circumventricular vascular organs of the lamina terminalis, including the subfornical organ and the subcommissural organ [43, 71]. In other terms, the so-called chordoid glioma is not a real glioma, and its originary cells are almost always located in the anterior wall of the 3rd ventricle, indicating that its origin is almost always the same.

Almost contemporarily, Hewer et al. reported another case of a TTF-1 immunopositive chordoid glioma, using both the 8G7G3/1 and SPT24 clones. These authors included in the study three new cases of spindle cell oncocytomas, all of which were immunoreactive for TTF-1 with both monoclonal antibodies [44]. In their experience, the immunoreactivity was of variable intensity with the 8G7G3 clone and diffusely strong with the SPT24 clone.

Again, Hewer et al. analyzed seven cases of subependymal giant cell astrocytomas of the tuberous sclerosis complex, which are tumors almost consistently located along the caudate nuclei in the lateral ventricles. They documented diffuse TTF-1 immunoreactivity in these tumors using both monoclonal antibodies. Based on these findings, they proposed a lineage-committed derivation from regional-specific cells related to the medial ganglionic eminence, the ventral septal region, and from the anterior wall of the 3rd ventricle of the developing basal brain, under the regulatory influence of TTF-1 [45].

In 2016, Wang et al. analyzed TTF-1 expression (with the 8G7G3/1 clone) in 3 chordoid gliomas, and included 11 cases of pituicytoma and 23 low-grade astrocytomas (16 pilocytic, 4 diffuse, and 3 pilomyxoid astrocytomas): all chordoid gliomas and all pituicytomas in this series were immunopositive, while all cases of astrocytomas were negative [46].

In 2017, Tauziède-Espariat et al. reported one additional case of a TTF-1 immunoreactive chordoid glioma [72] (the clone used was not available to us), and Garcia-Garcia et al. also reported another case which was TTF-1 immunonegative; again, the clone used was not stated by the reporting authors [73].

Additionally, based on our own (PubMed) and others’ computerized search [74, 75] and review of the literature, about 104 cases of chordoid gliomas have been recorded thus far, excluding the series of 16 cases of Rosenberg et al. [76] included in a molecular study, which, at least in part, may have been included in the other cited series of Bielle et al. [43].

However, no TTF-1-expressing CNS embryonal tumor had been reported until now.

Case 1 of this report, concerning the PNET in a 6½-year-old girl, is the only one among 24 embryonal CNS neoplasms analyzed in a study to exhibit TTF-1 immunoreactivity, this was prompted by the unexpected expression of TTF-1 in a unique case of monophasic stromal Wilms’
tumor metastasis to the lung, which was paradoxically TTF-1+/WT1- [29]. This TTF-1 immunopositive PNET was the only suprasellar tumor in our series as well.

In case 2, the embryonal neoplasm in a 36-year-old female was incidentally found, and was also located in the ventral brain at the level of the diencephalon. This TTF-1-expressing embryonal tumor in an adult should alert neurosurgical pathologists about the risk of misdiagnosis, that is, misinterpreting a TTF-1 immunopositive PNET as a metastasis to the brain from any site, especially the lung. As a matter of fact, caution should be taken by the pathologist, especially when dealing with small biopsies, the expression of TTF-1 in a malignant small round cell tumor may obscure the correct diagnosis of an embryonal tumor, primarily in favor of a small-cell (neuroendocrine) carcinoma; this can occur with the auxiliary support of other immunopositive markers of both neuroendocrine nature (such as synaptophysin [77], NSE [78], and neurofilament proteins [79]) and a stem cell marker (such as nestin [80]), which are well-known to be expressed in pulmonary as well as extrapulmonary small cell carcinomas, as they were actually expressed in this case.

Furthermore, in regard to the cytoplasmic reactivity for WT1 as seen in case 2, we would like to remark herein that a cytoplasmic reactivity was repeatedly seen by Bisceglia and Galliani when analyzing this expression in a large series of 100 cases of embryonal soft tissue, visceral (other than nephroblastomas), and CNS tumors. This WT1 cytoplasmic reactivity was observed in 53.8% of peripheral neuroblastomas, 19.2% of peripheral PNETs, 35.7% of medulloblastomas, 50% of pleuropulmonary blastomas, 60% of desmoplastic small round cell tumors, 50% of central PNETs, 40% of hepatoblastomas, and even (out of the rubric of embryonal tumors) in 22.2% of synovial sarcomas, with the conclusion in favor of interpreting such a finding as non-specific [53].

The expression of TTF-1 in both these cases was evaluated with the 8G7G3/1 clone.

In summary, as far as primary conventional brain tumors are concerned (Table 1), TTF-1 expression is often seen in high-grade (astrocytic or oligodendrocytic) gliomas when detected with the monoclonal SPT24 antibody, while low-grade astrocytomas and oligodendrogliomas do not express TTF-1 regardless of the antibody clone used.
Table 1 Primary Brain Tumors investigated for TTF-1 (from the Literature^).

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<td><strong>Monoclonal Ab</strong></td>
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<td><strong>8G7G3/1</strong></td>
<td><strong>SPT24</strong></td>
<td><strong>8G7G3/1</strong></td>
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<td>h.g. Astrocytic &amp; Oligodendrocytic Glial Tumors</td>
<td>0/33 (g. IV)</td>
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<td>14/28 (g. IV)</td>
<td>0/28 (g. IV)</td>
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<td>Ependymomas</td>
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<td>Subependymomas</td>
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<td>Embryonal tumors (cPNET &amp; congeners)</td>
<td>0/7 (mb)</td>
<td>1**/25 (14 mb; 4 s.t. &amp; 1 i.m. PNET; 3 s.t. nb; 2 pb; 1 rb)</td>
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<td>0/1 (mb)</td>
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<td>Central neurocytomas</td>
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<td>Pineocytomas</td>
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<td>I.g. Mixed Tumors (g.g. &amp; g.n. t.)</td>
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<td>Meningiomas</td>
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<td>0/9</td>
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<td>Schwannomas</td>
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**Abbreviations:** Ab= Antibody; h.g.= high grade; l.g.= low grade; cPNET= central PNET (primitive neuroectodermal tumor, currently CNS embryonal tumors); g.g.= ganglioglioma; g.n. t.= glioneuronal tumor; mb= medulloblastoma; s.t.= supratentorial; i.m= inframedullary; s.t. nb= supratentorial neuroblastoma; pb= pineoblastoma; rb= retinoblastoma; CNS: central nervous system.

**Symbols:** ^= Excluded from the Table are the TTF-1^+ primary brain tumors of uncertain histogenesis (i.e., chordoid glioma and subependymal giant cell astrocytoma of tuberous sclerosis complex) as well as the TTF-1^+ tumors of the posterior hypophysis; += immunopositive; *= intra[3rd]ventricular ependymoma; **= suprasellar embryonal tumor (PNET); = this study aimed to investigate TTF-1 and WT1 in 100 central and peripheral PNET (from soft tissue, visceral [other than nephroblastoma], and CNS cases) [53]; = this study, including the previous one, and extended to 117 sury embryonal tumors, analyzed the results restricted to TTF1 only. [47]

**Parenthetically:** In the cited study by Galliani et al. [47] of 92 embryonal tumors from extra-CNS sites (from soft tissue, visceral [other than nephroblastoma]) and 25 primitive neuroectodermal tumors of the CNS (cPNET / CNS embryonal tumors) none was TTF-1 immunoreactive, except for case 1, herein reported, with suprasellar location. Instead, of the 48 cases of nephroblastoma (Wilms tumor) studied for TTF-1 expression, 8 (16.6%) of them were positive. [29]
Ependymomas demonstrate occasional immunoreactivity when tested with the SPT24 clone, while no immunostaining is observed with the other clone.

Based on very limited data, subependymomas are negative for TTF-1 (0+ of four investigated with the 8G7G3/1 clone).

Meningiomas are usually negative with either clone.

Embryonal tumors of the brain (central PNETs, including medulloblastomas) do not express TTF-1 with either clone (nine cases studied with the SPT24 clone and 40 cases studied with the 8G7G3/1 clone), except for those arising in the diencephalic region as attested by the two cases presented herein.

Differentiated tumors of neuronal lineage (central neurocytomas and pineocytomas) can occasionally express TTF-1 immunopositivity with the SPT24 clone (1+ of 7), while they are negative with the 8G7G3/1 clone (0+ of 12).

Low-grade mixed glioneuronal or ganglioglial brain tumors do not express TTF-1 (0+ of 4 studied with the SPT24 clone and 0+ of 11 with the other clone).

Instead, regarding the entire spectrum of TTF-1 immunopositive retrohypophyseal (and infundibular) tumors as well as the so-called chordoid glioma of the 3rd ventricle and SEGAs of the tuberous sclerosis complex, we assert that TTF-1 is consistently detected with both SPT24 and 8G7G3/1 clones, and stronger and more diffuse immunoreactivity is observed with the former clone.

Notably, the notion of TTF-1 immunopositive sellar tumors is not widespread. Several recent such cases have not been so tested [63, 69, 70, 81, 82].

We categorically agree with the concept [36] that expression of TTF-1 in brain tumours appears to be a site-specific biomarker, rather than a non-specific tumor marker associated with tumor dedifferentiation.

4. Conclusions

Brain tumors (both low-grade and high-grade) of either astrocytic or oligodendrocytic lineage fail to express nuclear TTF-1 immunoreactivity as detected by the 8G7G3/1 clone.

Primary tumors of the posterior pituitary and infundibulum consistently express TTF-1 with both clones.

The so-called chordoid glioma of the 3rd ventricle as well as the SEGA of the tuberous sclerosis complex (notably with limited data for the latter tumor) constantly express TTF-1 with both clones.

TTF-1 expression in suprasellar or periventricular diencephalic embryonal tumors (PNET) is a novelty.

TTF-1 immunopositivity in both primary brain and posterior hypophyseal tumors likely reflects a lineage marker.

TTF-1 expression in a suprasellar, parasellar, diencephalic, or circumventricular (3rd) primary brain tumors should be taken into consideration when interpreting metastatic (brain) tumors of uncertain origin.
Acknowledgments

Case 1 of this report was previously presented in the session of “Neuropathology” at the 6th SIAPEC National Congress of the Italian Anatomic Pathology Society, held in Palermo, Italy: 27-29 Ottobre, 2011, and was published in abstract form in the proceedings of the Congress (Pathologica. 103 (04):206-207).

Author Contributions

Michele Bisceglia, MD, Tullio Parracino, MD, and Carlos A. Galliani, MD, were responsible for the study of case 1; Stefano Pizzolitto, MD, Giovanna De Maglio, PhD, and Serena D’Agostini, MD, were responsible for the study of case 2; Elena Minenna, MD, was fully responsible for the bibliographic search and relevant statistical data.

All the authors participated in the study design, and contributed effectively to the writing and/or illustrations of the manuscript.

Competing Interests

The authors have declared that no competing interests exist.

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Angiocentric Glioma: A Review of Clinicopathologic Features

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1. Introduction

Along with focal cortical dysplasia, hippocampal sclerosis and remote infarcts, tumors are a well known cause of medically intractable or pharmacoresistant epilepsy [1-3]. Most of the tumors encountered in this setting represent low grade glioneuronal neoplasms, particularly gangliogliomas and dysembryoplastic neuroepithelial tumors. In 2005, Wang and colleagues reported on 8 tumors which they designated as so-called “monomorphous angiocentric gliomas” [4]. The title of their paper suggested that these tumors were epileptogenic (all eight patients had epilepsy) and displayed features of infiltrating astrocytoma and ependymoma. During the same month, Lellouch-Tubiana and coworkers reported on a series of 10 children with refractory epilepsy who had similar appearing neoplasms which they referred to as “angiocentric neuroepithelial tumors” [5]. The term “angiocentric glioma” was used in reference to these
tumors in a series of eight tumors reported by Preusser et al in 2007 [6] and the entity was included under this designation in the 2007 World Health Organization (WHO) Classification of Tumours of the Central Nervous System [7].

The purpose of this article is to briefly review the salient clinical and pathologic features of this relatively new entity.

2. Clinical Presentation

Due to the rarity of this tumor, the precise incidence of this neoplasm is not known. The majority of cases have been described as arising in children. In the first three reported series [4-6], a total of 26 cases were presented; all but six of them were diagnosed in childhood. Almost all of the six adult cases presented with seizures in childhood [4-6]. Only anecdotal cases of this tumor have been described in geriatric adults over the age of 65 years [6, 8]. There appears to be no gender predilection in the tumor.

Angiocentric glioma has been described to arise in a variety of locations in the brain with an apparent predilection for superficial cerebrocortical locations. The most common sites in early reported series were fronto-parietal lobes followed by the temporal lobe [4-6]. Tumors have been described involving the occipital lobe, as well as rarely the brainstem [9-11].

As previously mentioned, the vast majority of angiocentric gliomas present with epilepsy. Often, patients have had surgery for several years and become refractory to pharmacologic management, necessitating surgical intervention. Occasionally, based on the location of the tumor, patients may present with other symptoms. Reports of progressive hand weakness [8], headaches and visual disturbance [9, 12], double vision and nausea [11], hemiparetic gait and facial nerve palsy [11], and psychotic symptoms [13] have been documented in the literature.

3. Neuroimaging

Imaging studies typically localize angiocentric glioma to superficial location with both cortical and white matter involvement [5]. The tumor is usually well circumscribed, solid, hyperintense and nonenhancing on fluid-attenuated inversion recovery (FLAIR) images [6]. On T1-weighted magnetic resonance (MRI) images, the tumor is marked in some cases by cortical rim-like hyperintensities; stalk-like extension toward the ventricle is noted in some cases on T2-weighted images and on FLAIR [5, 6]. Descriptions of the gross pathology are sparse in the literature. Based on anecdotal personal experience, tumors may show focal blurring of the gray-white interface, similar to what is encountered in diffuse astrocytomas or oligodendrogliomas.

4. Histopathology

The most salient microscopic feature of angiocentric glioma is the presence of fairly monomorphic appearing, bipolar spindled cells which arrange themselves around blood vessels forming perivascular pseudorosette-like structures (Figure 1). The number of tumor cells which surround blood vessels may range from a single layer to multiple layers. Cells may be arranged radially or longitudinally around vessels. Satellitosis around neurons in the cortex is a relatively infrequent finding. Individual cells typically have rounded to elongated nuclei, a finely granular nuclear chromatin pattern and inconspicuous nucleoli. Some cells may have a more epithelioid
appearance with more eosinophilic cytoplasm and more defined cell boundaries. Focally, the tumor may demonstrate microcystic changes (Figure 2). In other areas, spindled tumor cells may be more compactly arranged, resembling a schwannoma (Figure 3). Rarely, calcifications (Figure 4) may be present. The tumor is generally not circumscribed and an infiltrative margin is common, reminiscent of a diffuse infiltrating astrocytoma (Figure 5). As tumor cells infiltrate and reach the cortical surface, there may be a piling up of tumor cells in this location, referred to subpial aggregation. Intermixed neurons are frequently encountered in cortical regions with infiltrative tumor. Mitotic activity is generally absent or rare; although rare instances of tumors with increased mitoses (11 mitotic figures in 50 high power fields) have been documented [4]. Vascular proliferative changes and necrosis are not features of these neoplasms. Rare cases of angiocentric gliomas with mixed features of ependymoma have been rarely described [4, 14, 15].

Figure 1 Angiocentric gliomas are characterized by a distinctive arrangement of tumor cells around blood vessels forming perivascular pseudorosettes (hematoxylin and eosin, original magnification 100X).
Figure 2 Focal microcystic areas may be seen in angiocentric gliomas (hematoxylin and eosin, original magnification 200X).

Figure 3 Area of the tumor may be marked by a moderately dense arrangement of bland spindled cells (hematoxylin and eosin, original magnification 200X).
Figure 4 Rarely, microcalcifications may be present in the tumor (hematoxylin and eosin, original magnification 200X).

Figure 5 The margin of the tumor often shows an infiltrative pattern reminiscent of an infiltrating diffuse astrocytoma (hematoxylin and eosin, original magnification 200X).

A subset of tumors also appear to demonstrate evidence of adjacent cortical architectural abnormalities or focal cortical dysplasia [9, 16, 17] (Figure 6). In one series of five cases of angiocentric glioma, four of four evaluable cases demonstrated focal cortical dysplasia changes resembling Internal League Against Epilepsy (ILAE) type I patterns [9]. In one of the 3 cases of coexistent focal cortical dysplasia reported by Liu et al, dysmorphic neurons and balloon cells were described [16]. Using the current ILAE classification of focal cortical dysplasia, these cases would
be classified formally at type IIIb dysplasias [18]. The association of this tumor with focal cortical dysplasia may in part explain the association with epilepsy. The exact nature or significance of this association is uncertain. It has been conjectured with other tumors that are known to be associated with focal cortical dysplasia and epilepsy (e.g. gangliogliomas and dysembryoplastic neuroepithelial tumors) that the association may be indicative of a developmental origin of these tumors, empirically supported by the fact that these tumors typically present in childhood.

![Figure 6](image-url) The adjacent superficial cortex of an angiocentric glioma marked by a disordered cortical architectural pattern, consistent with focal cortical dysplasia (ILAE type IIIb) (hematoxylin and eosin, original magnification 200X).

Ultrastructural examination of these tumors have demonstrated that cells contain intermediate filaments and have basement membranes where they interface with blood vessels [4]. Intercellular microlumens with microvilli and “zipper-like” junctions have also been noted [4, 6]. Cilia have not been identified. It has been suggested that the cell of origin for these tumors may be bipolar radial glia that are prevalent during brain development and that these cells may share similar features to cells which give rise to ependyma [5].

Immunohistochemical analysis of the tumor has shown that tumors demonstrate positive staining with antibodies to glial fibrillary acid protein (GFAP), S-100 protein and vimentin [6]. Tumors do not stain with neuronal markers such as NeuN, chromogranin or synaptophysin [4]. A dot-like pattern of epithelial membrane antigen (EMA) immunoreactivity has been described, similar to what is seen in ependymomas (Figure 7) [4, 6]. Other ependymoma markers such as CD99 and D2-40 are variably expressed [6]. p53 and isocitrate dehydrogenase-1 (IDH-1) (R132H) immunostaining has not been observed [4].

Generally, angiocentric gliomas demonstrate low rates of cell proliferation, as evidenced by cell proliferation markers such as Ki-67 or MIB-1. Wang et al noted Ki-67 indices ranging from <1% up to 5% in initially resected tumors [4]. Similar low labeling indices were reported by others [6, 9]. One recurrent case in the series reported by Wang et al showed an index of 10% [4]. Only a few
other anecdotal cases of tumors with indices in the 8-10% range have been reported [19-21]; the significance of the elevated indices in these cases is uncertain.

![Figure 7](image)

**Figure 7** Dot-like immunoreactivity may be observed in some tumors with epithelial membrane antigen (EMA) staining (original magnification 400X). This staining pattern is not observed in low grade astrocytic neoplasms that are typically in the differential diagnosis, including pilocytic astrocytoma and diffuse astrocytoma.

Differential diagnostic considerations from a morphologic standpoint include ependymomas, diffuse astrocytomas and pilomyxoid/pilocytic astrocytomas. Ependymomas are usually discrete masses which are intraventricular in location, and typically not parenchymal based. The presence of true rosettes, a feature of some ependymomas, has not been described in angiocentric gliomas. The infiltrative areas of an angiocentric glioma can resemble a diffuse astrocytoma. Although some diffuse astrocytomas may show perivascular satellitosis of tumor cells, the layered perivascular pseudorosette pattern of an angiocentric glioma is not seen in diffuse astrocytoma. Many of the molecular features which may be encountered in diffuse astrocytoma (IDH mutations, p53 staining) are not seen in angiocentric glioma. Pilomyxoid and pilocytic astrocytomas typically are more circumscribed lesions and have characteristic imaging findings. The angiocentric arrangement of tumor cells in the pilomyxoid astrocytoma is usually looser and less salient. Rosenthal fibers and eosinophilic bodies, common findings in pilocytic astrocytomas, are not present in angiocentric glioma.

5. **Genetics/Molecular Findings**

Unlike oligodendrogliomas and a subset of diffuse fibrillary astrocytomas, angiocentric gliomas do not demonstrate evidence of IDH mutations [4, 22-24]. BRAF mutations are also not a feature of angiocentric glioma [23, 24]. In a genomic analysis of 19 angiocentric gliomas, all tumors showed gene fusions involving the MYB locus, a proto-oncogene, with most showing in frame MYB-QKI fusions (fusion of exon 15 of MYB with exon 5 of QKI) [25]. Another study substantiated
this finding in a majority (87%) of 15 tumors evaluated [26]. Two fairly recent studies also substantiated this finding in brainstem tumors [27, 28]. These fusions were not commonly seen in other glioma types, suggesting that these fusions may be specific and sensitive for angiocentric glioma. Using chromosomal comparative genomic hybridization, Preusser et al found a loss of chromosomal bands 6q24-q25 in one of eight tumors examined; they also found a copy number gain of two adjacent clones from chromosomal band 11p11.2 in one of three tumors evaluated by a high resolution screen by array-comparative genomic hybridization [6].

6. Treatment and Outcomes

Given that the majority of these tumors have an indolent clinical course and behave in a low grade fashion, they have been designated by the WHO as grade I neoplasms [7]. They are generally amenable to surgical resection. Rare cases of recurrence in tumors which have been initially incompletely excised have been documented; the recurrence may demonstrate features of a higher grade tumor [4, 14]. In most cases, gross total resection has been associated with good seizure control [9]. There is no need for postoperative radiation therapy or chemotherapy in the typical angiocentric glioma.

7. Summary

Angiocentric gliomas are a relatively uncommon tumor, most frequently encountered in children and young adults and often presenting with epilepsy. Recent work has suggested an association of this tumor with focal cortical dysplasia, suggesting a developmental basis for its origin. Tumors are generally fairly well circumscribed and morphologically marked by the presence of bipolar spindled cells which arrange themselves around blood vessels. The tumors behave as low grade neoplasms and have been designated by the WHO as grade I. A number of recent studies have noted the presence of MYB-QKI gene fusions in these tumors, a finding that is unusual in other low grade gliomas.

Author Contributions

The author has completed all the work.

Competing Interests

The author has declared that no competing interests exist.

References


Case Report

A Case Series of Temozolomide in the Management of Refractory Prolactinomas

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Abstract

Objective: To report three cases of refractory prolactinomas treated with Temozolomide (TMZ).

Background: Prolactinomas account for 40% of pituitary adenomas. Dopamine agonists (DA) are the first line of treatment followed by surgical resection and radiation. TMZ is an oral chemotherapeutic agent used in gliomas, which has been given to patients with prolactinomas refractory to conventional treatments.

Methods: Retrospective chart review was conducted for refractory prolactinoma patients treated between 2008 and 2018 at UT Southwestern Medical Center (UTSW). Three patients with refractory prolactinomas received oral TMZ at UTSW.

Results: All three patients demonstrated improvement in symptoms upon TMZ treatment, markedly decreased serum prolactin levels (SPRL), as well as radiographic decrease in tumor size.

Conclusion: TMZ is well tolerated and is a potentially effective treatment for refractory prolactinomas.

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Keywords
Refractory prolactinomas; temozolomide; pituitary adenomas; stereotactic radiosurgery; serum prolactin level

1. Introduction

Prolactinomas comprise 40% of pituitary adenomas [1]. They cause headaches, visual field defects, hypogonadism, galactorrhea and infertility. Most prolactinomas respond to Dopamine agonists (DA) [2]. Surgery is indicated for patients who failed DA treatment or are intolerant to DA. Prolactinomas unresponsive to DA or surgery may need radiosurgery.[3]. Despite multimodal treatments, a subset of prolactinomas progress with rapid growth and invasion into surrounding tissue. TMZ is an oral alkylator used in malignant gliomas which readily crosses the blood brain barrier. Nausea, vomiting, constipation, thrombocytopenia and leucopenia are side effects. Successful management of prolactinomas with TMZ was first reported in 2006 [4, 5]. Approximately 50% of prolactinomas respond to treatment with TMZ.[6]. A case series evaluating use of TMZ in refractory prolactinomas reported a response in 4 out of 9 patients. [7]. A recently published large study of 166 patients with aggressive pituitary tumors treated with TMZ of which majority were corticotroph tumors confirmed that TMZ is an effective first line treatment of aggressive pituitary tumors and carcinomas. In this retrospective review, patients received TMZ for multiple subtypes of pituitary tumors. Patients with functioning tumors and those who received TMZ concurrently with radiation therapy had a better response rate [8]. This study is our institutional experience in management of refractory prolactinomas.

2. Methods

Retrospective chart reviews were conducted as part of an Institutional Board review exempt study and three refractory prolactinoma patients treated between 2008 and 2018 at UTSW were identified. All patients had at least 3 relapses prior to starting TMZ treatment. They consented to publication of their clinical data. TMZ was dosed at 150 to 200 mg/m²/day for five days during each 28-day cycle. All patients had monthly serum prolactin level (SPRL) and bimonthly gadolinium enhanced brain magnetic resonance imaging (MRI) scans.

Patient: 1:
A 40-year-old female diagnosed with a prolactinoma in 1996 failed treatment with DA, surgery, Cyber knife® and Gamma Knife® radiosurgery. In May 2013, she developed diplopia. Brain MRI showed enlargement of the prolactinoma measuring 3.1x3.4x4.0 cm (anteroposterior by transverse by craniocaudal (APxTRxCC) with SPRL of 3044.8 ng/ml (normal range in non-pregnant females: 5-40 ng/ml or 106-850 mIU/L). On completion of 18 cycles of monthly TMZ, her tumor decreased to 2.3 cmx1.7cmx1.8 cm APxTRxCC. She was monitored with SPRL and brain MRIs every 2 months off TMZ. TMZ was restarted in October 2016 for progression (Figure 1 C). She completed 21 cycles of TMZ by February 2018 and has stable disease with a SPRL of 8.3 ng/ml. (Figure 2).
Patient 2:
A 70-year-old female was diagnosed with a refractory prolactinoma in 2008. She had failed previous treatment with Dopamine agonists, stereotactic radiosurgery and surgical resection. In June 2014, her SPRL was 3591 ng/ml (normal range in non-pregnant females: 5-40 ng/ml or 106-850 mIU/L). She completed 14 cycles of TMZ treatment in August 2015. Her tumor has remained stable off TMZ with normal SPRL in October 2017.

Patient 3:
A 76-year-old male underwent resection and Cyber Knife® radiosurgery of a prolactinoma in October 2009. He was not given DA due to a history of paranoid schizophrenia. He completed 9 cycles of TMZ at a reduced dose of 100 mg/m²/day due to pancytopenia. His tumor has remained stable off TMZ with normal SPRL in July 2017.

3. Discussion

Our series confirmed the reports of previous patients with refractory prolactinomas having good responses and acceptable side effects from TMZ. Optimum dosage of TMZ and duration of treatment (number of cycles) is not known. In our institution, dosage of TMZ was based on the treatment regimen for glioblastoma also known as the “Stupp Protocol” [9]. Patients with refractory prolactinomas in our institution underwent bimonthly gadolinium enhanced brain and pituitary MRI and SPRL measurements every month. In patient 1, after successful completion of TMZ treatment for 12 months, the patient opted to continue TMZ treatment for an additional 6 months given her excellent clinical and radiological response. At recurrence while off TMZ therapy, the patient opted to receive a total of 24 cycles of TMZ treatment and had completed 21 cycles at the time of preparation of this report. Patient 2 opted to discontinue TMZ treatment after 14 cycles due to fatigue. Patient 3 received a reduced dose of TMZ due to pancytopenia with eventual discontinuation after 9 cycles due to several delays and dose reductions of TMZ. All three patients demonstrated clinical stability, decrease in tumor size radiologically and decreasing SPRL. Previous studies have published favorable responses of corticotroph tumors with TMZ treatment, however, the optimal dose, and the number of cycles is unclear for these other subtypes as well. Long-term TMZ treatment could be tolerated and may be necessary for some refractory prolactinomas, as Patient 1 recurred on surveillance after 18 monthly cycles of TMZ, and again had a good response to TMZ re-challenge, hence making this is a unique case as previously published case series have shown that response to retreatment of refractory pituitary tumors with TMZ is poor. [8]. In addition, for patients with refractory prolactinomas with concurrent paranoid schizophrenia who cannot receive DA, TMZ may be considered earlier in the treatment course. The tumor tissue was not tested for MGMT status in our patient cohort and therefore is a limitation of this study. Larger prospective studies are needed to ascertain whether earlier treatment with TMZ may spare standard treatment modalities, such as radiation.
Figure 1 Patient 1 Images A to C: Contrast-enhanced coronal brain MRIs. A: 10 years after initial diagnosis. The patient failed prior DA therapy, surgery and radiosurgery. B: Decrease in tumor size 6 months into TMZ therapy. C: Extension of left suprasellar enhancement during TMZ holiday.

Figure 2 Graph showing SPRL for patient 1 over time since diagnosis. 1. SPRL after DA treatment; 2. Failed surgery and Cyber Knife; 3. SPRL prior to starting TMZ; 4. Completion of 18 cycles of TMZ; 5. SPRL on surveillance; 6. Radiographic recurrence; 7. After 2 cycles of TMZ retreatment; 8. Completion of 21 cycles of TMZ. Note: SPRL from 1996 is unavailable. Normal SPRL in non-pregnant females: 5-40 ng/ml or 106-850 mIU/L.

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Author Contributions

All authors contributed equally to the conception or design of the work, data collection, data analysis and interpretation, drafting the article, critical revision of the article and final approval of the version to be published.
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Abstract
(1) Background: Current imaging standard for detecting and assessing glioblastoma multiforme (GBM) progression depends on contrast-enhancement on brain magnetic resonance imaging (MRI). Isolated foci of diffusion restriction have been observed to precede enhancement in GBM. The aim of our study was to investigate the frequency of isolated diffusion restriction that precede corresponding enhancement and to investigate the association between isolated diffusion restriction and survival in patients with GBM.
(2) Methods: MRI of the brain, including diffusion weighted images (DWI) and apparent diffusion coefficient (ADC) maps were retrospectively examined in 102 consecutively treated patients with histopathologically confirmed GBM. Images were assessed for the presence of isolated diffusion restriction in GBM and identified lesions were monitored for enhancement on follow-up MRI. Data were collected on the length of time for enhancement to appear, normalized ADC, and overall survival of patients.
(3) **Results:** Forty patients (39.2%) showed areas of isolated diffusion restriction. Ten patients (25%) developed corresponding enhancement on follow-up imaging after an average of 145 days after the index imaging. In patients with isolated restricted diffusion, the mean ADC was $721.4 \pm 117.2$ mm$^2$/s compared to $888.7 \pm 85.2$ mm$^2$/s in the normal appearing-white matter (NAWM) in contralateral hemisphere ($p<0.001$). On survival analysis, the overall survival was longer ($p=0.036$) in patients with isolated restricted diffusion and these patients had survival benefit ($p=0.006$) in the early follow-up period.

(4) **Conclusions:** Isolated restricted diffusion in GBM precedes corresponding enhancement in a subset of patients with GBM and was associated with early survival benefit.

**Keywords**
- Diffusion weighted imaging
- glioblastoma multiforme
- apparent diffusion coefficient

1. **Introduction**

Patients with glioblastoma multiforme (GBM) have poor prognosis with an overall survival time of less than twelve months despite aggressive treatment [1]. GBM’s highly variable response to existing therapies coupled with its short survival times [2], underlines the need for additional imaging biomarkers capable of identifying earlier signs of disease progression to augment treatment management and accelerate decision-making. The current imaging standards for detecting and assessing tumour progression in patients with GBM depend heavily on changes in contrast-enhancing abnormalities on brain magnetic resonance imaging (MRI) [3, 4]. Contrast enhancement on gadolinium enhanced MRI is based on the immature leaky capillaries in the region of the GBM. Contrast enhancing abnormalities are examined and characterized to estimate prognosis and also to guide management of these malignant brain tumours [5].

Diffusion weighted imaging (DWI), a MRI method based on Brownian motion of water molecules, has become an important tool in the characterization of brain tumours [5, 6]. In tumours, restricted diffusion is seen in areas showing very high cellularity [5, 7, 8]. The increased cellularity leads to the reduction of interstitial space resulting in restricted diffusion. Diffusion restriction is usually identified in GBM in the area of contrast enhancement. Gupta et al have shown that the increased cellularity associated with restricted diffusion can be seen before enhancement on T1-weighted (T1W) post gadolinium images [3]. The aim of our study was to investigate the frequency of isolated diffusion restriction that precede corresponding enhancement and to investigate any association between isolated diffusion restriction and survival in patients with GBM. The hypothesis of our study was that isolated diffusion restriction preceding enhancement is associated with survival in patients with GBM.

2. **Methods**

*Patient selection:* Consecutive patients over a 3-year period were identified from our institutional brain tumour database with confirmed diagnosis of GBM on biopsy or resection and their data were retrospectively analyzed. The study was approved by the institutional research ethics board. Patients with the following set of axial images on their first diagnostic MRI, before
any medical or surgical treatment, were included in our study: 1) DWI; 2) ADC (apparent diffusion coefficient) maps; 3) axial post gadolinium T1W images; and 4) axial fluid-attenuating inversion recovery (FLAIR) images. Additional information on patients’ demographics as well as clinical, surgical, follow-up, and survival data was obtained from the brain tumour database. Karnofski performance score, which defined the functional status of the patient, was also obtained from the brain tumor database. All procedures performed in studies involving human participants were in accordance with the institutional ethical standards.

**Image acquisition:** MRI were performed on a 1.5 T magnet scanner (Singa, GE Healthcare) with the following tumor protocol: DWI, pre and post gadolinium T1W images, T2 and FLAIR image of the brain. The DWI were acquired using single-shot echo-planar imaging with 8000 ms TR, 73.6 ms TE, 260-mm FOV, 160x192 matrix size, 5-mm section thickness with 1.5 mm intersection gap, and 1000 and 0 mm 2 /s b-values obtained in 3 orthogonal directions. FLAIR images were acquired as fast spin echo images using 8000 ms TR, 120 ms TE, 220-mm FOV, 256x254 matrix size, 5-mm section thickness with 1.5 mm intersection gap. T2 images were acquired as fast spin echo images using 8000 ms TR, 120 ms TE, 220-mm FOV, 256x254 matrix size, 5-mm section thickness with 1.5 mm intersection gap. Both pre and post-contrast T1W images were acquired as fast spin echo images using single-shot echo-planar imaging with 500 ms TR, 22.8 ms TE, 220-mm FOV, 320 x 192 matrix size, 5-mm section thickness with 1.5 mm intersection gap.

**Image analysis:** Regions with low ADC signifying restricted diffusion both within and outside of the contrast-enhanced tumour were identified by one of the coauthors (AB) under the guidance of a fellowship trained neuroradiologist (JS) with more than 7 years of experience as Neuroradiology staff. Restricted diffusion was defined as hyper-intensity on DWI and hypo-intensity on ADC map compared to the normal-appearing white matter (NAWM) in the contra-lateral hemisphere. These areas of restricted diffusion were first identified on visual inspection of the images and then by quantification on the ADC maps. Patients with regions of isolated restricted diffusions (defined as regions with low ADC without corresponding enhancement on the post-gadolinium T1W images) were identified (Figure 1). These regions of isolated restricted diffusion with no enhancement on first MRI, were monitored for enhancement on follow-up MRI. The final cohort of patients included those who demonstrated: 1) isolated restricted diffusion or diffusion restriction larger than enhancement and 2) no corresponding hemorrhage on their first MRI and those with no immediate postoperative changes in the region of isolated restricted diffusion, on their follow up MRI.

The degree of restriction was quantified as the lowest ADC value within each region of restricted diffusion. The degree of restriction was calculated by manually outlining the region of restricted diffusion (using ‘Freeform mark up’ tool) on the slice it was seen. ADC was also measured in the NAWM in the contra-lateral hemisphere using ‘Freeform mark up’ tool with an area of at least 10 mm². This was used to calculate the normalised ADC (nADC, i.e., ratio of minimum ADC in the tumor to that in contralateral NAWM). The ADC values (in mm²/s) were recorded on a PACS work station and not on an MRI console. These two values have been shown to be comparable and not statistically different [9]. The total volume of enhancing component of the tumor was calculated by manually outlining the abnormality (using ‘Freeform mark up’ tool) on all the slices. The sum of the areas on each slice was multiplied by the slice thickness and the inter-slice gap to get the volume. Volume of tumour, time interval for enhancement to appear, and overall survival of patients from the time of first imaging were recorded.
Figure 1 Patient with GBM with heterogeneously enhancing tumor on post-gadolinium T1 axial images (a) showing heterogeneous pattern of diffusion restriction on diffusion weighted images (DWI)(b) and apparent diffusion coefficient (ADC) map (c). Superior to the enhancing lesion, there was no enhancement on post-gadolinium T1 weighted images (d) but with isolated restricted diffusion on DWI (e) and ADC map (f). On follow up 2.5 months later, enhancement (g) was seen on post-gadolinium T1 axial images, in the same area of isolated restricted diffusion on DWI (h) and ADC map (i).

Statistics- Descriptive statistics were performed. The two group of patients with and without isolated restricted diffusion were compared using Wilcoxon signed-rank test to analyze any significant difference. Kaplan Meier survival statistics were performed in Graph-pad statistical package using Log-rank and Gehan-Breslow-Wilcoxon tests to assess the difference in the survival pattern between the two groups. In comparison to the Log-rank test, the Gehan-Breslow-Wilcoxon test places more weight on deaths at early time points, and hence is more sensitive to the detection of early survival differences.

3. Results

Over 3 consecutive years, a total of 155 patients had a pathologically confirmed GBM in our institution. Of these, 102 patients fulfilled our imaging inclusion criteria. Other patients were excluded since they did not have DWI at the time of diagnostic MRI. Restricted diffusion within or adjacent to enhancing tumour was seen in 97 patients (95.1%) with 40 patients (39.2%) with isolated restricted diffusion (Table 1). Of these 40 patients with isolated restricted diffusion, 10 (25%) developed corresponding enhancement on follow-up examinations after an average of 144.75 ± 110.48 days from the index imaging. The longest time interval for enhancement on
follow-up imaging was after 359 days in one patient. Another 10 of the 40 patients (25%) did not have follow-up MRI and another 4 patients (10%) did not have ADC maps on follow-up MRI. In 6 patients (15%), post-surgical changes interfered with analysis of DWI. In these patients with isolated restricted diffusion, we were not able to assess the follow-up enhancement. The remaining 10 patients (25%) had regions of isolated restricted diffusion that did not show corresponding enhancement on available follow-up imaging. In patients with isolated restricted diffusion, the mean ADC was $721.4 \pm 117.2 \text{mm}^2/\text{s}$ compared to $888.7 \pm 85.2 \text{mm}^2/\text{s}$ in the NAWM ($p < 0.001$) (Figure 2).

Table 1: Demographic, imaging, treatment and survival characteristics of the GBM patients with and without isolated restricted diffusion.

<table>
<thead>
<tr>
<th></th>
<th>With isolated restricted diffusion (Mean ± SD)</th>
<th>Without isolated restricted diffusion (Mean ± SD)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>40 (41.2%)</td>
<td>57 (58.8%)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>59.98 ± 10.38</td>
<td>62.8.10 ± 13.88</td>
<td>0.26</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>21/19</td>
<td>30/17</td>
<td>0.99</td>
</tr>
<tr>
<td>KPS at diagnosis</td>
<td>76.58 ± 16.5</td>
<td>73.56 ± 14.95</td>
<td>0.48</td>
</tr>
<tr>
<td>Degree of resection (1/2/3)</td>
<td>31.4/17.1/51.4</td>
<td>54.4/8.7/36.9</td>
<td>0.12</td>
</tr>
<tr>
<td>Volume of tumor (mm³)</td>
<td>23048.91 ± 21789.92</td>
<td>29431.53 ± 21964.34</td>
<td>0.16</td>
</tr>
<tr>
<td>Survival (days)</td>
<td>486 ± 363.5</td>
<td>291.9 ± 344.3</td>
<td>0.036</td>
</tr>
</tbody>
</table>

Note: N-number; M-Male; F-Female; SD-standard deviation; KPS-Karnofski performance score; Degree of resection: 1-biopsy; 2-partial resection; 3-gross total resection.

Figure 2: Apparent Diffusion Coefficient (ADC in $\text{mm}^2/\text{sec}$) in the region of isolated restricted diffusion (shown as lesion ADC) compared to that in the normal appearing white matter (NAWM) in the contralateral hemisphere in 40 patients with glioblastoma multiforme.

We had clinical follow up for all patients and the overall survival was significantly longer ($p=0.036$) in patients with isolated restricted diffusion after index MRI than those without (Table 1). On survival analysis (Figure 3), the Gehan-Breslow-Wilcoxon test showed significant ($p=0.006$) survival benefit in the early follow-up period reflecting longer survival (median survival of 456 days...
vs 173 days) in patient with isolated diffusion restriction. However there was no significant difference (p=0.134) in the overall survival in these two groups of patients on Log-rank (Mantel-Cox) test.

![Survival proportions: Survival of Data 1](image)

**Figure 3** Kaplan-Meir curve showing the early survival benefit in patients showing the isolated restricted diffusion (Gehan-Breslow-Wilcoxon test, p=0.006) but no overall survival benefit (Log-rank Mantel-Cox test, p=0.134). DWI- represent those with isolated restricted diffusion; No DWI- represent no evidence of isolated restricted diffusion.

Among patients with isolated restricted diffusion, those who developed enhancement on follow-up had a trend (p=0.059) towards higher average survival (474.1 ± 369.8 days) compared to those who did not progress to enhancement (729.3 ± 404.7 days). However the small numbers in each group did not allow for any meaningful analysis. The patients with isolated restricted diffusion progressing to enhancement (214.6 ± 120.2 days) had a trend towards shorter follow up (p= 0.059) compared to those who did not progress to enhancement (429.8 ± 308.9 days). Thus a shorter follow-up period was not responsible for the lack of corresponding enhancement.

4. Discussion

In patients with GBM, the regions of isolated restricted diffusion, on their diagnostic MRI, is likely a focus of tumour, even in the absence of enhancement. This is an important concept to consider since the current definition of a tumour on imaging only includes the contrast enhancing component of lesions (RANO criteria) [10]. Foci of isolated restricted diffusion at the time of imaging could be the focus of recurrent or residual tumor. It should also be noted that these foci of restricted diffusion could be seen long before corresponding contrast enhancement and could be separate from the enhancing component in patients with new or recurrent GBM. Earlier detection of these unrecognized foci of GBM could lead to more timely and suitable treatment that may improve prognosis.

In our study, isolated restricted diffusion was identified in 39.2% of GBM patients and in more than 20% of them restricted diffusion preceded corresponding enhancement. On an average, enhancement appeared five months after detection of isolated restricted diffusion on the
diagnostic MRI. Gupta et al found isolated restricted diffusion in a similar proportion (40.3%) of their patients (27 out of 67). They found enhancement at the site of restricted diffusion in 85.2% patient after a median of 3.0 months [3]. By recognizing these foci of isolated restricted diffusion on diagnostic MRI, as potential foci of tumour, a significant delay can be avoided in targeted treatment.

Survival data showed that isolated restricted diffusion was associated with longer short-term survival. We hypothesize that the patients with GBM showing isolated restricted diffusion may have different molecular signatures compared to those without. This hypothesis remains to be tested as we did not have molecular signature available in these patient for analysis. We plan to verify this in a prospective study. The patients with isolated restricted diffusion progressing to enhancement had a trend towards shorter follow up (p= 0.059) compared to those who did not progress to enhancement. This suggested that a shorter follow-up period was not responsible for the lack of corresponding enhancement. This may also suggest that progression to enhancement was a poor prognostic factor in terms of overall survival. However the number of patients in these subgroups was small to reach any meaningful conclusion.

Diffusion imaging is a part of routine brain tumor MRI. Our findings highlighted the added value of isolated restricted diffusion without corresponding enhancement on the diagnostic MRI in patients with GBM. Recognizing these areas of restricted diffusion may provide a more comprehensive approach to the diagnosis and treatment monitoring of GBM without any additional resources. Moreover, inclusion of isolated restricted diffusion in treatment planning of GBM, both on surgery or subsequent radiotherapy, may result in more predictable patient outcomes. Investigators continue to assess the role of isolated restricted diffusion as a predictor of GBM progression and prognosis [11]. Recognizing isolated diffusion restriction on post-treatment follow-up MRI may also be useful to gauge treatment efficacy and to identify recurrent or residual foci even in the absence of corresponding enhancement. For example, tumors treated with anti-angiogenic agents (e.g. bevacizumab) have been reported to reduce contrast enhancement, resulting in an overestimation of treatment response on conventional imaging [12]. Nevertheless, this interesting and important imaging phenomenon in GBM still requires rigorous clinical validation, which warrants further studies in larger patient populations.

Limitations: This study was limited in terms of its small number of patients and its retrospective nature. Our study was also limited due to the lack of data on the molecular markers and inconsistent follow up imaging in these patients. However our study generates an important hypothesis in sequential patients that remains to be tested in a larger prospective study. The study was also limited by the missing imaging data points in 14 patients. Of these, 10 patients died and did not have any follow up images. Another 4 patients had follow up images but did not have DWI done on follow up. The 10 patients who died on follow up were censored. The remaining 4 out of the 40 patients (10%) did not have diffusion images on follow up. These patients did not give any information on the length of time for appearance of enhancement. These patients were still included on survival analysis as we had clinical information on survival in all of them.

5. Conclusions

Isolated restricted diffusion preceded the corresponding enhancement in a subset of patients with GBM and was associated with early survival benefit. Recognition of this important imaging
phenomenon in GBM may foster its uptake into routine treatment planning, which may enable more predictable outcomes in the future. Prospective study in larger patient cohort is warranted to confirm our observations.

Author Contributions

Adil Bata - collected the data and wrote the first draft.
Namita Sinha - Intellectual contribution to the manuscript and editing the final draft.
Jai Shankar - Original idea, data analysis, final manuscript editing and submission.

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Competing Interests

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

References


Review

Craniopharyngiomas: An Appropriate Surgical Treatment based on Topographical and Pathological Concepts

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Abstract:
The optimal treatment of craniopharyngiomas (CPs) represents a neurosurgical challenge, the major reason being their close relationship with the hypothalamus and the third ventricle (3V) boundaries. Nevertheless, CPs are generally defined as “suprasellar” lesions, an inexplicit and frequently defective term. Despite being heterogeneous lesions, CPs are actually characterized by repeating pathological patterns which depend on their point of origin along the pituitary-hypothalamic axis. Surgical plan in tailoring the best treatment strategy needs to take in consideration some critical aspects, including the understanding of the accurate CP-hypothalamus relation. Without firm consensus and proposed guidelines for the appropriated management of CPs, radical removal and conservative resection remain the two opposing therapeutic strategies. However, an increasing leading of opinions stress out these two strategies should be replaced by a tailored plan to pursue the maximal tumor resection while minimizing the likelihood of hypothalamic injury in each case. Based on the
accurate definition of the CP-third ventricle floor relationship, our topographical scheme has been proven useful to predict the degree of hypothalamic distortion and adherence to the tumor. This is an essential information to predict the surgical risk associated with radical tumor removal. Apart from the sellar-suprasellar category of CPs which is formed by tumors growing below a non-distorted third ventricle floor (TVF), four major CP topographies can be considered: i) suprasellar-pseudointraventricular CPs, that grow below an intact but upward displaced TVF; ii) suprasellar-secondary intraventricular CPs, which originate below the TVF but break through it and invade the 3V; iii) not-strictly intraventricular or infundibulo-tuberal CPs, which originate within the TVF itself and predominantly expand into the 3V; and iv) strictly intraventricular CPs that wholly develop within the 3V, above an intact TVF. The most extensive and strongest CP-hypothalamic adhesions occur in the secondary intraventricular and infundibulo-tuberal categories. A hypothalamic position around the middle portion of the tumor, an amputated pituitary stalk and an elliptical tumor shape on preoperative MRI are reliable signs to predict a high-risk CP adherence severity level. In the cases with these radiological signs a limited removal is strongly advocated.

Keywords
Craniopharyngioma; topography; hypothalamus; third ventricle; adherence; adhesion; infundibulo-tuberal; treatment; trans-lamina terminalis approach; endoscopic transsphenoidal approach

1. Introduction

Defined by the World Health Organization as “benign, partly cystic epithelial tumours”, craniopharyngiomas (CPs) are widely regarded as rare brain tumours and considered as challenging intracranial tumours, despite their histological classification. Such a major neurosurgical challenge is due to their intimate relation with the hypothalamus and third ventricle (3V) boundaries. Nevertheless, analysis of the underlying CP pathological and topographical features related to this type of tumors is scarce in medical literature. On the one hand, most studies assume the inaccurate concept of a primary suprasellar position for most CPs, even though this heterogeneous type of lesion may develop at any point along the pituitary-hypothalamus axis, from the sella turcica to the 3V. Moreover, it should be kept in mind that almost half of CPs develop at the level of the third ventricle floor (TVF), within the infundibulum and/or the tubercinereum, regions including the vital hypothalamic nuclei [1-5]. On the other hand, the extreme heterogeneous CP morphology has definitely contributed to thwart a clear identification of the topographical and pathological CP features which allow the definition of those subgroups of cases associating a high surgical risk. The thorough assessment of 3,705 documents relating to CPs, including books, individual well-reported cases, surgical case series and historical case series such as Cushing’s series recorded in the Brain Tumor Registry (n=124) or the pathological series housed in the Narrenturm, Vienna (n=25), has allowed us to study over 5,000 CPs, including brain specimens from non-operated tumors and surgically treated lesions. We have been able to affirm that CPs, despite representing a morphologically heterogeneous type of tumor, are characterized
by repeating topographical and pathological patterns that can be differentiated in anatomical studies [4, 6, 7]. As hypothalamic dysfunction is the major cause of death and serious morbidity following CP surgery, an accurate preoperative definition of the specific anatomical CP-hypothalamus relationship is essential to plan the most appropriate surgical approach and degree of excision for each case. Regarding this issue, we have recently showed that conventional preoperative MRI studies can reliably predict the accurate CP topography and CP-hypothalamus adhesion pattern to be found in the surgical procedure [8, 9]. The aim of this review is to present a critical overview of gathered knowledge on topographical and pathological concepts regarding to CPs that should be considered when planning surgical treatment of these complex lesions.

2. Craniopharyngiomas: A Heterogeneous Group of Epithelial Lesions Developed along the Hypothalamic-Pituitary Axis

2.1 Pathological Definition of Craniopharyngiomas: Historical Background

The morphological and pathological heterogeneity of CPs has notably contributed to the difficulty in classifying these lesions throughout history. In the Nineteenth Century, a vast list of ambiguous terms such as medullary sarcoma, carcinoma of the pituitary gland, ependymal papilloma, cystosarcoma, cholesteatoma, and teratoma, were used to refer to lesions growing in the vicinity of the suprasellar area and showing gross pathological features typical of what we currently known as CPs today [10-12]. This extremely varied terminology has generated a lot of confusion. The first comprehensive classification of tumors related to the pituitary gland was presented in 1893 by Sir Rupert William Boyce (1863-1911), an assistant professor of Pathology at University College of London [13]. Based on the systematic study of 3,000 brain autopsies, he was able to differentiate the category of “pituitary strumas” or glandular neoplasms, from the remaining hypophyseal or infundibular tumors, such as granulomas, teratomas and carcinomas. Boyce used the term “pituitary cyst” for lesions resembling CPs owing to his erroneous assumption that these tumors developed from an excess of pituitary secretion [13].

The pathological entity of CPs was first systematically defined a decade later, in 1904, by the Austrian pathologist Jakob Erdheim (1874-1937) [14]. The confluence of several fortunate circumstances contributed to Erdheim’s definition of CPs as a separate tumor entity. Carl Benda had recently introduced new staining methods for pituitary gland cells that allowed the differentiation of pituitary “strumas” (adenomas) [15]. In addition, Erdheim had shown a particular interest in the pathological alterations involving the pituitary gland since the beginning of his career and was able to study in detail the collection of old brain specimens with pituitary tumors gathered at the Pathological Anatomical Museum in Vienna, stored at the Narrenturm (the former hospital for insane patients in Vienna) [16, 17]. The methodical study of a few sets of cystic lesions developed at the infundibulum in these brain specimens eventually led him to define the new pathological category of “hypophysengangsgeschwülste” (hypophyseal duct tumor), a term under which he grouped the heterogeneous epithelial growths developing either from the pituitary stalk or from the infundibulum (presumably from the pars tuberalis, the thin layer of glandular adenohypophyseal tissue wrapping around the pituitary stalk and infundibulum) [14, 16]. Erdheim showed great originality in relating the presence of squamous epithelial cell nests within the pars tuberalis, with the embryological development of the pituitary gland and growth of CPs [14]. With brilliant insight, he proposed that these tumors could originate from epithelial cell
remnants of Rathke’s pouch (the embryonic primordium of the pituitary gland) or, more accurately, from non-involuted remnants of the craniopharyngeal duct (Rathke’s pouch’s path of migration connecting the embryo’s mouth and TVF at the earliest stages of pituitary gland development) that was included within the pars tuberalis [14, 16]. Although CP pathogenesis is still controversial, recent molecular studies support Erdheim’s embryological theory that CPs are thought to originate from displaced embryonic stem cells of the primitive stomodeum located along the pituitary-hypothalamic axis [17, 18]. In his monumental masterpiece, Erdheim not only defined the CP entity but also characterized the two major histological variants with distinct morphological and molecular features currently considered in the WHO classification: adamantinous (ACP) and the squamous-papillary (PCP) types, for which he suggested a common embryonic pathogenesis [20, 21]: The adamantinous type consisted of lesions similar to adamantinomas or odontogenic jaw tumors, characterized by strands of a multistratified squamous epithelium with peripheral palisading of nuclei that enclosed sheets of “stellate reticulum” and cysts filled with cell debris and cholesterol particles. The squamous-papillary type included lesions similar to papillomas of the oral mucosa, typically formed of a unilocular cyst, whose inner wall was lined with a squamous epithelium forming wart-like or cauliflower-like excrescences [16]. Finally, Erdheim also pioneered the relationship between the damage of the of the infundibulo-tuberal area by CPs and the presence of obesity observed in many patients [22-24].

Three decades later, in 1929, the founding father of scientific neurosurgery, Harvey Cushing (1869-1939), introduced the easy-to-remember term “craniopharyngioma” [25]. Despite these lesions not originating from the pharynx but rather from an embryo’s primitive buccal cells migrating with Rathke’s pouch/craniopharyngeal duct, the term “craniopharyngioma” eventually triumphed over Erdheim’s more accurate term of “hypophyseal duct tumors” [12, 25]. The heterogeneity of CPs regarding their location and their varied extent of adhesion to the surrounding brain structures, commonly led to either failed craniotomies and/or to a high rate of death or life-ruining hypothalamic symptoms, that puzzled Cushing throughout his career. For this reason, he defined CPs as the “most baffling problem which confronts the neurosurgeon” [26]. One century later, CPs remain one of the most formidable and challenging intracranial tumors faced by practitioners.

2.2 Craniopharyngioma: Not a Suprasellar but a True Hypothalamic Tumor

CPs have been classically claimed to represent a suprasellar, extra-axial type of epithelial lesion. Unfortunately, the term “suprasellar” remains invariably linked to the concept of CPs, and it still dominates the neurosurgical and neuroradiological literature on these tumors [20, 27, 28]. The widespread use of the term “suprasellar” for CPs dates back to the early 20th Century, when the identification of dense shadows above the sella turcica represented the major radiological sign to ascertain the preoperative diagnosis of these lesions [25, 26]. The problem posed by this imprecise term is that it may cause an inaccurate topographical definition of the tumor, consequently leading to an incorrect surgical decision, due to the fact that the term suprasellar misses the fundamental point: “the tumor involvement of the vital hypothalamus”. The systematic study of anatomical CP relationships in both surgical and pathological cases published in the literature has proved that this tumor type predominantly involves the 3V and encroaches upon the hypothalamus [3, 6, 7].
Percival S. Bailey (1892-1973), one of Cushing’s most talented assistants, had a particular interest in neuropathology and was the first author to define CPs as true hypothalamic tumors [29]. The methodical study of Cushing’s vast collection of CP specimens, in addition to Bailey’s interest in hypothalamus physiology, led the latter to notice that most CPs developed within the hypothalamic region and predominantly extended into the 3V [10, 29]. Bailey also recognized that diabetes insipidus, adiposogenital dystrophy (Fröhlich’s syndrome) and hypersomnia were all typical symptoms in CP patients that revealed hypothalamic involvement by the tumor [29]. Comprehension of the pathological and physiological close relation between CPs and the hypothalamus was crucial for further developing surgical strategies aimed at minimizing the likelihood of devastating hypothalamic injury associated with CP surgery. In 1938, Norman M. Dott (1897-1973), another Cushing fellow who worked with Bailey in Boston, reported the first account of the successful surgical treatment of CPs centered at the hypothalamus [30, 31]. Dott proposed a novel strategy, including a two-stage approach (a subfrontal route followed by a transcortical-transventricular approach) to remove both the suprasellar and the intraventricular components of the tumor under direct view. He also warned about leaving untouched the basal portion of the tumor strongly adhered to the TVF in order to avoid undue injury to the adjacent hypothalamus [30].

The following generation of British neurosurgeons, headed by Douglas Northfield (1902-1976), contributed to emphasize the concept of a true hypothalamic, tuberal topography for a large number of CPs. Based on the ischemic/hemorrhagic lesions found within the hypothalamus in the autopsy of patients who died following surgical attempts at removal of CPs originated in the infundibulo-tuberal region, Northfield warned about the impossibility of resecting those lesions growing in the basal hypothalamus [32]. Recent studies focusing on the meningeal relationships between CPs and the pituitary-hypothalamic axis in a large series of tumors reported by Song Tao Qi et al. found that a subarachnoid-subpial position of the lesion within the TVF, without any intervening membranous layers between the tumor and the hypothalamus, occurred in 30% and 50% of pediatric and adult patients, respectively [5, 33, 34]. In light of the aforementioned information, the generalized and misleading term “suprasellar” should be abandoned and substituted by an accurate description of CP topography.

3. Topographical Classifications of Craniopharyngiomas

Currently there are numerous classification schemes for CP topography that have been proposed. Table 1 summarizes the major twenty-six topographical schemes published in the literature since this entity was defined in 1904 to the present. These series include a total of 2,480 CP cases. In most of them, surgical evidence represented the principal source of information [35-58]. Only six classifications were based on the pathological evidence of the relationships between the tumor and adjacent intracranial structures identified on brain autopsy specimens [1, 4-6, 14, 32-34, 59-62]. Among these schemes, the one presented by Juraj Steno in 1985 deserves special attention because it is the first modern topographical classification based on the anatomopathological features of 30 brain specimens of non-operated CPs [1]. Stenos’ scheme takes into consideration the degree of TVF involvement by the lesion, classifying CP topography into three major types: i) purely extraventricular lesions developed in the sellar and/or suprasellar compartments outside the 3V; ii) extraventricular-intraventricular CPs that grow in both the
suprasellar cistern and the 3V cavity, in which the TVF remnants are located around the mid-third of the tumor; and iii) purely intraventricular CPs wholly developed within the 3V above an intact TVF [1]. Shortly afterwards, Ivan S. Ciric published the only CP classification based on the embryological development of the leptomeningeal layers and the relative position of the epithelial remnants of Rathke pouch cells [63]. Ciric’s scheme included a depth axis from the outer meningeal layers to the inner nervous tissue and distinguished three groups of lesions: i) extrapial-intrarachnoid CPs, separated from the TVF tissue by the arachnoid and pia layers; ii) partially intrapial CPs, growing inside the nervous tissue of the TVF; and, iii) completely intrapial CPs, developing purely inside the 3V above an intact TVF. Finally, several topographical schemes are mainly based on MRI studies, including the two French classifications discussed below [64-67].

In the early 1990s, the neuroradiologist Charles Raybaud differentiated four major anatomic regions that could be occupied by the tumor: the sellar; suprasellar; infundibulo-tuberal; and third ventricle compartments. He observed that the majority of CPs primarily develop in the infundibulo-tuberal region, and his classification was the first MRI-scheme supporting the concept that CPs represent a type of lesion primarily involving hypothalamus [64], as had been previously found in the autopsy studies led by Erdheim and Steno [1, 14]. More recently, Christian Sainte-Rose and Stephanie Puget introduced a topographical CP scheme based on the appearance of the hypothalamus on preoperative MRI scans, which also took into consideration patient outcome [66, 67]. This scheme distinguished three major degrees of hypothalamic distortion caused by the tumor: i) grade 0, which corresponds to cases without hypothalamus distortion; ii) grade 1, included tumors causing hypothalamus displacement; and iii) grade 2, formed by tumors encroaching upon the hypothalamus, which cannot be identified on preoperative MRI [66, 67]. Saint-Rose and Puget’s scheme, however, has the drawback of assuming that the 3V involvement depends on the progressive expansion of a tumor theoretically originated at the sellar/suprasellar region, without taking into consideration the multiplicity of CP locations along the pituitary-hypothalamic axis. Furthermore, the appearance of the TVF on preoperative MRIs cannot be used as a valid sign to reliably ascertain the anatomical integrity of this structure. For example, an unidentifiable TVF does not necessarily signify its anatomical disruption by the tumor. In a recent study analyzing the anatomical CP relationships for a series of 17 intraventricular CPs, it was found that 15% of the cases whose TVF could not be identified on preoperative MRIs actually corresponded to purely intraventricular CPs presenting a small pedicle attachment to the intact TVF, a low-risk adhesion type allowing a safe radical removal [5].

3.1 Topographical Craniopharyngioma Schemes: Anatomical Reference Axes Used

According to the anatomical axis considered for CP classification, four major types of topographical CP schemes can be distinguished (Table 1):

Topographical classifications considering one vertical axis. A total of 12 series considered the occupation of the compartments along the vertical sellar-3V axis (sellar, suprasellar, infundibulo-tuberal and 3V) as the main criterion for CP classification [14, 32, 41-43, 45-54, 57, 59, 64-67]. The problem with many of these schemes is that they may lead to the erroneous idea that all CPs originate within the sella/suprasellar area and then progressively expand towards the 3V as they enlarge. Rather, different potential primary sites of CP development should be contemplated.
Table 1 Summary of the major topographical classifications of craniopharyngiomas.

<table>
<thead>
<tr>
<th>Author, year [Ref]</th>
<th>Number (children)</th>
<th>Classification model Source</th>
<th>Anatomical structure of reference</th>
<th>Major locations considered</th>
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<td></td>
<td></td>
<td>Vertical</td>
<td></td>
<td>II: SS-pseudo-3V (18%)</td>
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<td></td>
<td></td>
<td></td>
<td>III: infundibulo-tuberal 3V (42%)</td>
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<td></td>
<td>IV: pure intraventricular (14%)</td>
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<td>Northfield, 1957 &amp; 1973 [32, 59]</td>
<td>49 (23)</td>
<td>Surgical &amp; Pathological</td>
<td>Hypothalamus</td>
<td>I: intrasellar (6%)</td>
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<td></td>
<td></td>
<td>Vertical</td>
<td></td>
<td>II: subtuberal (30%)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>III: tuberal/3V (63%)</td>
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<td>Rougerie 1962 &amp; 1979 [35, 36]</td>
<td>140 (92)</td>
<td>Surgical</td>
<td>Sella, Chiasm</td>
<td>I: Intrasellar (11%)</td>
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<td></td>
<td>II: intra-SS prechiasmatic (51%)</td>
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<td></td>
<td>III: intra-SS retrochiasmatic (36%)</td>
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<td>IV: giant (2%)</td>
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<td>Pertuiset 1962 &amp; 1975 [37, 38]</td>
<td>-</td>
<td>Surgical</td>
<td>Vertical &amp; AP Sella, Chiasm</td>
<td>I: intrasellar</td>
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<td>IIa: SS-prechiasmatic (15%)</td>
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<td>IIb: SS-subchiasmatic (50%)</td>
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<td>IIc: SS-retrochiasmatic (35%)</td>
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<td>IId: SS-pseudo-3V</td>
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<td>III: intraventricular</td>
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<td>II: SS-prechiasmatic (25)</td>
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<td>III: SS-retrochiasmatic 3V (46%)</td>
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<td>II: SS-prechiasmatic (13%)</td>
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<td></td>
<td>III: intra and extraventricular (46%)</td>
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<td>IV: intraventricular (26%)</td>
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<td>Steno 1985 [1]</td>
<td>30 (10) A</td>
<td>Pathological</td>
<td>Meningeal layers, 3V</td>
<td>I: intrasellar-SS (13%)</td>
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<td></td>
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<td>Vertical &amp; Meningeal-brain depth*</td>
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<td>II: SS-extraventricular (13%)</td>
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<td>III: intra and extraventricular (46%)</td>
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<td>IV: intraventricular (26%)</td>
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<tr>
<td>Ciric 1987 [63]</td>
<td>-</td>
<td>Embryological</td>
<td>Meningeal layers, infundibulum</td>
<td>I: extrapial-extra-arachnoid</td>
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<td></td>
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<td>Vertical &amp; Meningeal-brain depth*</td>
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<td>II: extrapial-extra/intra-arachnoid</td>
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<td>III: extrapial-intra-arachnoid</td>
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<td>IV: extrapial-intrapial</td>
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<td>V: intrapial-intraventricular</td>
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<td>Sweet 1988 [44]</td>
<td>43 (21)</td>
<td>Surgical</td>
<td>Vertical &amp; AP Chiasm, 3V</td>
<td>I: intrasellar (3%)</td>
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<td>II: SS-prechiasmaic</td>
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<td>III: SS-subchiasmatic</td>
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<td></td>
<td>IV: retrochiasmatic-3V (58%)</td>
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<td></td>
<td></td>
<td></td>
<td>V: intraventricular</td>
</tr>
<tr>
<td>Raybaud 1991 [64]</td>
<td>23 (17)</td>
<td>Preop MRI</td>
<td>Vertical Sella, TVF</td>
<td>I: intrasellar-pre/retrachiasmatic (34%)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>II: infundibulo-tuberal (43%)</td>
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<tr>
<td>Study</td>
<td>N</td>
<td>Study Type</td>
<td>Approach</td>
<td>Location</td>
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<td>Maira 1995, 2000, 2004 [45-47]</td>
<td>92</td>
<td>Surgical &amp; preop MRI</td>
<td>Vertical</td>
<td>Sella, 3V</td>
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<tr>
<td>Samii 1995 [48]</td>
<td>24 (10)</td>
<td>Surgical</td>
<td>Vertical</td>
<td>Sella, 3V</td>
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<tr>
<td>De Vile 1996 [65]</td>
<td>75 (16)</td>
<td>Postop MRI</td>
<td>Vertical</td>
<td>TVF</td>
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<tr>
<td>Yasargil 1996 [49]</td>
<td>162 (80)</td>
<td>Surgical</td>
<td>Vertical</td>
<td>Diaphragma sellae, 3V</td>
</tr>
<tr>
<td>Fahlbusch 1999 &amp; Hofmann 2012 [50, 51]</td>
<td>221 (40)</td>
<td>Surgical &amp; Preop MRI</td>
<td>Vertical</td>
<td>Sella, 3V</td>
</tr>
<tr>
<td>Zhang 2002, 2008 [68, 69]</td>
<td>202 (202)</td>
<td>Surgical &amp; Preop MRI</td>
<td>AP &amp; Lateral</td>
<td>Pituitary stalk, Chiasm</td>
</tr>
<tr>
<td>Pascual 2004, 2011, 2013 [4, 6, 62]</td>
<td>224 (63)</td>
<td>Surgical &amp; Preop MRI &amp; Pathological</td>
<td>Vertical &amp; Meningeal-brain depth*</td>
<td>TVF</td>
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<td>Reference(s)</td>
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<td>92</td>
<td>Surgical (E-TS)</td>
<td>Vertical &amp; Lateral &amp; Pituitary stalk</td>
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AP: anteroposterior; Meningeal-brain depth*: depth axis from the outer meningeal layers to the inner nervous tissue; E-TS: endoscopic transsphenoidal route; TVF: third ventricle floor; SS: suprasellar; Preop MRI: preoperative Magnetic Resonance Imaging; Postop MRI: postoperative MRI; 3V: third ventricle.
**Topographical classifications considering one horizontal or antero-posterior axis.** Two classic surgically-based schemes used the optic chiasm to classify the position of CPs as this was the main anatomical structure seen through the frontotemporal or pterional approach [35, 36, 39, 40]. These schemes distinguished three major CP topographies: i) prechiasmatic CPs—for tumors originated below the chiasm which expand between both optic nerves; ii) subchiasmatic CPs—for the tumors displacing the optic nerves and chiasm upwards; and iii) retrochiasmatic CPs—for lesions hidden behind the optic chiasm and pushing this structure forward, most of them expanding within the 3V. The problem with the term “retrochiasmatic” is that it does not provide any information about the accurate relationship between the tumor and the 3V-hypothalamus in these schemes.

**Topographical classifications considering two axes: one vertical or lateral and one horizontal.** Five CP classifications are based on the vertical and antero-posterior axes [37, 38, 44, 55, 56, 60, 61], and two schemes consider the lateral position of the tumor relative to the pituitary stalk in addition to either an antero-posterior axis [68, 69] or a vertical axis [58]. For example, William H. Sweet (1910-2001), who advocated the pterional-trans-lamina terminalis route for CPs, proposed a scheme based on tumor position relative to the optic chiasm and 3V. Very recently, Amin K. Kassam [55] and Bin Tang [58], two experts in the endoscopic extended transsphenoidal approach, used the pituitary stalk and infundibulum as the structures of reference to define CP topography due to the fact that they are the first ones identified when using the transsphenoidal route. Specifically, Kassam's scheme considers four major CP types [55]: i) preinfundibular CPs, or lesions located in front of the stalk-infundibulum complex, just behind the optic chiasm; ii) transinfundibular CPs, tumors expanding into the pituitary stalk itself; iii) retroinfundibular CPs, corresponding to those lesions growing behind the stalk-infundibulum complex which can expand either into the 3V cavity or towards the suprasellar-interpeduncular compartments; and iv) intraventricular CPs, situated above an intact pituitary stalk. The removal of this last type through an endonasal transsphenoidal route necessarily compels breaking through an anatomically intact TVF.

**Topographical classifications considering two axes: one vertical and one depth axis through the leptomeningeal-brain tissue complex forming the TVF.** Four studies classified the tumor location along the vertical pituitary-hypothalamic axis in addition to considering how deeply the tumor extended into the leptomeningeal layers and nervous tissue [1, 3-6, 33, 34, 62, 63, 70, 71]. Steno’s scheme was the first to highlight that the presence of an intervening meningeal layer between the tumor and the outer-pial aspect of the TVF in CPs developed beneath an intact TVF allowed a straightforward and safe removal of these tumors except in recurrent cases [1, 2]. A safe radical removal could be also achieved in purely intraventricular CPs in which the TVF was found to be anatomically intact below the lesions. On the contrary, he noticed that the highly prevalent group of CPs with an extra-intraventricular position, formed by lesions centered at the TVF, was characterized by direct contact between the tumor and the hypothalamus. The strong adhesions between these tumors and the surrounding TVF remnants explained the highest surgical risk of hypothalamic injury when attempting a radical removal of this CP topography [1]. The recent surgical classifications proposed by Pascual et al. and Qi et al. [4-6, 33, 34, 62] integrate the pathological concepts evidenced in Steno’s seminal study [1] with the embryological theory proposed by Ciric [63]: that those CPs developing within the neural tissue of the infundibulum/tuber cinereum are originated from Rathke’s pouch cell remnants coming into
contact with the diencephalic vesicle floor before the pia mater is formed and covers the basal brain surface.

### 3.2 Topographical Craniopharyngioma Classification Based on the Assessment of the Third Ventricle Floor Status: Surgical Implications of Our Model

The most important aspect when planning CP surgery is to well understand the anatomical position of the hypothalamus relative to the tumor, with the aim of predicting the likelihood of achieving a safe resection. In this regard, in 2004, our group presented a comprehensive classification scheme for CPs based on the definition of the accurate anatomical relationships between the tumor and the TVF, the region containing hypothalamic nuclei. This anatomical area is essential for monitoring vital functions such as food intake, body water homeostasis, primary emotional reactions of aversion/craving, as well as the control of the sleep-wakefulness cycle [6].

Four major CP topographies depending on the specific type of CP-TVF relationship were identified:  

i) **suprasellar-pseudointraventricular CPs**: the category of lesions usually originated at the junction between the pituitary gland and stalk that expand within the suprasellar cistern while causing an upward displacement of the TVF, mimicking an intraventricular position;  

ii) **sellar/suprasellar-secondary intraventricular CPs**: lesions initially develop under the 3V but at later stages invade and occupy the 3V cavity after breaking through the TVF;  

iii) **infundibulo-tuberal or not-strictly intraventricular CPs**: tumors developed at a subpial position within the neural layer of the infundibulum or tuber cinereum and expanding predominantly into the 3V(These lesions grow in close contact to the hypothalamic nuclei [3, 4]);  

iv) **strictly-intraventricular CPs**: tumors primarily developed within the 3V, above an intact TVF [4, 6, 62]. An additional topographical category of CPs without 3V involvement has to be considered, the group of **sellar-suprasellar CPs** that includes those tumors originated at the dorsal surface of the pituitary gland, beneath the sellar diaphragm, which exclusively occupy the sellar and/or suprasellar compartments (Figure 1).  

This scheme is relatively simple and has the major advantage of providing essential information about the type of hypothalamic-CP relationship that will be found during the surgical procedure. Furthermore, the relative position of the hypothalamus and its degree of anatomical distortion can be easily ascertained on preoperative MRI studies by defining the CP topography according to these categories [8, 9]. In our topographical scheme, the category of sellar-suprasellar CPs is the one associating the lowest risk of hypothalamic injury, whereas the surgical risk is maximal for the secondarily-intraventricular and infundibulo-tuberal topographies that are characterized by wide and strong adhesions to the hypothalamus [7].
Figure 1 Topographical classification scheme for craniopharyngiomas based on the original point of development along the hypothalamic-hypophyseal axis [3, 6]. **Left:** Illustrative mid-sagittal schematic of the hypothalamic-hypophyseal complex. The lower part of the hypothalamus surrounding the infundibular recess (IR) corresponds to the infundibulum. CPs may originate at four different points along this vertical axis: i) at the dorsal surface of the pituitary gland (1); ii) from the pars tuberalis covering the infundibular stem or pituitary stalk (2); iii) at a subpial position within the neural tissue of the median eminence (ME) and the tuber cinereum (TC) of the third ventricle floor (3); iv) wholly within the third ventricle, from beneath the ependymal layer of the infundibulum (4). A: arquate nucleus; Ch: optic chiasm; DM: dorsomedial nucleus; ds: diaphragma sellae (in blue); M: medial mammillary nucleus; MB: mammillary body; P: posterior nucleus; PD: pars distalis; PI: pars intermediate; PN: pars nervosa; PV: paraventricular nucleus; SO: supraoptic nucleus; TM: tuberomammillary nucleus; VM: ventromedial nucleus; 3V: third ventricle. **Right:** Tumor-hypothalamus anatomical relationships for the five major CP categories considered in our topographical classification: i) sellar-suprasellar; ii) sellar/suprasellar pseudointraventricular (S-SS-Pseudo 3V); iii) sellar/suprasellar secondary intraventricular (S-SS-Secondary 3V); iv) not-strictly intraventricular (Not-strictly 3V) or infundibulo-tuberal; v) strictly intraventricular (strictly 3V). Column A shows the tumor-hypothalamus relationships as observed on mid-sagittal MRI scans. Column B illustrates these relationships as seen in coronal trans-infundibular sections. The position of the hypothalamus (in red) relative to the tumor is indicated for each topographical category. The color of the frame for each topographical category corresponds to the number of the point of origin along the hypothalamic-hypophyseal axis.

### 3.3 The Category of Infundibulo-tuberal Craniopharyngiomas: Clinical Assessment and Surgical Risks

The category of not-strictly intraventricular or infundibulo-tuberal CPs deserves special address due to the fact that it is the most prevalent topography in both adults and children, with an overall
rate between 30 and 50% of cases, and high surgical risk [1, 6, 33, 42, 52-54, 72]. Infundibulo-tuberal CPs originate in the basal region of the hypothalamus within the TVF, an anatomical area formed by the infundibulum (Figure 1), the hollow and funnel-shaped proximal portion of the neurohypophysis, and the tuber cinereum, the adjacent grey matter enclosed between the optic tracts and the mamillary bodies [30, 75]. Mortality rates reported for this topography range between 4 and 15%, and postoperative morbidity due to hypothalamic injury, including lethargy, apathy, progressive obesity, severe memory loss, and behavioral or psychiatric disturbances may occur in up to 50% of patients, whose quality of life becomes notably hampered [76]. The underlying reason for such a high risk of hypothalamic damage is the subpial origin of this CP category leading to the close contact between the tumor capsule and the hypothalamic nuclei without any protective intervening meningeal layer.

The median eminence (Figure 1), located along the midline of the infundibulum, contains the arcuate nucleus, which plays a fundamental role in the regulation of feeding behavior linked to energy balance. In addition, the lower ventromedial, periventricular and tuberoinfundibular nuclei, as well as the supra-optic-hypophyseal and tuberoinfundibular tracts included within the infundibulo-tuberal region are involved in the regulation of water metabolism, control of sleep cycles and sexual functions through the monitoring of circulating sexual hormones and the control of hypophyseal secretion of gonadotropins [30, 77-81]. Apart from a thorough assessment of MRI studies, the preoperative identification of symptoms caused by dysfunction of these hypothalamic basal structures is essential to predict the anatomical and functional status of the infundibulum and tuber cinereum. The infundibulo-tuberal syndrome can be observed in patients with a functional impairment of the infundibulum and tuber cinereum, and is manifested by symptoms such as abnormal somnolence, diabetes insipidus and/or adipose-genital syndrome [82]. This adipose-genital syndrome, also known as Fröhlich’s syndrome, consists of sexual infantilism and abnormal obesity [23]. Despite the surgeon’s experience and clinical skills, any forcible attempts at radical CP removal in patients with symptoms of the infundibulo-tuberal syndrome can lead to a high risk of irreversible hypothalamic injury and severe postoperative morbidity or even patient death [4, 65, 82].

The infundibulo-tuberal CP topography makes the greatest controversy regarding the feasibility of achieving a radical resection. Some surgeons, led by William H. Sweet (1910-2001) considered the layer of gliosis developed around these tumors as a viable cleavage plane for a safe and radical removal [44, 84]. On the contrary, histological/pathological evidence has shown a true fusion between the tumor and the surrounding hypothalamus, rendering a total excision impracticable. The generation of a sticky layer of reactive gliosis at the TVF remnants around the tumor additionally contributes to the strong, extensive adhesions usually found in this subgroup [83]. Both Bailey and Northfield emphasized that extreme adhesiveness of the glial layer was a sign indicating that the cleavage plane was positioned within the normal hypothalamus [29, 59]. Actually, the overall rate of total resection for the infundibulo-tuberal category is only about 60%, a percentage notably lower than that reported for other CP categories. This indicates the difficulty of releasing the tumor from the hypothalamic adhesions and/or the neurosurgeon’s determination to leave untouched the tumor fragments strongly attached to the floor/walls of the 3V in these cases [76]. In support of such a conscious decision, Kubota’s et al. analysis of the brain-tumor interphase in 6 whole CP specimens predominantly growing inside the 3V found that the layer of peritumoral gliosis had an irregular thickness and, in some areas, no effective distance for
a safe dissection could be identified between the tumor and the adjacent hypothalamic nuclei [73]. Likewise, several authors have evidenced the presence of tumor finger-like protrusions penetrating into the adjacent hypothalamus [85-87], a decisive histological finding suggesting the cautious assessment of CP-hypothalamus adhesions and warning against the indiscriminate radical removal of infundibulo-tuberal CPs.

4. Craniopharyngioma Adherence: From a Neurosurgeon’s Subjective Notion to an Objective Definition of Adhesion Pattern and Severity

Apart from the neurosurgeon’s skill, patient outcome following CP removal mainly depends on the type and strength of tumor adhesion to the hypothalamus [7, 88]. The pervasive uncertainty regarding the extent and degree of adhesion in each case is a source of great distress for the neurosurgeon. The extreme heterogeneity of tumor adhesions has surely contributed to the lack of a reliable description of this pathological feature in most modern CP surgical series. We have recently been able to address this issue through the methodical examination of 500 well-described CPs, including non-operated whole tumors from brain autopsy specimens as well as surgically treated cases. We were able to identify repetitive patterns of CP adhesion associated with specific tumor topographies and to present the first comprehensive classification of CP adherence [7]. Furthermore, our methodology allows for an accurate, reliable assessment of the pattern and strength of CP adhesions employing conventional MRI studies [9]. This model provides an objective way of grading the type of CP-hypothalamus adhesions into five major levels of severity, each one associating an increasing risk of hypothalamic injury during surgery. A critical understanding of surgical risks based on the severity of CP adhesions to the hypothalamus defined on preoperative MRI may be a fundamental tool for guiding the neurosurgeons’ decision regarding the safest approach to the tumor and extent of removal to be carried out in each patient [7, 9].

4.1 The Three Components of Craniopharyngioma Adherence

The type of tumor adherence can be objectively defined according to three components:

Anatomical structures involved in the adhesion. This component defines the structure of the hypothalamic-pituitary axis to which the tumor shows the predominant or primary adhesion. The maximal adhesion is usually observed at the anatomical structure where the CP originally develops. According to the anatomical structure involved, the following types of attachment can be differentiated (Figure 2): i) attachment to the pituitary gland and/or sella turcica, below the diaphragm sellae; ii) attachment to the pituitary stalk and outer surface of the infundibulum; iii) attachment to the entire thickness of the TVF; iv) attachment to the 3V floor and walls; v) attachment to the ependymal lining of the 3V; vi) attachment to the whole hypophyseal-hypothalamic axis.

Morphological pattern of CP adhesion. This component defines the extent and shape of the CP adhesions, and six patterns can be distinguished (Figure 3): i) pedicle-like attachment, formed by a narrow fibro-vascular stem between the tumor base and the ependyma of the 3V, usually observed in purely or strictly intraventricular CPs; ii) sessile or “patch-like” attachment, in which a limited area of the tumor surface forms the adhesion, usually to the pituitary stalk and/or infundibulum; iii) “cap-like” adhesion, involving the upper half of the tumor, usually visible in large CPs pushing the 3V upwards (pseudointraventricular CPs); iv) ring-like attachment, in which a band
of adhesion encircles the tumor’s central portion, usually observed in tumors invading the 3V (secondarily 3V CPs) and also in CPs developed within the TVF itself (infundibulo-tuberal CPs). As this band of adhesion is usually formed by the nervous tissue remnants of the TVF, it represents a close, hazardous-to-touch plane contact between the CP and the hypothalamus; v) “bowl-like” adhesion, in which the tumor’s bottom part is attached to the TVF, observed among CPs expanding within the 3V; vi) circumferential adhesion, the most extensive type of attachment in which the surrounding brain tissue wraps around the entire tumor surface, typical of infundibulo-tuberal CPs.

![Image of craniopharyngioma adherence patterns](image)

Figure 2 First component of craniopharyngioma adherence: intracranial structures involved in the attachment [7]. Six major adhesion patterns can be considered: A. Sella-Gland: tumor attachment occurs within the sella turcica to the pituitary gland and/or the sellar structures below the diaphragm sellae. B. Outer aspect of the Stalk-Infundibulum: the CP attachment occurs at the solid portion of the pituitary stalk and the outer aspect of the infundibulum. C. Third ventricle floor (TVF): the tumor is adhered to the entire thickness of the TVF. D. TVF-3V walls: CP attachment involves the floor and walls of the third ventricle. E. 3V (inner lining): the tumor is attached to the ependymal lining of the third ventricle cavity. F. Global (Sella to 3V): the CP attachment involves all the structures of the hypophyseal-hypothalamic axis.
Figure 3 Second component of craniopharyngioma adherence: morphology or extent of the attachment [7]. Six major patterns can be distinguished: **A. Pedicle**: the tumor is attached through a narrow fibrovascular stem. **B. Sessile**: small patch of adhesion. **C. Cap-like**: wide adhesion involving the upper half of the tumor. **D. Ring-like**: a circular band of brain tissue (the TVF) is encircling the central portion of the tumor surface. **E. Bowl-like**: wide adhesion to the infundibulo-tuberal region involving the lower half of the tumor. **F. Circumferential**: the entire tumor surface is attached to the surrounding brain tissue (hypothalamus).

**Strength of CP adhesion.** This component describes the resistance offered by the attachment to surgical release and can be classified in four types (Figure 4): i) *loose* or easily dissectible adhesions; ii) *tight* adhesions, for which a sharp dissection is required to preserve the anatomical structure attached; iii) CP-brain or CP-pituitary stalk *fusion*, when there is no identifiable cleavage plane between the tumor and the adjacent nervous tissue; iv) *replacement*, the strongest and most dangerous degree of adhesion occurs when the structure involved in the attachment is no longer recognizable because it has been replaced by tumor growth. The infundibulum and tuber cinereum of the TVF are usually replaced by large infundibulo-tuberal CPs as well as by secondarily 3V lesions encroaching upon the 3V.
Figure 4 Third component of Craniopharyngioma adherence: adhesion strength [7]. Four major patterns can be identified: A. Loose: easily dissectible adhesion. Note the arachnoid layer (arrows) interposed between the tumor (t) and the normal brain; B. Tight: No meningeal layer is interposed between the tumor (t) and the anatomical structure. Sharp tumor dissection is mandatory to preserve the brain structure attached. PG: pituitary gland; PS: pituitary stalk; C. Fusion: a cleavage plane between the tumor and the adjacent brain tissue cannot be identified; D. Replacement: the structure involved in the attachment is no longer recognizable, as it has been encroached upon and replaced by the tumor. Ch: optic chiasm; MB: mammillary body.

4.2 Craniopharyngioma Adhesions Classified into Five Levels of Adherence Severity

Taking into consideration the three components of tumor adhesion, the severity of CP adherence can be classified into 5 major levels—mild, moderate, serious, severe, and critical (Figure 5)—each associating an increasingly higher risk of hypothalamic injury and a worse postoperative outcome [7]. The least severe type of hypothalamic adhesion (level I or mild) occurs more often in wholly intrasellar or sellar-suprasellar CPs separated from the TVF by a meningeal layer, either the dura mater of the diaphragm sellae or the arachnoid membranes of the chiasmatic cistern. Level II or moderate severity is typically observed in strictly intraventricular CPs with a pedicle or a sessile attachment to the ependymal lining of the 3V, a type of adhesion which can usually be released straightforwardly. Level III (serious) adhesion is found in suprasellar CPs tightly adhered or fused to the pial surface of the infundibulum and/or tuber cinereum, without any intervening arachnoid layer. Attempts at releasing the tumor from these adhesions to the outer surface of the median eminence/basal hypothalamus associated a poor outcome in about one tenth of the cases. Level IV (severe) occurs in CPs fused to the TVF nervous tissue or those replacing the infundibulo-tuberal region. These extensive adhesions to the hypothalamus are usually observed in infundibulo-tuberal CPs and in tumors invading the 3V. Severe CP adhesions usually show a bowl-like, ring-like or circumferential morphology, and forceful attempts to separate the tumor from the adjacent hypothalamus led to a poor outcome in about 25% of cases.
due to irreversible hypothalamic damage. Finally, level V, the most severe (critical) degree of hypothalamic adherence occurs in large and aggressive CPs extending from the sella to the 3V roof that encroach upon all the structures of the pituitary-hypothalamic axis. The invasion and replacement of the TVF led to a rate of poor outcome/death as high as 40% in this group [7].

**Figure 5** Severity levels of craniopharyngioma adherence to the hypothalamus. The first row shows autopsy specimens of CPs (t) for the five adherence severity levels (I to V) considered. The middle and lower rows display preoperative and postoperative MRI scans, respectively, of tumors presenting similar adherence severity levels to those shown in the autopsy specimens. The red arrows point to the position of the hypothalamus relative to the tumor. The table at the bottom summarizes the specific three components of CP adherence that define each level of severity. **Level I - Mild: A1.** Midsagittal brain section showing a sellar-suprasellar CP (t) which displaced the third ventricle floor upward (red arrow). MB: mamillary body; 3V: third ventricle. **A2.** Preoperative midsagittal section showing a sellar-suprasellar CP (t). Note the hypothalamus (red arrow) is above the tumor. Ch: optic chiasm. **A3.** Postoperative scan demonstrating the anatomical integrity of the third ventricle floor (TVF) following radical tumor removal through a pterional route. **Level II - Moderate: B1.** Coronal autopsy specimen showing a strictly intraventricular papillary CP (t) with a small basal attachment (blue arrow) to the TVF (red arrows). **B2.** Preoperative MRI scan of a solid strictly intraventricular CP (t). Note the pituitary stalk (PS) and the pituitary gland (PG) are intact below the tumor. **B3.** Postoperative scan demonstrating an intact TVF following a complete tumor resection through a pterional trans-lamina terminalis approach. **Level III - Serious: C1.** Sellar-suprasellar pseudointeraventricular CP (t) beneath an upwardly displaced TVF (red arrow). **C2.** Preoperative midsagittal MRI scan showing a pseudointeraventricular CP (t) pushing the TVF upward and stretching the
chiasm (Ch). C3. Contrast-enhanced areas can be observed at the upper pituitary stalk (PS) and the outer side of the infundibulo-tuberal area (arrows), a sign of residual tumor/gliotic scarring after blunt dissection of a tightly attached tumor which was approached through a pterional route. Level IV - Severe: D1. Not-strictly intraventricular or infundibulotuberal CP (t) with a mixed solid-cystic consistency. Note the MB is the only visible TVF structure. The pituitary stalk (PS) has been amputated by the tumor and the pituitary gland (PG) is intact beneath the tumor. D2. Coronal preoperative MRI scan showing an infundibulo-tuberal CP (t). The hypothalamus is located around the mid-third portion of the tumor (red arrows). D3. Postoperative scan revealing a hypointense sign on the left hypothalamus (arrow), indicating hypothalamic injury after radical trans-lamina terminalis tumor resection. Level V - Critical: E1. Sagittal section showing a suprasellar-secondary intraventricular CP (t) that has broken into the third ventricle. E2. Preoperative MRI scan showing a large multilobulated secondary intraventricular CP (t). The hypothalamus (red arrows) is around the mid-third portion of the tumor. E3. Postoperative contrast-enhanced T1-weighted MRI scan, following a radical transcallosal removal, shows a breached TVF (asterisk), a sign suggesting tumor replacement of the tuber cinereum. The hyperintense sign at the TVF remnants (arrow), indicates the presence of gliotic scarring and/or tumor remnants.

5. Diagnosis of CP Topography: MRI Predictors of the Tumor-Hypothalamus Relationship

Since the introduction of MRI in the late 1980s, this technology represents the gold standard tool for the preoperative diagnosis of CPs, despite CT remaining more useful to depict tumor calcifications. MRI allows an accurate assessment of the tumor’s anatomical relationships in the three spatial dimensions [89]. Preoperatively recognizing the true hypothalamus position regarding the tumor, as well as the anatomical distortion of this structure, represents essential information for the neurosurgeon to anticipate the risks of hypothalamic injury and to plan accordingly the most suitable surgical procedure. Our group has recently demonstrated that an accurate definition of CP topography (Figure 6) and the type of CP-hypothalamus adhesion (Figure 7) can be reliably achieved with conventional MRI by examining the T1- and T2-weighted sequences [8, 9]. Midsagittal and coronal-transinfundibular MRI sections are the two most important ones for a proper assessment, as CPs originate along the midline structures that form the pituitary-hypothalamic axis. By assessing a small set of MRI signs, it is possible to achieve a correct preoperative definition of CP topography and hypothalamic adhesion severity in a high rate of patients, 85% and 90% of cases respectively [8, 9]. Preoperative MRI evaluation must include the occupation by the tumor of the intracranial anatomical compartments surrounding the pituitary-hypothalamic axis (sella turcica, chiasmatic-or suprasellar- cistern and 3V), in addition to the anatomic distortion of the pituitary-hypothalamic structures (pituitary gland, pituitary stalk, infundibulum, tuber cinereum and third ventricle walls).

5.1 Tumor Occupation of the Third Ventricle and the Chiasmatic Cistern

The compartments whose occupation provide the most reliable information to define CP-hypothalamus relationships are the 3V and the chiasmatic-or-suprasellar cistern. No occupation of
the 3V is only observed in the sellar-suprasellar topography, the one associating the lowest risk of hypothalamic injury (Figure 6. A1). Although extremely important, accurately distinguishing between the four remaining topographical categories involving the 3V is more difficult, and the occupation of the chiasmatic cistern represents one of the most valuable radiological signs. A wholly tumor-free chiasmatic cistern strongly points to the strictly intraventricular topography (Figure 6. E1), whereas its partial occupation (“belly-like” protrusion) is typical of the infundibulo-tuberal category (Figure 6. D1). Complete obliteration of the chiasmatic cistern may be observed in tumors originated below the TVF (suprasellar-pseudointraventricular or secondary intraventricular topographies) (Figure 6. A1, B1, C1) [8].

5.2 Pituitary Stalk Distortions

The anatomical distortions of the pituitary-hypothalamic axis, in particular the appearance of the pituitary stalk and the anatomical position the hypothalamus relative to the tumor, are the two most useful MRI signs to define the type of CP-hypothalamus relationship. The appearance of the pituitary stalk is the radiological sign showing the strongest correlation with the type of CP topography [8]. Four major patterns of stalk distortion can be differentiated on preoperative MRI scans: intact or wholly visible; thickened; amputated; and not visible. Amputation of the upper infundibular portion of the stalk by the tumor is usually observed in the not-strictly intraventricular or infundibulo-tuberal topography (Figure 6. D1). This sign strongly suggests the presence of a severe (level IV) CP adhesion to the hypothalamus. On the contrary, a normal pituitary stalk appearance points to the strictly intraventricular topography with a moderate (level II) adherence to the hypothalamus (Figure 6. E1). Inability to identify the pituitary stalk on preoperative MRI is a less specific sign, as this can occur in any of the three CP topographies that originate beneath the TVF and occupy the chiasmatic cistern: the sellar-suprasellar, pseudointraventricular and secondary intraventricular topographies, all of which encroach upon the whole pituitary stalk (Figure 6. A1, B1, C1).

5.3 Hypothalamus Position Relative to the Tumor

The level of the hypothalamus relative to the tumor is the most informative MRI sign to predict the severity of CP adherence [9]. This variable is best assessed on coronal-transinfundibular MRI sections, and three major positions can be considered: hypothalamus located at the bottom of the tumor area; around its mid-third portion; or above the top pole of the lesion. When the hypothalamus is located around the mid-third or central portion of the tumor, the presence of extensive and tenacious adhesions to the hypothalamus, with either a ring-like or a circumferential pattern (high-risk adherence levels, IV-severe or V-critical), should be expected (Figure 7. D2, E2). On the contrary, when the hypothalamus is located either around the lower third (Figure 7. B2) or upper third of the tumor (Figure 7. A2, C2), low-risk adherence levels (I-mild, II-moderate, or III-serious) are likely to be found [9]. A hypothalamus positioned at the lower third of the tumor is typically observed in the strictly intraventricular topography (Figure 6. E2), whereas a hypothalamus situated above the tumor pole generally occurs in the sellar-suprasellar (Figure 6. A2) or pseudointraventricular (Figure 6. B2) topographies [8].
Figure 6 MRI signs defining the five major topographical craniopharyngioma categories. Midsagittal (A1 to E1) and coronal-transinfundibular (A2 to E2) MRI sections representative of each category. Red arrows point to the relative position of the hypothalamus in relation to the tumor. Blue angles formed by the intersection of a plane tangential to the base of the mamillary bodies (MB) with the plane tangential to the fourth ventricle floor forms the mamillary body angle. Ch: chiasm; t: tumor; 3V: third ventricle. The table below summarizes the specific MRI signs observed for each topographical category.

<table>
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<th>S/SS-Secondary 3V</th>
<th>Not-Strictly 3V (Infundibulo-Tuberal)</th>
<th>Strictly 3V</th>
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</thead>
<tbody>
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<td>Stretched upward</td>
<td>Stretched forward</td>
<td>Compressed forward</td>
<td>Round</td>
</tr>
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<td>Not visible</td>
<td>Not visible</td>
<td>Amputated, infiltrated</td>
<td>Tumor free</td>
</tr>
<tr>
<td>Hypothalamic level</td>
<td>Upper third</td>
<td>Upper third</td>
<td>Mid third</td>
<td>Mid third</td>
<td>Lower third</td>
</tr>
<tr>
<td>Mamillary body angle</td>
<td>60-89°</td>
<td>&gt;90°</td>
<td>30-59°</td>
<td>&lt;30°</td>
<td>30-59°</td>
</tr>
<tr>
<td>Chiasmatic cistern</td>
<td>Occupied by tumor</td>
<td>Occupied by tumor</td>
<td>Occupied by tumor</td>
<td>Partially occupied</td>
<td>Occupied by tumor</td>
</tr>
<tr>
<td>Chiasmatic cistern</td>
<td>Occupied by tumor</td>
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<td>Chiasmatic cistern</td>
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<td>Occupied by tumor</td>
<td>Occupied by tumor</td>
<td>Partially occupied</td>
<td>Occupied by tumor</td>
</tr>
</tbody>
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5.4 Craniopharyngioma Shape

Some macroscopic CP features, such as tumor size, consistency, shape, or presence of calcifications, provide key information about the extent and degree of CP adhesions, and should also be defined on preoperative neuroradiological studies. Among these features, tumor shape has proven particularly useful to predict the severity of CP-hypothalamus adhesions. A pear-like tumor outline is typical of CPs originated within the sellar compartment which have pushed the diaphragm sellae upward against the optic chiasm as they grow (Figure 7. A2). The presence of this layer of duramater interposed between the tumor and the TVF prevents the development of tight adhesions to the hypothalamus in these cases, associating the lowest risk of hypothalamic injury. Quite the opposite, a multilobulated morphology indicates that the tumor originated along the PS, at the level of the chiasmatic -suprasellar- cistern, and that it expanded into the arachnoid spaces, giving rise to more extensive and sticky adhesions to the hypothalamus (Figure 7. E2). Finally, the elliptical shape is associated with the tumors showing the most severe degrees of hypothalamic attachment (levels IV and V) (Figure 7. D2) [9]. Two additional, highly informative MRI signs that should be evaluated on preoperative MRI studies are the mamillary body angle and the anatomical distortion of the optic chiasm, both of which will be described below.
Figure 7 Surgical findings and MRI signs defining the five increasing severity levels of craniopharyngioma adherence. The bottom table summarizes the specific MRI signs associated with each CP adherence severity level. **Level I - Mild: A1.** Right pterional view showing the arachnoid layer (arrow) between the tumor (t) and the surrounding structures. ICA: internal carotid artery; rON: right optic nerve. **A2.** Midsagittal MRI scan showing a craniopharyngioma with a pear-like morphology (t) expanding in the sellar and suprasellar compartments below the hypothalamus (red arrow). The pituitary stalk cannot be identified. **Level II - Moderate: B1.** Surgical view through a right pterional approach showing the narrow pedicle attachment (arrow) of a papillary CP (t) to an intact third ventricle floor (TVF) below the tumor. **B2.** Midsagittal MRI scan of a round solid tumor (t) purely located in the third ventricle. Note the hypothalamus (red arrow) is below the tumor and the pituitary stalk (PS) has a normal appearance. **Level III - Serious: C1.** Right pterional view showing a tight adhesion (arrow) between the tumor and the upwardly displaced chiasm and third ventricle floor. **C2.** MRI scan showing a dumbbell CP (t) that displaces the chiasm and third ventricle floor (red arrow) upward, mimicking an intraventricular position. **Level IV - Severe: D1.** Intraoperative view through a right pterional approach showing a CP (t) fused (arrows) to the inner surface of the infundibulum (IF). **D2.** Coronal MRI scan showing an elliptical tumor (t) that predominantly expands into the third ventricle and partially occupies the chiasmatic cistern. Note the pituitary stalk (PS) has been amputated. The hypothalamus is positioned around the mid-third of the tumor. **Level V - Critical: E1.** Intraoperative view through the lamina terminalis after complete tumor removal. The basilar artery (B) and the right posterior cerebral artery (P1) are observed through the broken TVF (asterisk). **E2.** Coronal MRI scan showing a large multilobulated CP (t) expanding from the sella to the third ventricle. The pituitary stalk is not visible and the hypothalamus is located at the mid-third portion of the tumor (red arrows).
5.5 The Mamillary Body Angle

Identification of the position and anatomical status of the whole TVF on conventional MRI studies from CP patients is quite difficult, particularly when CPs are larger than 3cm because the infundibulum and tuber cinereum are severely distorted, and they cannot usually be distinguished from the CP capsule. The compact structure of the mamillary bodies (MBs), as they remain the only identifiable component of the TVF in many cases. Based on this observation, our group defined in 2013 the mammillary body angle (MBA) as a useful radiological tool to ascertain the position of the TVF regarding the tumor [62]. The MBA is defined as the angle formed by the intersection of the plane tangential to the base of the mamillary bodies with the plane tangential to the fourth ventricle floor (Figure 8). This angle can easily be measured on midsagittal MRI scans (Figure 6. A1-D1). In healthy individuals without intracranial pathology, the normal MBA value usually ranges between 50° and 70°. CP growth causes a progressive displacement and distortion of both the TVF and the MBs, and a change in the MBA value can be correlated with the TVF status, depending on the original site of tumor development along the pituitary-hypothalamus axis (Figure 8). Thus, this radiological sign has proven useful to predict CP topography. Tumors originated below the TVF at a sellar/suprasellar position typically cause an upward displacement of the mamillary bodies, shifting the MBA towards an obtuse value (>90°) (Figure 6. B1). An obtuse MBA is a strong predictor of the pseudointraventricular CP topography. By contrast, an acute MBA (<90°) is usually observed in intraventricular CPs displacing the TVF downwards as they expand within the 3V. The MBA value becomes even more reduced (hyperacute, <30°) in cases of CPs originating within the TVF itself (not-strictly intraventricular or infundibulotuberal CPs) (Figure 6. D1), as these tumors push the MBs against the midbrain while expanding within the tuber cinereum [62]. Consequently, a hyperacute MBA strongly suggests a severe CP-hypothalamic adhesion, usually between the central portion of the tumor and the remnants of the TVF.

**Figure 8** The mamillary body angle (MBA) and its relation to craniopharyngioma topography. A: scheme illustrating how the MBA is measured on a midsagittal section. The MBA is formed by the intersection of the plane tangential to the base of the mamillary bodies (MBs) with the plane tangential to the floor of the fourth ventricle. Normal MBA ranges from 30° to 60°. B: Not-strictly intraventricular or
infundibulotuberal CPs (t) developed in the TVF itself typically show hyperacute MBAs as the MBs are displaced towards the midbrain during tumor enlargement. C: Pseudointraventricular CPs (t) originated in the suprasellar cistern push the TVF upward towards the fornices and roof of the 3V leading to an increase in the MBA value (obtuse MBA).

5.6 Optic Chiasm Distortions

Visual impairment is one of the major and earliest presenting symptoms among CP patients. This deficit is due to the progressive distortion of the optic chiasm by the tumor. The direction and severity of optic chiasm distortion is mainly related to the original site of tumor development, and will significantly influence the severity and outcome of visual deficits. Our group systematically analyzed these distortions in a cohort of well described CP cases, and we were able to identify repetitive chiasm distortion patterns associated with specific tumor topographies [90]. The chiasm distortion types showed a strong correlation with the severity of visual deficits and their reversibility after tumor removal. Two major types of chiasm distortion could be identified on midsagittal MRI sections: compression and stretching (Figure 9). Compressed chiasms are characterized by a crescent and swollen appearance, a deformation that is typically caused by CPs originated within the TVF at the same level or slightly above the horizontal plane of the chiasm (Figure 9. B). In contrast, stretched chiasms show an elongated and thinned appearance after being pushed upwards by a tumor growing below or in front of the chiasm plane (Figure 9. C). In addition to the shape alteration, the optic chiasm may be displaced in a preferential direction by the tumor growth. Among the compressed chiasms, two major subgroups can be distinguished: i) chiasm compressed downward, when the chiasm is slightly pushed and displaced beneath the lower pole of the tumor (Figure 9. B1); ii) chiasm compressed forward, when this structure is displaced towards the tuberculum sellae and smashed against this bony structure (Figure 9. B2). Among stretched chiasms, three major displacements may occur: i) stretched forward, when the chiasm is elongated along the anterior margin of the tumor (Figure 9. C1); ii) stretched upward, when it is elongated over the tumor dome (Figure 9. C2); and, iii) stretched backward, when it is elongated along the posterior margin of the tumor (Figure 9. C3) [90].

The importance of identifying these optic chiasm distortion patterns on preoperative MRI scans is to assess their correlation with the presence and severity of specific visual deficits and with the postoperative visual outcome [90]. For example, vision is not impaired in 80% of CP patients whose optic chiasm shows a normal appearance or a downward compression. These cases also associate the best visual outcome. On the contrary, more than 80% of patients with either forward compressed or stretched distortions have visual deficits. The upward stretched pattern associates a rate of severe visual disturbances as high as 75%, as well as the worst visual outcome. About one third of CP patients with a chiasm stretched upward will not experience any visual improvement following tumor removal. This poor visual outcome is probably the result of an irreversible axonal degeneration caused by the effect of three simultaneous mechanical deformations: elongation of the fibers; compression of the optic nerves at the level of the sharp bone edges of the optic foramina; and strangulation of the dorsal surface of the chiasm by the anterior communicating artery complex (Figure 9. C). The type of optic chiasm distortion is also a valuable MRI sign to predict the CP topography [90]. Strictly, intraventricular CPs typically do not cause any deformation to the optic chiasm (Figure 9. A1), or they only slightly compress it downwards
(Figure 9. B1). On the contrary, the forward compressed distortion points to the not-strictly intraventricular category (Figure 9. B2), chiasms stretched forward suggest a secondary intraventricular position (Figure 9. C1) and the upward stretched alteration is usually observed in the pseudointraventricular and sellar-suprasellar topographies (Figure 9. C2) [90].

**Figure 9** Optic chiasm distortions caused by craniopharyngiomas as relates to tumor topography. The upper row shows schematic drawings of the three major types of chiasm distortions found in CP cases. The lower panels show midsagittal MRI scans of representative cases. **A. Normal chiasm:** a normal chiasm shape and position is typically found in tumors (t) with a strictly intraventricular topography (A1). **B. Compressed chiasm:** a chiasm with a swollen appearance is generally found in intraventricular-retrochiasmatic CPs (t) that push the chiasm towards the planum sphenoidale (arrows). The compressed distortion is subclassified into downward displaced chiasm at the antero-inferior margin of the tumor (B1), and forward displaced chiasm, pushing against the tuberculum sellae (B2). **C. Stretched chiasm:** elongation of the chiasm generally occurs with CPs (t) originated beneath the third ventricle floor. Arrows indicate the three mechanisms involved in damaging the optic fibers: i) compression of the optic nerves by the sharp bone edges of the optic foramina (grey arrows); ii) elongation of the chiasm (white arrows); and iii) strangulation of the chiasm dorsal surface by the A1-anterior communicating artery complex (black arrows). Three subgroups can be identified depending on the displacement direction: forward (C1); upward (C2); and backward (C3).
6. The Impact of Topography and Adherence on Craniopharyngioma Recurrence

Together with hypothalamic injury, CP recurrence represents a formidable challenge posed by these complex tumors. This is mainly due to the difficulty in predicting such recurrence, in addition to the high morbidity and mortality rates associated with surgery of recurrent tumors [91]. Moreover, the rate of patients affected by CP recurrence is rather high, as one third overall of these tumors recur after a 3-year postoperative interval. For these reasons, there has recently been a growing interest in using molecular changes present in CP cells as a potential tool to predict the likelihood of tumor recurrence [18]. Unfortunately, there is no agreement on the actual reliability of molecular alterations to predict CP recurrence. Lack of clear conclusions are probably due to the fact that most molecular studies have the shortcoming of analyzing the CP molecular profile without taking into consideration the individual type of treatment and pathological features of each tumor, among them CP topography and tumor adhesion pattern [18].

Most authors agree that an incomplete tumor removal is the major predictor of CP recurrence [91]. Apart from the surgeons’ skills, accurate CP topography and type of tumor adhesion are the major factors that will determine the possibility of achieving a complete tumor removal. A correct definition of these pathological factors must be established prior to any attempt at analyzing the impact of the molecular changes in CP recurrence. For example, it should be noted that the wide and strong adhesions to the surrounding brain observed in infundibulo-tuberal CPs, as well as the common microscopic finding of finger-like tumor protrusions into the hypothalamus that characterize this topography, are two causes explaining why tumor remnants can be left behind unintentionally even after an apparently gross total removal [4, 91]. On the contrary, strictly intraventricular CPs, particularly those with a squamous papillary histology, are generally characterized by a clear and loose cleavage plane between the tumor and the hypothalamus, except at the point of a small pedicle/patch attachment to the TVF, that generally allows for a complete removal [5, 6, 34]. Thus, the molecular changes in these two topographical categories are not comparable, and a stratified analysis of the molecular alterations for each topography and degree of tumor removal is necessary.

7. CP Topography: The Fundamental Variable in Selecting a Surgical Approach

A wide range of surgical approaches have been employed for CPs, such as the pterional, subfrontal, transsphenoidal, transcallosal, frontal-trans-ventricular and interhemispheric. Overall, the surgeon’s experience and preference have normally dictated the particular chosen approach. Nevertheless, different CP topographies and adhesion patterns support the concept that certain routes are more suitable than others for specific CP-hypothalamus relationships. Therefore, an essential aspect of CP surgical planning is to decide “where to begin”. The optimal surgical route is that which provides a direct and broad view of the tumor-brain cleavage plane, particularly of the expected areas of maximal adhesion to the hypothalamus [1, 33, 42, 49, 55, 58, 84].

The upper trans-ventricular routes can only be used safely in strictly intraventricular CPs, as in these cases the hypothalamus is situated below the tumor (Figure 6. E2). These lesions typically present small attachments to the inner side of the TVF (Figure 7. B1) that can usually be released straightforwardly through a transcallosal or frontal-transventricular approach [6, 46, 92, 93]. However, these routes associate a high risk of iatrogenic hypothalamic injury when used to remove any of the remaining topographical CP categories involving the 3V [2, 38, 66, 94]. The
reason is that upper trans-ventricular approaches do not offer a direct view of the basal hypothalamus, the region to which most not-strictly intraventricular and secondary intraventricular CPs are strongly attached. The trans-lamina terminalis approach, either through a pterional, subfrontal or interhemispheric route, provides a direct view of the TVF where the attachments of any intraventricular CP are located, and is therefore suitable to remove either strictly or not-strictly intraventricular tumors [5, 6, 31, 46, 92, 95]. Some authors such as Yasargil and Steno favored the use of combined basal and upper approaches to have a complete view of the tumor-hypothalamus surface [2, 49].

In the last ten years, the use of endoscopic endonasal transsphenoidal routes to remove CPs has been expanding [55, 57, 58]. The major advantage of this route is that it fully exposes the tumor-hypothalamus adhesion plane present in tumors developing within the infundibulum (infundibulo-tuberal CPs) and in suprasellar lesions invading the 3V (secondary-intraventricular CPs). Nevertheless, the advantage of performing a sharp dissection under direct view should be counterbalanced by the high risk of cerebrospinal fluid fistula due to the direct connection which will remain between the 3V and the nose, particularly when this approach is used by non-experts [96, 97].

8. CP-Hypothalamus Adherence Severity: The Fundamental Factor in Deciding the Degree of Tumor Removal

The debate about the solid decision of tumor removal is still highly controversial among experts on CP treatment. Most neurosurgeons agree that a complete tumor resection is the optimal way of reducing the chances of tumor recurrence and the hazardous procedures for treating recurrent CPs, but many others advocate a conservative, incomplete removal to reduce the risk of hypothalamic damage and the long-term associated invalidating sequelae [66, 98-100]. Nevertheless, we firmly believe that the decision regarding the degree of tumor removal should not be based on a specific technique or opinion but rather on the individual CP topography and its pattern of adherence. Thus, aside from the surgical approach, the second aspect of the surgical planning to be considered, is “when to stop”. An accurate preoperative definition of the risk of hypothalamic injury for each case is essential to take the proper decision about the degree of tumor removal most suitable. This aspect of surgical planning should also be part of patient counselling.

Conventional MRI studies can correctly identify the CPs presenting the most severe adhesions to the hypothalamus (levels IV and V) in approximately 90% of cases [9]. The MRI signs that predict hypothalamic adherence with a high surgical risk are the position of the hypothalamus around the mid-third of the tumor, the amputation of the pituitary stalk and an elliptical tumor shape. In most of these cases a radical removal would inevitably lead to irreversible hypothalamic injury, and therefore an incomplete tumor removal is strongly advocated.

9. Conclusions

The widespread, inaccurate use of the term “suprasellar” to define CP topography should be abandoned and replaced by an accurate definition in each case. Most CPs actually involve the 3V, and a rate as high as 40% originate in the infundibulum or tuber cinereum, growing in close contact with the hypothalamus. An accurate definition of tumor position is the fundamental first
step in planning a proper surgical treatment. Likewise, a generalized treatment policy for CPs is not valid nowadays. CP heterogeneity regarding tumor location and adhesion to adjacent structures support a tailored treatment based on a careful preoperative MRI assessment of topography and CP-brain relationships. CP topography and the pattern of CP-hypothalamic adhesions expected to be found during the surgical procedure can be reliably predicted with conventional MRI studies in most cases. The highest risk of hypothalamic injury during surgical removal is associated with tumors originated within the TVF itself (infundibulo-tuberal or not-strictly intraventricular CPs) or in suprasellar lesions that have eventually invaded the 3V (secondary-intraventricular CPs). These two CP categories are characterized by a direct contact between the tumor tissue and the hypothalamus without any intervening meningeal layer to be used as a safe dissection plane. In these cases, in which the hypothalamus is usually positioned around the mid-third or central region of the tumor and the pituitary stalk has an amputated appearance on preoperative MRI, an incomplete tumor removal is strongly recommended to avoid iatrogenic hypothalamic injury.

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Review

**Contribution of PET Imaging to Clinical Management of Gliomas**

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**Abstract:** Gliomas originating from glial cells comprise about 30% of all primary central nervous system tumors and 80% of malignant brain tumors. Gliomas differ in their biological activity and are categorized according to grades, from benign to malignant with high recurrence rates. For diagnosis, location and extent of the tumor is assessed by CT and MRI, but for grading, additional parameters are necessary: contrast enhanced CT and MRI reveal damage of the blood–brain barrier, perfusion-weighted MRI shows regional blood supply, and MR spectroscopy permits insight into regional metabolism. Positron emission tomography (PET) of glucose metabolism as well as amino acid and nucleoside uptake can assess tumor grade and invasive growth, indicate effects on function of tissues outside of the tumor, demonstrate treatment efficacy, detect recurrences, and yield prognostic information. Coregistration of PET and MRI combines high-resolution morphological information with biological information. This imaging technology is optimized in hybrid MRI/PET by which morphologic, functional, metabolic, and molecular information is assessed simultaneously in the human brain.
Keywords
Gliomas; tumor recurrence; morphologic imaging; functional imaging; molecular imaging; PET

1. Introduction

Gliomas are the most frequently occurring primary tumors affecting the central nervous system (accounting for 29%), with an incidence of 20.5 per 100,000 people each year. Within this group, 54% are malignant gliomas with an incidence of 6.6 per 100,000 [1]. Survival rates are dependent on the grade of gliomas: 35.7% at 1 year and 4%–7% at 5 years for glioblastoma, 60%–80% at 1 year and 26%–46% at 5 years for astrocytomas and oligodendrogliomas of WHO grade III, and 94% at 1 year and 67% at 5 years for astrocytomas and oligodendrogliomas of WHO grade II [2]. Patients with malignant tumors have an average survival of 15–20 months despite surgical resection followed by radiotherapy and chemotherapy [3]. Imaging plays an integral and decisive role in the grading of gliomas, therapeutic management, and in the development of new treatment strategies. Furthermore, multimodal and molecular imaging may have an especially significant role in detecting and defining new targets for research [4-6].

Morphological imaging by CT and especially MRI detects the location of brain tumors and is improved by contrast enhancement, due to damage of the blood–brain barrier. MR spectroscopy may provide additional information on the tissue, including the presence or level of malignancy. Information detected by MRI is limited since brain tumor tissue may extend into tissue outside the areas affected by a blood–brain barrier disruption. After radiation therapy, a blood–brain barrier disruption may persist in non-tumorous tissue. MR spectroscopy is unreliable in certain areas, specifically close to the skull or the ventricles. Nuclear medicine procedures can detect physiological and metabolic changes in the involved brain tissue [7, 8]. Notably, positron emission tomography (PET) can quantify tumor metabolism, proliferation rate, and invasiveness and demonstrate the effects on functional networks as well as monitor changes after therapy [9-12].

In this review, which is an update of a previously published paper [13], clinically relevant results studying glucose metabolism, amino acid uptake, and cell proliferation with PET tracers are summarized. Other tracers specifically applied for research topics [4, 14] are not discussed.

Energy for the brain is nearly exclusively supplied by glucose. For the measurement of the cerebral metabolic rate of glucose, 18F-labeled deoxy-d-glucose (FDG) is used, which becomes phosphorylated to FDG-phosphate and accumulates in proportion to local metabolism. Regional quantitative metabolic rates of glucose (rCMRGlc) are determined from the tissue activity measured by PET and the arterial input function [15].

Amino acid uptake in gliomas is increased due to altered overexpressed L-type amino acid carriers [16, 17]. This tracer indicates the different metabolic activities in the tumor cell. Amino acid uptake in gliomas is higher than in white matter, and also often higher than in the normal cortex. The most frequently used amino acid for tumor brain imaging with PET is (methyl-11C)-L-methionine (MET) [18, 19], but this tracer suffers from a short half-life of 11C. Amino acids labeled with 18F-fluorine as O-(2-[18F] fluoroethyl)-L-tyrosine (FET) and 3, 4-dihydroxy-6-[18F]-fluoro-L-phenylalanine (FDOPA) showed comparable results to MET. However, since FET is not further
metabolized it can only reflect transport in tissue. FDOPA is a substrate for the enzyme aromatic amino acid decarboxylase in dopaminergic neurons, which is responsible for FDOPA uptake by the basal ganglia and might therefore interfere with tumor delineation. On the other hand, MET is incorporated into proteins, used for methylation, and is needed for DNA translation.

As nucleosides are involved in cellular proliferation, they can indicate histologic grade. 3'-deoxy-3'-[18F] fluorothymidine (FLT) accumulation correlates with activity of thymidine kinase-1, which is expressed during the DNA synthesis phase [20]; this makes it a suitable tracer of tumor proliferation [21]. Accumulation of FLT depends on blood-brain barrier (BBB) permeability, and high FLT uptake is found in tumors with impaired BBB; therefore, FLT is not useful in low grade gliomas with intact BBB [22-24].

PET studies during functional activation show the effect of tumors on brain tissue outside the tumor and on eloquent areas. Blood flow tracers such as 15O-water are frequently used for this purpose, but functional changes can be also recorded by FDG. Accurate anatomic localization can be achieved by coregistration and fusion with MRI [25]. However, perfusion MRI is the method used most in the clinical setting for demonstration of functional activation.

2. Diagnosis, Grading and Prognosis

The World Health Organization (WHO) classification of gliomas distinguishes four grades of gliomas: I and II are benign; grade III is anaplastic; and grade IV, glioblastoma multiforme, is the most malignant with the worst prognosis [26]. The WHO classification of gliomas has been updated recently [27], combining molecular parameters such as the IDH mutation and 1p/19q co-deletion with histology, which defines five subgroups. However, since nearly all imaging studies were done on the basis of the previous classification, the prior classification was used in this review for the description of imaging data. Gliomas are frequently heterogeneous [28] and therefore, histologic grading may not be representative for further prognosis/development. Anatomic imaging is still the first step in diagnosis, but it does not yield all the information on tumor pathology essential for individualized treatment. PET can supplement conventional CT and MRI information on tumor grading, necrosis, proliferative activity, and vasculature.

3. Glucose Metabolism

The first PET study in oncology [29] already showed increased glucose consumption in brain tumors and the effect of radiation necrosis, especially in malignant gliomas. FDG uptake levels in low-grade tumors is the same as in white matter, and uptake in high-grade tumors can be in the range of that of normal gray matter. Regions with low and high uptake can be near each other in a single tumor. This variability must be taken into account when tumors are graded. Usually, uptake ratios of tumor to white matter greater than 1.5 or of tumor to gray matter greater than 0.6 are used for distinction of benign tumors (grades I and II) from malignant tumors (grades III and IV) (Figure 1) [30]. Additionally, delayed PET after injection may help to distinguish tumors from normal gray matter [31].
Figure 1 Typical patterns of glucose (FDG) uptake in gliomas of different grades: Astrocytoma WHO grade 2 shows low uptake in relation to gray matter and can hardly be differentiated (a), in malignant gliomas (grade 3 (b) and grade 4 / glioblastoma (c)) glucose uptake is significantly increased and above the level of gray matter; in the glioblastome (c) a central necrosis (reduced FDG uptake) is visible.

In a primary glioma, FDG uptake correlates with histologic tumor grade [32], cell density [33], and survival [34]. Additionally, metabolism in normal brain tissue is impaired and the reduction is related to prognosis [35]. FDG-PET is therefore of value in patient management [36]. Necrotic compartments in glioblastoma often cause heterogeneous FDG uptake. In pilocytic astrocytoma with metabolically active fenestrated endothelial cells, uptake can be rather high, but prognosis is good. The similarity of FDG uptake in tumors to that in the cortex and the partial-volume averaging by PET limit the detection of small gliomas. This limitation can be overcome by coregistration with MRI. Coregistered MRI/FDG-PET can help in the selection of the most metabolically active tumor part for stereotactic biopsy [37]. Other malignant tumors in the brain, especially medulloblastomas, often show high FDG uptake [38, 39]. It should be considered that the Response Assessment in Neuro-Oncology (RANO) guidelines emphasize superiority of amino acid PET over FDG–PET [40]. Therefore, the use of FDG for brain tumor imaging has been widely abandoned in PET centers with access to radiolabeled amino acids.

4. Amino Acid Uptake

Uptake of MET in gliomas is 1.2 to 6.0 times above that in normal tissue and correlates to cell proliferation, Ki-67 expression, nuclear antigen expression, microvessel density, and angiogenesis [41]. MET-PET has a sensitivity of 76% and a specificity of 87% for the diagnosis of brain tumors [42]. Due to the low uptake in normal brain tissue and the accumulation despite the unimpaired
blood–brain barrier, amino acid tracers are especially suited for detection of low-grade tumors (Figure 2). Even tumors not visible on FDG-PET can be detected with MET [43]. Amino acid uptake was correlated to histological tumor grade in high- and low-grade gliomas [44]. Additionally, the invasion of malignant cells into the surrounding brain could reliably be distinguished by MET-PET [45], demonstrating the importance of integrating this technique into radiation therapy planning [46]. In some gliomas, MET-PET is superior to contrast-enhanced MRI [47]. MET-PET can help determine prognosis in gliomas and is superior than FDG-PET and MRI in predicting survival in low-grade gliomas [48]. Recent reviews have summarized the value of MET-PET for imaging of gliomas [4, 49-51].

**Figure 2** (11C)-MET-PET in low and high grade gliomas: uptake of (11C)-MET is increased in relationship to grade in astrocytoma and glioblastoma as well as in oligodendrocytoma.

MET uptake differs with tumor type: in oligodendrogliomas, uptake tends to be higher than in astrocytomas of the same histological grade, although they are less aggressive [52]. In oligodendrogliomas, 11C-choline PET may be useful in evaluating the potential malignancy, but MET-PET is superior in detecting “hot lesions” [53]. MET uptake is increased in other malignant intracranial tumors, but also in benign neoplasias, such as meningiomas (for review see [38]).

The disadvantage of MET is the short half-life of 11C (20 min). Therefore, 18F-labeled aromatic amino acid analogs O-(2-[18F] fluoroethyl)-L-tyrosine (FET) and [18F]-fluoro-dihydroxy-phenylalanine (FDOPA)) with similar brain uptake as MET were introduced for tumor imaging [18, 19, 54-56]. Uptake of FET is related to grade and prognosis [57, 58]; specifically, the volume of increased FET uptake correlated to survival of patients with glioblastoma [59]. Despite significant differences between high- and low-grade gliomas, sensitivity and specificity for detection were 87% and 68%, respectively [60]. As with MET, coregistration with MRI [61] and combination with MRS [62] improves diagnostic accuracy: high FET accumulation was related to neuronal cell loss indicated by MRS [63]. The early phase of FET uptake appears to be most informative for grading...
[64, 65] and prognosis [66]; tracer accumulation is slow in low-grade gliomas, for which late scans provide the best contrast [67]. The FET uptake kinetic analysis independently predicted overall and progression-free survival [68]. Dynamic FET studies with inclusion of early and late scans therefore improved differentiation and grading performance [69-72]. Kinetic analysis of dynamic FET uptake parameters is of special prognostic value in diffuse low-grade gliomas [51].

FDOPA was more sensitive and specific than FDG for differentiating high- and low-grade tumors, and uptake was related to proliferation [73]. FDOPA studies have demonstrated association with tumor grade [74], progression-free survival [75], and overall survival in recurrent tumors [76, 77]. High uptake (an SUV of more than 1.75) was a predictor of progression in low-grade gliomas [78]. A direct comparison of FDOPA and FET in high-grade gliomas revealed no significant difference in patterns, but uptake ratios were 10-15% higher for FET than for FDOPA [79].

5. Nucleoside Uptake

Imaging of nucleoside uptake adds another dimension to the assessment of the biological activity of tumors, specifically in regards to cellular proliferation (Figure 3)[80]. The tracer FLT achieves high tumor to normal tissue ratios of grade III and IV gliomas [81], which was not observed in grade II gliomas. FLT uptake was correlated to Ki-67 expression [82]. For preoperative tumor grading, FLT-PET was superior to MRI and MRS for differentiation between grades III and IV [83]. FLT-PET was superior to MET-PET in tumor grading and assessment of proliferation in some gliomas; notably, the combination with MET-PET added significant information [84]. A direct comparison in primary and recurrent low-grade gliomas showed low FLT uptake (SUV = 1.8) but high uptake of FDOPA (SUV = 5.75) and FDG (SUV = 8.5), and tumor to normal tissue ratios of 2.3 ± 0.5 for FDOPA, 1.8 ± 0.9 for FLT, and 1.0 ± 0.6 for FDG, confirming the value of FDOPA for evaluation of low grade gliomas [85]. In high-grade gliomas, FLT-PET predicted overall survival of patients [86].

![Characterization of Target Tissue](image)

**Figure 3** Comparison of Gd-Ti-MRI, FDG-, MET- and FLT-PET in glioblastoma: disruption of blood brain barrier and peritumorous edema visible on MRI, increase of glucose turnover in tumor and reduction in surrounding tissue, increased MET-uptake in tumor core, infiltration detected by FLT-PET.
6. Effects on Surrounding and Remote Brain Areas

The rim of reduced metabolism in normal tissue around malignant tumors might be partly due to edema and infiltrating tumor tissues [87]. Additionally, function of the non-tumorous brain is affected: cortical centers are displaced and functional activations are reduced or occur at atypical locations, even in contralateral areas which indicate reorganization of functional networks; the knowledge of these altered networks is important for planning tailored surgery. In the motoric network, activation of secondary motor areas and of the motor cortex ipsilateral to the paretic limbs has been observed [88]. Functional MRI is the most important procedure to determine functional anatomy in patients with gliomas [6].

In patients with brain tumors in the dominant hemisphere, a considerable reorganization of the language-related network is observed [89], dependent on the speed of the development of the brain lesion: a verb generation paradigm analysis showed increased activation area beyond the primary language regions to the left frontal medial gyrus, the orbital inferior frontal gyrus, the anterior insula, and the left cerebellum (Figure 4), as well as the contralateral functional network. Successful resection of a left frontotemporal tumor improved aphasia and restored left hemisphere dominance, suggesting a reversible disinhibition by removal of the primary functional damage. The hierarchy of the functional network for recovery in an individual patient as shown in these examples should be taken into account in tailored surgery.

![Figure 4](image_url) Activation of H215O PET by verb generation coregistered to MRI. Primary speech centers are affected by the tumor, temporal Wernicke center and frontal Broca center are shifted and language activation pattern is shifted to the right hemisphere.

7. Monitoring Treatment Effects

PET studies can follow the efficiency of therapy in brain tumors [90]. Via FDG-PET, reduction of the tumor is visible after only a few weeks of radiation and chemotherapy [91], and recurrence is
indicated by progressive hypermetabolic regions [92]. The early assessment of therapy efficacy by PET can help to optimize therapy of gliomas: FDG accumulation was measured before and after 14 days of temozolomide chemotherapy and tumor response after 8 weeks was analyzed [91]. Pretreatment FDG uptake was higher in responders than non-responders, and responders showed a greater than 25% reduction of metabolic rate in tumor regions after 8 weeks [91]. FDG-PET also predicted response to temozolomide (TMZ) versus TMZ plus radiotherapy (RT) in recurrent malignant glioma [93]. Changes in tumor glucose metabolism were observed also with everolimus or rapamycin in combination with RT and TMZ [93, 94]. However, hypermetabolism is sometimes mimicked by the infiltration of macrophages, especially after radiotherapy. This disadvantage makes FDG-PET not the optimal method for the evaluation of treatment [95].

Amino acid and nucleoside tracers do not possess this disadvantage and several studies indicated that patients can benefit from treatment studies based on MET- or FET-PET [96-100]. Differentiation of recurrent tumors and necrosis is detected by MET-PET with high sensitivity and specificity (~75%), and progression was detected early (Figure. 5) [101-104]. In many patients, information supplied by MET-PET affected further treatment management [105].

The changes observed by amino acid PET [106-109] indicate deactivation of amino acid transport as an early sign of response to chemotherapy. FET-PET was more efficient (sensitivity 80%, specificity close to 100%) [110] than MRI in showing effects of multimodal treatment [111, 112]. Early changes of FET uptake of > 10% after postoperative radiochemotherapy predicted a significantly longer disease-free and overall survival in patients with glioblastomas [113]. Similar results were obtained with FET- and FDOPA-PET [114, 115]. FET-PET was also useful to assess pseudoprogression in glioblastomas [116] and could also demonstrate treatment effects in recurrent tumors [115, 117-121].

Figure 5 Decrease of 11C-Methionin uptake in PET demonstrated response to chemotherapy with favorable prognosis.
FLT-PET was successful in the prediction of the prognosis of responders and non-responders to a combination therapy and also in predicting survival. An SUV decrease of more than 25% or less than 25% distinguished responders and non-responders, respectively [122]. Additionally, the responders survived 3 times as long as non-responders. Notably, the kinetics of FLT uptake are closely related to prognosis, early efficacy of treatment, and outcome [123-126], and can serve as early surrogate markers of longtime survival.

These results indicate that coregistration of various PET and MRI modalities are useful for evaluating new treatments, e.g. targeting proliferating cells [127], angiogenesis [114], or applying gene therapy vectors [128].

FET-PET was also useful to assess pseudoprogression in glioblastomas [116] and could also elucidate treatment effects in recurrent tumors [115, 117-120]. Antiangiogenic treatment with bevacizumab causes a rapid decrease in T1 contrast-enhancing tumor parts, which suggests radiographic response rates are related to pseudonormalization of abnormal blood-brain barrier permeability [129]; the distinction between anti-vascular and true antitumor effects by MRI criteria is difficult, therefore, the RANO criteria were developed [130]. On the other hand, transiently-increased permeability of the vasculature may be a consequence from irradiation and can be enhanced by temozolomide [131]. Pseudoprogression as well as pseudoresponses after therapy can be differentiated from actual tumor response by FET-PET [70, 132].

With FLT-PET, a distinction between responders and non-responders to a combination therapy was possible: FLT-PET at 2 and 6 weeks predicted survival better than MRI [122]. FLT uptake investigated at different time points in the course of treatment was able to differentiate between responders and non-responders by a SUV decrease of more than 25% or less than 25%, respectively, and the responders survived 3 times as long as non-responders [122]. As shown by several groups [123-126], the kinetics of FLT uptake are closely related to prognosis, early efficacy of treatment, and outcome. These therapeutic effects may be related to neovascularization by bevacizumab and/or permeability changes by chemotherapy [123, 133] and can serve as early surrogate markers of longtime survival [11]. Similar results were obtained with FDOPA-PET [134].

Multimodal imaging, including various PET and MRI modalities, will have a great impact on the development of new therapeutic strategies, such as targeting proliferating cells [127], angiogenesis [114], or applying gene therapy vectors [128].

8. Residual Tumors, Recurrences and Necrosis

The capacity of PET to identify tumor compartments that differ in activity is especially important for the detection of residual or recurrent tumors after resection, and for differentiation between treatment-induced changes (such as necrosis) and active proliferating tissue. After tumor resection, normal postsurgical changes do not show increased FDG uptake. Therefore, a hypermetabolic activity after surgery is highly suspect of residual tumor, and FDG-PET can be performed within a few days after surgery [92]. While normalization of glucose metabolism in the surrounding area of the resected tumor might be related to edema and increased intracranial pressure, a newly detected hypermetabolism weeks after therapy indicates a recurrent tumor and progression from low-grade to high-grade glioma [37, 92, 135, 136]. One of the most important applications of PET tracers after treatment of gliomas is the differentiation between radiation-induced changes, like necrosis, or recurrent or residual tumors after radiation therapy [29, 137].
Generally, the question, “Tumor or necrosis?” is an oversimplification as in most cases both tumor and necrotic tissue can be found next to each other in patients or may even overlap [138]. Sensitivity of FDG-PET was 75% at a specificity of 81% for the detection of recurrent tumor versus radiation necrosis [17, 139]. However, there is a specific overlap in FDG-uptake in recurrent tumor and radiation necrosis [138] (review in [140]). Stereotactic biopsy based on FDG-PET improves the detection of tumor tissue when compared to anatomical imaging alone [37]. A disadvantage of FDG-PET is that accumulation of FDG may occur from macrophages that potentially infiltrate the sites having received radiation therapy [91, 141]. Therefore, radiation necrosis may be indistinguishable from a recurrent tumor.

Necrosis and gliosis after therapy show a reduction of amino acid uptake in contrast to uptake in recurrent or residual tumor growth. Therefore, MET-PET successfully differentiates between recurrent tumor growth and radiation necrosis with the detection of a recurrent tumor at a high sensitivity and high specificity (Figure 6). Again, MET-PET is more sensitive than FDG-PET for differentiation between recurrent tumor and radiation necrosis (Figure 7) [102, 104, 142] and provided high accuracy [143], despite its limitations in tumor grading [144], and is especially effective in combination with MRI [145, 146]. Even in brain lesions that did not show increased uptake in FDG-PET, a sensitivity between 89% (tumors) and 92% (gliomas) with a specificity of 100% was obtained [43] (for detailed review see [147]). A recent systematic review and meta-analysis [148] demonstrated that FET-PET has good sensitivity (82%) and average specificity (76%) for diagnosis of brain tumors; it allows discrimination between infection and tumoral lesions and also between tumor recurrence and radionecrosis [149]. MET-PET and FET-PET differentiated tumor tissue and treatment-related changes with a sensitivity of 91% and a specificity of 100%. In adequately equipped centers, amino acid PET is the method of choice for differentiation between necrosis and progressive disease [40]. Fusion of FDOPA-PET with MRI provides precise anatomic localization of tracer uptake [150], and FDOPA-PET better identifies large tumor volumes than perfusion-weighted MRI [151]. FDOPA-PET is also superior to FDG-PET in detection of recurrences [152] and is able to differentiate glioma recurrence from treatment-related changes.

FLT-PET had a moderately better overall accuracy for diagnosing glioma recurrence than FDG-PET [153, 154]. The FLT influx rate differentiated recurrence from radionecrosis, but the SUV did not [155]. For monitoring treatment effects and the differentiation between necrosis and recurrence, multimodal imaging is most effective [12].
**Figure 6** Differentiation between recurrent tumor and radiation necrosis by FDG-PET.

**Figure 7** Uptake of glucose (FDG) and methionin (MET) in the course of a patient with glioblastoma: After tumor resection and radiation therapy the uptake of FDG and MET is initially reduced; MET-PET indicates the recurrency long before it can be proven by FDG-PET.
9. The Future: Hybrid PET/MRI Systems

Coregistration of PET and MRI data requires positioning the patient in different scanners, often under different conditions and at different times. Hybrid PET/MRI systems combine high-resolution MRI (including spectroscopy, functional MRI, diffusion- and perfusion-weighted imaging) with the molecular, biochemical, and functional imaging properties of PET. In contrast to PET/CT data acquisition, a hybrid PET/MRI system analyzes simultaneously due to PET detectors [156] in the MRI gantry. After the feasibility of simultaneous PET/MRI recording was shown [157], dedicated brain PET/MRI scanners tested some promising applications [158]. In the first clinical studies, the hybrid system demonstrated simultaneous high-resolution structural, functional, and molecular imaging in tumor patients [159].

Besides the time-saving benefits and patient management advantages, hybrid MRI/PET improves the presurgical diagnosis of patients with focal epilepsy, where small lesions, hypoplasias, or heterotopies can be delineated [160, 161]. As previously stated, hybrid MRI/PET has great advantages in the differential diagnosis of brain tumors, grading of gliomas, assessment of progression, and the distinction between necrosis and recurrence of tumors [162-166]. Additionally, it is an important tool for the selection of sites for biopsies and in the evaluation of treatment effects [160, 167-176]. Adding diffusion tensor imaging/fiber tracking, fMRI, PWI, MRS, and activation-PET to multimodal imaging can enhance the assessment of a tumor on the functional networks in the brain [177-181] and show anaerobic changes as well as the effect on efferent and connecting fiber tracts and on task-related activation patterns.

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