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Correlation between bevacizumab therapy in meningioma and tissutal biomarkers of response

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Abstract

Meningioma is the most common primary central nervous system tumor. The majority of meningiomas present with a non-aggressive behaviour, but a fraction of them relapses repetitively and determines increased morbidity and mortality. The standard treatment of symptomatic meningioma is surgical resection, aimed at achieving gross total resection followed by radiotherapy, when indicated.

No preferred standard of care is available for systemic therapies in case of relapse/recurrence or unresectable disease, not eligible for surgery and/or radiotherapy; a variety of therapies has been studied, with controversial results.

Meningiomas have historically been systemically treated with hydroxyurea, but recent data have shown promising results supporting the use of bevacizumab in this setting. So far no biomarkers to select and personalize treatment for patients are available in clinical practice.

Based on such knowledge, a retrospective study comparing hydroxyurea and bevacizumab in recurrent meningioma, previously treated with surgery and radiotherapy, was conducted.

Tissutal molecular data were collected and analyzed to investigate possible biomarkers of response to treatment.

1. Introduction

a. Epidemiology and risk factors

Meningiomas are the most common primary central nervous system tumors. They have the highest incidence rate, 37.6%, among all primary central nervous system (CNS) tumors. [1] Malignant meningiomas, diagnosed as grade 2 or 3, represent up to 20% of all meningioma and they are characterized by high incidence of recurrence and/or metastases outside the brain, consequently determining a poor prognosis. [2] Meningioma can have an intracranial location or a less frequent spinal location (accounting for approximately 1.2–12.7% of all meningiomas). [3] (*Figure 1*)

They have an estimated age adjusted incidence of 9.1 per 100.000 population per year, increasing to 57.3 per 100.000 in adults over the age of 85. [4] In patients aged 40+ years, incidence is 18.69/100.000 while in age 0–19 years it is 0.16/100.000. In patients aged 40+ years, aged 15–39 years, and age 0–14 years, meningiomas make up to 43.6%, 15.6%, and 1.7% of all CNS tumors, respectively. The median age at diagnosis is 66 years old. There is a female:male positive ratio, the incidence rates being 2.33 and 1.12 respectively. [5]

The main risk factors for the development of meningiomas are ionizing radiations, especially directed to the neural tissue such as in the treatment of childhood leukemia and brain tumors, and type 2 neurofibromatosis (NF2). [1,6] Other genetic syndromes, such as schwannomatosis, Gorlin syndrome, Cowden syndrome with *PTEN* mutations, multiple endocrine neoplasia type 1 (MEN1), Rubinstein-Taybi syndrome and Werner syndrome, can predispose at developing meningioma. [7]

NF2 is a neurocutaneous syndrome and accounts for 3% of all types of neurofibromatoses. It has an autosomal dominant inheritance pattern and an incidence of 1 in 25.000 people. [8] NF2 is caused by alterations in the gene encoding for the protein merlin, which is usually located at 22q12.2 chromosome. [8]

Patients affected by NF2 can develop different neoplastic CNS manifestations, such as schwannomas, ependymomas, meningioangiomatosis, hamartomas and meningiomas. Intracranial meningiomas are diagnosed in 40-45% of individuals with NF2 and spinal meningiomas in 20% of cases. In this setting lesions tend to be multiple, cells have a more mesenchymal spindle-like aspect and higher mitotic rate and therefore grade, usually resulting in atypical or anaplastic meningiomas. [9]

b. Pathology and classification

Meningiomas originate from non-neuroepithelial progenitor cells, the arachnoid cap cells. [10] They represent a distinct and highly metabolic active subgroup of arachnoidal cells and are involved in the

resorption of cerebrospinal fluid. [10] They are situated at the apex of Pacchionian bodies and are exposed to venous blood flow, often within a dural sinus. [10]

Meningioma cells are present in the pia mater, arachnoid mater, and the trabeculae and septae of the subarachnoidal space. [5] They make up a monolayer covering of the meninges and are connected via tight junctions, gap junctions, and desmosomes, providing an interface between neuronal tissue and the cerebrospinal fluid (CSF). [5]

The most recent classification for CNS tumors, and therefore meningioma, is the 5th edition of the World Health Organization (WHO) 2021. [11]

WHO 2021 considers meningioma one type of CNS tumor, further divided in 15 subtypes, graded from WHO grade 1 to 3. The latest classification underlines that criteria defining atypical (grade 2) or anaplastic (grade 3) meningioma should be applied regardless of the underlying histological subtype. [11]

Currently, grade 1 includes meningothelial, fibrous, transitional, psammomatous, angiomatous, microcystic, secretory, lymphocyte rich and metaplastic subtypes; grade 2 includes clear cell and chordoid subtypes; grade 3 includes rhabdoid and papillary subtypes. [12]

According to the Central Brain Tumor Registry of the United States (CBTRUS), relative meningioma grade frequencies are: 80% grade 1, 20% grade 2/atypical, 1-2% grade 3/anaplastic. [13]

Up to now, the role of meningioma grading has been of great relevance, because the most reliable clinical factors associated with recurrence in meningiomas are in fact the grade of the tumor and the extent of tumor resection at surgery. [14]

Grading is attributed based on different criteria, among which mitotic activity is one of the key ones:

- WHO grade 2: 4-19 mitotic figures/10 high power field (HPF) or brain invasion or 3 minor criteria among increased cellularity, small cell with high nucleus/cytoplasm (N/C) ratio, large and prominent nucleoli, patternless or sheet-like growth, foci of ‘spontaneous’ or geographic necrosis
- WHO grade 3: ≥20 mitotic figures/10 HPF or frank sarcomatous/carcinomatous histology
- WHO grade 1: absence of criteria for grade 2 and grade 3 [12]

Mitoses should be identified by careful review of good quality haematoxylin&eosin (H&E)-stained sections (3–5 microns in thickness), with counts obtained in consecutive HPFs (×400, a combination of ×40 objective lens with a ×10 eyepiece lens) and counted in HPFs of 0.16 mm2 each. [15]

Brain invasion is defined as the infiltration of tumor cells through the leptomeninges into the subjacent brain parenchyma, and it has been shown in some studies to portend aggressive behavior with shorter recurrence-free survival, but other studies do not support this. [15]

Benign meningiomas exhibit stagnancy on serial imaging or an average growth rate of 1–1.5 mm per year when they do grow. Significant growth rate beyond this harbingers a more aggressive behavior and pathologic grade. [7]

WHO 2021, in contrast to previous versions, has introduced molecular markers as grading criteria. [1] Molecular biomarkers (oncogenic variants in the *TERT* promoter and homozygous deletion of *CDKN2A/B*) represent now independent diagnostic biomarkers for CNS WHO meningioma grade 3. [15] Therefore according to the latest guidelines, any meningioma with *TERT* promoter mutation and/or *CDKN2A/2B* homozygous deletion should be allotted to WHO grade 3, irrespective of histology. [1]

To the current date, the mutations identified in meningioma have not been shown to be tightly correlated to patient outcome. [14]

For other types of CNS tumours, molecular profiling has identified distinct subtypes with characteristic aberrations. Many of these subtypes are associated with prognosis or provide targets for treatment, and therefore help clinical decision making—eg, epigenetic subgroups in medulloblastoma and ependymoma, or isocitrate dehydrogenase status in diffuse glioma.[16]

After the publication of the new classification, the consortium to inform molecular and practical approaches to CNS tumor taxonomy-not official WHO (cIMPACT-NOW) Steering Committee convened a working group to provide clarification and assess the evidence of possible novel genetic and epigenetic criteria to consider for future directions (as shown in *Figure 2*) [15]

c. Molecular aspects

Evidence suggests that molecular data (eg, copy-number variations [CNVs], DNA methylation profiling [DNAMP]) can identify lower- or higher-risk meningioma cases among morphologically ambiguous meningioma samples. [15]

According to c-IMPACT-NOW, in cases with possible but not definite brain invasion (a criteria for grade 2), additional molecular testing is advised and should also be incorporated into the grading of a brain-invasive meningioma otherwise benign (sometimes referred to as a BIOB) meningioma. A new recommendation exists, assigning a BIOB meningioma to CNS WHO grade 2 in case of 1p deletion combined with concurrent 22q deletion/*NF2* alteration, regardless of grading. [15]

For BIOB meningiomas, other molecular markers of high-risk (DNA methylation, RNA subgroups) may also qualify as a criterion for CNS WHO grade 2 designation (or CNS WHO grade 3 in case of *CDKN2A/CDKN2B* homozygous deletion, *TERT* promoter oncogenic variant, or possible upcoming other molecular criteria for CNS WHO grade 3).[15] Consequently, a BIOB meningioma in which no testing could be performed should be termed “meningioma, not otherwise specified” with the CNS WHO grade left off. [15]

Mutations in *TERT* promoter can be found in all grades of meningiomas regardless of *NF2* status but more frequently in high-grade meningiomas. [7] *TERT* gene alterations are associated with earlier recurrence and significantly shorter overall survival. [7]

Oncogenic variants in the *TERT* promoter and *CDKN2A/CDKN2B* deletions are genetic alterations observed in a small (<5%) subset of meningiomas, typically those with oncogenic variants in *NF2* and/or loss of chromosome 22q. These alterations are associated with a high risk of recurrence and dramatically shorter progression free intervals, regardless of other histological features, thus, they are considered significant independent prognostic factors in meningiomas, warranting a CNS WHO grade 3 designation. [15] Oncogenic variants in the *TERT* and *CDKN2A/CDKN2B* deletions should be tested for in CNS WHO grade 2 (atypical) and grade 3 (anaplastic) meningiomas to identify patients at increased risk of recurrence and progression. [15]

Loss of 9p21, which includes *CDKN2A* and *CDKN2B* (*CDKN2A/B*) is an independent poor prognostic factor in meningiomas. While WHO 2021 classification recognizes and incorporates homozygous loss of *CDKN2A/B* as a grave prognostic marker for aggressive behavior in meningiomas, also heterozygous loss of *CDKN2A/B* has been suggested to also be prognostic in meningioma even if rare. [15]

Recently, due to the wider availability of wide next generation sequencing (NGS) platforms, other gene mutations have been discovered and studied in meningioma; their role is variable, some can be correlated to tumor location, others to specific subtypes, others have a prognostic value.

i. NF2

NF2 encodes for the tumor suppressor protein Merlin on the q12.2 band on chromosome 22 and it is also the most recurrently mutated gene in sporadic and radiation-induced meningiomas, especially recurrent high-grade. [17] About 60% of sporadic meningiomas have *NF2* inactivation. [7] The presence of *NF2* mutations in low-grade and high-grade meningiomas suggests its role in meningioma tumorigenesis. [7] The prevalence of meningioma in Neurofibromatosis type 2, with 40–60% of patients developing a meningioma in their lifetime, led to identifying the Neurofibromatosis type 2 gene. [7]

NF2 codes for the protein merlin. [18] The merlin protein (also known as schwannomin) is a member of the 4.1 family of proteins which link integral membrane proteins to the cytoskeleton, and that are involved in the regulation of cell growth, proliferation and motility. Meningioma-associated *NF2* mutations commonly result in a truncated, non-functional protein, which may lead to abnormal cell growth and motility through destabilization of adherens junctions. [19] *NF2* acts as a tumor suppressor gene via blocking the contact inhibition pathway, suppressing the YAP-mediated Hippo signaling pathway, and downregulating *PI3K/AKT/mTOR* pathway. [7,20] (*Figure 3*)

Additionally, loss of merlin activity has been associated with increased levels of ErbB receptors in primary Schwann cells, which regulate downstream mitogenic signaling pathways (e.g. *Ras/Raf/MEK/ERK* and *PI3K/AKT*); altogether, these findings support a relevant role of merlin in tumorigenesis in meningiomas. [19]

The *Pi3K-Akt-mTOR* (mammalian target of rapamycin) pathway is currently the most relevant intracellular signaling pathway target in meningiomas, in particular in high grade meningioma. *NF2* gene and its protein were shown to interact with *mTOR*. [18]

ii. Other alterations

While *NF2* alterations are the most frequent oncogenic variants in meningioma, another set of recurrent somatic oncogenic variants that are mutually exclusive with *NF2*, including *TRAF7, AKT1, KLF4, SMO, PIK3CA,* and *POLR2A*, is associated with tumor location, morphology, and clinical outcomes. [15] Based on the prominent biological differences between *NF2* and non-*NF2* driver alterations, some have suggested dividing meningiomas into NF2 (harboring *NF2* variants), TRAKLS (with *TRAF7, AKT1, KLF4*, and *SMO* alterations), and not otherwise classified (NOS) molecular tumor types. [15] The so-called *TRAKLS* genotype is highly associated with histologic grade 1 meningiomas.[15] On univariable analysis, individual gene alterations of *TRAF7* and *KLF4*, as well as the *TRAKLS* genotype, have been shown to be statistically associated with improved progression-free survival (PFS) compared to *NF2*-altered meningiomas.[15]

Tumor necrosis factor receptor (TNF-R)-associated factor (*TRAF*) proteins are signaling adaptors downstream of TNF-R. Interestingly, *TRAF7* mutations were mutually exclusive with *NF2* mutations and frequently co-occurred with Kruppel-like factor 4 (*KLF4*) K409Q or *AKT1* E17K mutations. Mutations in both *TRAF7* and *KLF4* occurred exclusively in secretory meningiomas. Unlike *KLF4* mutations that are primarily present in secretory meningiomas, *TRAF7* mutations were also found in high-grade meningiomas. A recent study has also shown KLF4 K409Q-mutated meningiomas upregulate hypoxia-associated genes and are susceptible to mTOR inhibitor both in vitro and in vivo. [7]

AKT1 (E17K) mutations were most frequently observed in non-*NF2* mutated tumors and are associated with skull base meningiomas.

Furthermore, mutations in *PIK3CA* were detected in 7% of non-*NF2*-mutant meningiomas and were mutually exclusive from *AKT1* mutation. A recent study analyzing outcome data from 469 meningiomas with known molecular subgroups identified that *PI3K*-activated tumors were associated with earlier recurrence. [7]

Genomic sequencing of meningiomas found *SMO* mutations occur in approximately 5% of non-*NF2* mutant meningiomas. [7] Smoothened encodes a G-protein-coupled receptor (*SMO)* downstream of

the tumor-suppressor gene Patched in the hedgehog (Hh) signaling pathway. [7] *SMO*-mutated meningiomas are predominantly of the meningothelial subtypes and grade I tumors but can also present in high-grade and progressive tumors. [7] Interestingly, meningiomas harboring recurrent *SMO L412F* mutations tend to occur at the olfactory groove. [7] Furthermore, targeting *SMO* by Sonidegib inhibited the proliferation of SmoM2-induced cells. [7]

Loss of cytoplasmic *MTAP* (methylthioadenosine phosphorylase) immunohistochemical staining may also serve as a potentially promising surrogate due to the close proximity of the respective gene to *CDKN2A/B*. [15]

Other alterations are loss of H3K27me3, *BAP1*, *SMARCE1*, *SMARCB1*, *ARID1A*, *FOXM1*.

Multiple investigations have demonstrated that loss of H3K27me3 is associated with a greater risk of meningioma recurrence on multivariable analysis and that its loss is especially predictive of recurrence in grade 2 tumors. [15] Hence, H3K27me3 status in CNS WHO grade 2 meningioma offers prognostic information (shorter progression interval and overall survival) but does not currently inform grading. [15]

BRCA1-associated protein (*BAP1*) is a ubiquitin carboxy-terminal hydrolase and is involved in maintaining genome stability, transcription factors regulation, chromatin modification, and double-strand DNA repair. Somatic *BAP1* inactivating mutations are associated with the rhabdoid and/or papillary morphology subtype of meningioma, particularly those with higher grades and poor prognosis, but not present in non-rhabdoid subtypes or low-grade meningiomas. [7]

SMARCE1 inactivation is strongly associated with clear cell meningioma, which primarily occurs in the cerebellopontine angle and spine, predominantly affecting younger individuals, including children and young adults, therefore this mutation is highly useful in establishing the diagnosis and grade. [15] It has an aggressive behavior, including recurrence and occasional cerebrospinal fluid seeding, leading to their designation as CNS WHO grade 2 tumors.[15] Additionally, these tumors cluster away from other meningioma subtypes on DNA methylation profiling, providing further evidence that they are unique. [15]

While *BAP1*-mutant meningiomas and *SMARCE1*-mutant meningiomas can occur sporadically, they can also arise in patients with pathogenic germline variants therefore, a relevant family history and/or young age of onset should prompt consideration of genetic counseling and germline testing. [15]

SWI/SNF (switch/sucrose nonfermenting) complex is an ATP-dependent chromatin remodeling complex frequently mutated in meningioma. [15] *SMARCB1(INI1)* of the *SWI/SNF* complex was found to be uniformly lost in malignant rhabdoid tumors. [15] Recent extensive sequencing studies of high-grade and recurrent meningiomas observed prevalent *ARID1A* (a subunit of the *SWI/SNF* complex) mutations across all grades and associated with a 7.4-fold increased hazard of death. [7]

A comprehensive genomic, epigenomic and RNA sequencing analysis of 280 human meningiomas identified *FOXM1* as a crucial transcription factor for meningioma cell proliferation and a biomarker for aggressive meningioma with poor survival. Upregulation of *FOXM1* expression secondary to the loss of the negative regulator *NF2* is a likely mechanism. Overexpressing *FOXM1* was also found to upregulate the expression of vascular endothelial growth factor A (VEGFA), aryl hydrocarbon receptor (AHR) and cytochrome P450 family 1 subfamily A member 1(CYP1A1) in meningioma cell lines. [7]

Comprehensive cytogenetic studies revealed that chromosomal instability with copy number alterations (CNA) is more frequently observed in high-grade meningiomas with *NF2* mutations but less common in atypical non-*NF2* meningiomas. CNVs can accumulate with the progression of *NF2*-mutant cases, and *NF2*-mutant meningiomas tend to harbor the greatest abundance of genomic instability. [15] In addition to chromosome 22 deletion, losses of chromosomes 1p, 14q, 10p/10q, 18q and 6p/q and less frequently on 2p/q, 3p, 4p/q, 7p/q, 8p/q, and 13p/q were clustered in atypical meningiomas. [7,15]

The prognostic significance of oncogenic variants in these genes occurring individually or in combination is evident on univariable analysis but has not been demonstrated on multivariable analysis to be prognostically independent of grade, molecular class, or methylation class, and therefore it can only be used to inform clinical decision-making in challenging cases. [15]

Although the specific CNVs can vary substantially among individual tumors, loss of 1p is the most frequently observed in the context of adverse clinical outcomes. Thus, oncogenic phylogeny models propose 1p loss as the first CNV after monosomy 22q in *NF2*-mutant, high-grade meningiomas, with rare cases that show 1p loss in the absence of 22q loss (and hence no reliable data on this subset). [15]

A significant proportion of cases of meningioma still display unpredictable behaviour. This not only makes it difficult to predict outcomes for individual patients, but also results in clinical trials which are confounded by variable biology and treatment responses. [21] To overcome subjective interpretation of histological criteria and of mutations, support might be gained from DNA methylation-based subtyping.

d. DNA methylation profile (DNAMP)

Epigenetic regulations such as DNA methylation changes are highly sensitive markers for meningioma identity and biological behavior and appear relatively stable over recurrences. [7,16] Of all the genome-wide molecular approaches that have shown promise in meningioma, DNA methylation has been the most widely used given its relative stability, its ability to infer copy number profiles, and its use in the widely applicable German Cancer Research Center pan-cancer classifiers. [21]

Methylation-based tumor typing is a suitable technique in meningioma due to their solid growth pattern and high tumor fractions. [15] The role of methylation classes in meningioma is acquiring growing relevance because the application of new treatments in this setting is still an unmet need and this difficulty might be due to a limited understanding of meningioma biology. [17] DNA methylation profiling is a powerful tool for biological discovery, but clinical adoption of this approach has been encumbered by a lack of medical indications, along with challenges that include availability, technical variation, and cost remain. [17] Landry et al. demonstrated that despite methylation class clustering being agnostic to outcome, group membership was found to be the strongest predictor of recurrence-free survival after adjusting for WHO grade, Simpson grade, age, sex, copy number alterations, location, and mitotic index, demonstrating the strong clinical value of methylation profiling. [21]

At least three independent methylation classifiers currently exist, each with a proved prognostic association between distinct methylation groups. [15]

In 2017, Sahm et al. published a multicentre, retrospective analysis, investigating genome-wide DNA methylation patterns in 497 meningiomas from 10 European neuro-oncology centres to identify distinct methylation classes of meningiomas. [16] The methylation classes were further characterised by DNA copy number analysis, mutational profiling, and RNA sequencing. [16] DNA methylation-based classes and WHO classification schema were compared and methylation classes were analysed for progression-free survival outcomes. [16]

Unsupervised clustering of meningiomas alone revealed two major epigenetic groups: groups A and B, with further subdivision into four subgroups in group A and two subgroups in group B. These six subgroups were designated as methylation classes. [16] Based on further molecular and clinical characteristics, the four methylation classes of group A were designated methylation classes benign 1 through 3 (MC ben-1, MC ben-2, and MC ben-3) and methylation class intermediate A (MC int-A); the two methylation classes of group B were designated methylation class intermediate B (MC int-B), and methylation class malignant (MC mal). [16] There was an enrichment of grade I tumours in MC ben-1, MC ben-2, and MC ben-3, and an enrichment of WHO grade III tumours in MC mal, and WHO grade II tumours were scattered across all methylation classes. [16] *(Figure 4)*

These methylation classes also have unique molecular features. For example, MC ben-1 has the majority of benign *NF2* mutated tumors with only chr22 loss; MC ben-2 has the tumors carrying *AKT1*, *SMO*, *KLF4*, and *TRAF7* mutations; MC ben-3 has both *NF2* mutated and *PIK3CA* mutated tumors with mostly chromosomal gains, particularly chr5. MC int-A has 53% *NF2* mutated tumors with losses of both chr1p and 22q; MC int-B and MC mal both have *NF2* mutated tumors, *SUFU* mutations, occasional *TERT* mutations, and losses of chr1p, 10, and 22 with a higher frequency of *CDKN2A* deletion in MC mal group. [16] Pathways related to the immune system were also observed in MC ben-1, whereas checkpoint inhibition PD-1/PD-L1 pathway was more enriched in MC ben-2 and MC mal. [16]

Based on custom next-generation sequencing, known recurrent mutations (most frequently *NF2*, followed by *TRAF7* and *AKT1*) were significantly enriched in some methylation classes. Within group A, *NF2* mutations were observed in 63% MC ben-1 tumours (p<0·0001). In the MC ben-2 subgroup, five mutations were enriched: *AKT1* (33%, p<0·0001), *SMO* (7%, p=0·0002), *KLF4* (15%, p<0·0001), and *TRAF7* (49%, p<0·0001). This subgroup of tumours rarely harboured *NF2* mutations (4%) and there are very few copy-number alterations. Only one *AKT1* mutation and five *KLF4* mutations were detected outside of MC ben-2. MC ben-3 showed *NF2* mutations in 32% (p=0·4902). MC int-A carried *NF2* mutations in 53% (p=0·4382). In group B, *NF2* mutations were found in 35% of MC int-B tumours (p=0·5479) and in 31% of 35 MC mal tumours (p=0·1841). *SUFU* mutations were confined to group B, with one (5%) of 20 MC int-B tumours (p=0·1841) and two (6%) of 35 MC mal tumours (p=0·0362) harbouring this mutation. Four of five *TERT* promoter mutations mapped to meningiomas in group B. [16]

Annotation of copy-number-variations (CNV) revealed that MCs are associated with distinct cytogenetic aberrations (Fig. 4A, B): MC ben-1 was associated with deletions of 22q (95 %) but otherwise virtually no CNV. MC ben-2 presented with absence of recurrent CNVs. Typical for MC ben3 were multiple chromosomal gains, most frequently affecting chromosome 5 (47 %). MC int-A frequently exhibited losses on 1p (70 %) and 22q (84 %). In Group B, MC int-B frequently exhibited losses on 1p (89 %), 10 and 22 (89 %), all features also shared with MC malignant. However, in MC mal, a higher frequency of CDKN2A deletion was apparent (70%). [16]

Choudhury et al. show meningiomas are comprised of 3 DNA methylation groups with distinct clinical outcomes, biological drivers, and therapeutic vulnerabilities. Merlin-intact meningiomas (34%) have the best outcomes and are distinguished by *NF2*/Merlin regulation lacking by susceptibility to cytotoxic therapy; immune-enriched meningiomas (38%) have intermediate outcomes and are distinguished by immune infiltration, HLA expression, and lymphatic vessels; hypermitotic meningiomas (28%) have the worst outcomes and are resistant to cytotoxic therapy. (*Figure 5*) [17] Only 17% of meningiomas in the DNA methylation group with the best outcomes had chromosome 22q copy number deletions of any size containing the entire *NF2* locus, compared to 76% or 98% of meningiomas in the DNA methylation groups with intermediate or poor outcomes, respectively. [17]

Nassiri et al. demonstrated the presence of two methylation classes, favorable and unfavorable in terms of recurrence free survival (RFS). [14]

Landry et al. demonstrated demonstrated that by integrating DNA methylation profiling, RNA sequencing, and whole exome sequencing and performing an unsupervised cluster-of-cluster analysis, four stable consensus molecular groups of meningioma emerged. Molecular group 1 (immunogenic) was associated with immune pathway activation, isolated chromosome 22q loss or *NF2* mutations, and portended the best clinical outcomes. Molecular group 2 (benign *NF2*-wildtype) was akin to MC ben-2, defined by neutral copy number profiles and canonical non-*NF2* mutations;

they were also associated with favourable outcomes. Molecular group 3 (hypermetabolic) tumours had intermediate to poor outcomes and were defined by activation of metabolic signaling pathways; molecular group 4 (proliferative) tumours were defined by activation of cell cycle pathways, a significant copy number and mutational burden, and were associated with the worst outcomes. [21]

All major DNAMP-based proposed classification schemes share a largely benign subgroup with *NF2*-wildtype meningiomas ("Merlin-intact," "benign-2"), a mostly benign subgroup with isolated *NF2*/22q-alteration lacking any other alterations ("immune-enriched/activated," or in systems with higher granularity "benign-1") and a highly aggressive subgroup with *NF2*/22q-alteration and several other changes on CNV (1p, 10, 14, CDKN2A/B) or sequence (*TERT* promoter) level ("hypermitotic," "proliferative," "malignant"). [15] Some schemes propose subgroups for the *NF2/*22q-altered cases ("intermediate-A/B," "hypermetabolic"). [15]

Based on the available evidence that methylation and CNV patterns outperform current grading based on morphological criteria, these data should nonetheless be leveraged for grading when available. Since limited independent prospective studies on DNAMP alone are available, it is desirable to rely only on a concordant DNAMP and CNV result (eg, both indicating a higher grade in a morphological CNS WHO grade 1 case or vice versa). If both DNAMP and CNVs unequivocally indicate a clinical behavior different from the morphological criteria, the meningioma should for now be graded as "CNS WHO grade 1, with molecular data suggestive of higher risk of recurrence" or "CNS WHO grade 2, molecular data suggestive of lower risk of recurrence." [15]

The importance of methylation status for grade 2 meningiomas was further demonstrated in a recent case-control study comparing 11 patients with more than two recurrences (Group Dismal) with matched 11 patients without any recurrence (Group Benign). Methylation profiling accurately separated Group Dismal from Group Benign and is independent of location and extent of resection. [7]

Berghoff et al. analyzed 126 meningioma samples (41.3% grade 1, 38.1% grade 2, 20.6% grade 3). The most frequently altered genes were *NF2* (30.9%), *TRAF7* (30.9%), *KLF4* (19.8%), *ARID* (14.3%), *AKT1* (4.8%), *TERT* promoter (3.2%), *SUFU* (1.6%) and *PIK3CA* (0,8%); *SMO* mutations were absent in this cohort. [22] In univariable analysis presence of *TRAF7* and *KLF4* mutation as well as the *TRAKL* mutation genotype were associated with improved PFS and OS prognosis, with 5-year PFS and OS of at least 90% and 95% respectively. *NF2* and *TERT* promoter mutations were associated with impaired PFS and OS prognosis with mPFS of 29 and 5 months respectively and 5-year OS rate of 63% and 25%. Methylation clusters showed better prognostic discrimination for PFS and OS. [22]

Data on the prognostic value of methylation classes were also collected in the EORTC-1320-BTG trial, confirming that methylation profiling is the only molecular marker independently associated with OS. [23]

Finally, Hergott et al.demonstrated that methylation signatures from cell-free DNA in plasma can be used to predict outcome in meningioma, which may be useful for tailoring management in patients who do not undergo surgery or for serial monitoring of risk profiles in the postoperative period. [21]

Overall, independent international efforts on large cohorts have demonstrated the presence of two benign meningioma molecular groups (one defined by non-NF2 mutations and neutral copy number profiles and the other by NF2 loss and immune activation), and at least one malignant subgroup with poor outcomes and multiple copy number alterations, which can be further divided into a hypermetabolic group and a proliferative group. This suggests that these molecular groups are highly robust and generalizable. [21]

The wide spectrum of clinical behavior among WHO grade 1 and 2 meningiomas points towards the limited prognostic power of the current classification, particularly at the border between grade 1 and 2. As a result, the basing of decisions about radiotherapy on the current grading scheme is heavily debated. [16]

e. Clinical presentation and diagnosis

The presentation of meningiomas, like other CNS tumors, depends upon their location. Intracranial meningiomas can develop in different areas of the skull, according to the location of the meninges (any intracranial or spinal dural surface) [24] Rarely, intraventricular meningiomas are identified. Some meningiomas can present as multiple lesions or incarcerate important intracranial structures. Many meningiomas are discovered incidentally on brain imaging, since they are typically not fast growing or infiltrative lesions and they have an insidious symptom onset. [25]

The most common symptoms are headache due to increased intracranial pressure, focal neurological (including cranial nerve) deficits or generalized or partial seizures caused by focal mass effect. Personality changes, confusion and altered consciousness can be seen, especially in anterior (frontal) or parasagittal meningiomas.[25]

The gold standard technique for meningioma is magnetic resonance imaging (MRI) with contrast medium (CM), which allows for the best tumor delineation, highlights the presence of intra- and trans-osseous extension and defines the tumor relationship to the underlying brain. [26]

Typical meningiomas appear as dural-based masses isointense to grey matter on T1 (60-90% of cases) and hyperintense (40% of cases)/isointense (50% of cases) on T2 weighted and FLAIR imaging, enhancing vividly and homogeneously on both MRI and CT. [26]

Meningiomas can present some typical radiologic signs, the most common of which is an enhancing dural tail at the perimeter of the tumor (visible in 60-72% of the meningiomas). [1,26]

More than half of the meningiomas present peritumoral oedema, assessed in FLAIR, which is tendentially correlated with more aggressive grade (2 or 3), even though oedema is often present also in the secretory type meningioma (grade 1). Higher grade is also associated with more heterogeneous contrast enhancement [1,26]

Additional but less used techniques are CT scans with bone window setting, useful to assess hyperostosis and intraosseous tumor growth and conventional angiography, useful to assess the invasion of major sinuses (lateral or superior sagittal sinuses). [1]

Furthermore, meningiomas express somatostatin receptors (SSTRs). Among all the receptors included in the family, SSTR2 is the most prevailing SSTR in human meningiomas and it is considered a reliable diagnostic biomarker for meningiomas. A higher expression of SSTR5 was found in WHO grade 2 meningiomas. The presence of SSTRs is useful to delineate meningiomas through PET after the injection of somatostatin analogs such as 68Ga-DOTATATE (DOTA-D-Phe1-Tyr3—octreotate) or 90Y-DOTATOC (DOTA-D-Phe1-Tyr3-octreoitde). [1,27]

f. Treatment

i. Surgery

For many patients affected by meningioma, in particular asymptomatic tumors, observation with routine surveillance imaging alone is an acceptable strategy. For tumors that are growing or causing symptomatology, maximal safe surgical resection remains the standard of care for therapeutic management of meningioma. However, the ability to achieve complete resection may be limited by tumor location, involvement of nearby dural venous sinuses, arteries, cranial nerves, and brain invasion into eloquent tissue. [25]

The extent of resection (EOR) represents an important prognostic factor and is commonly defined as gross total resection (GTR) (no residual tumor) or subtotal resection. [1]

Rates of recurrence for surgically treated meningiomas are impacted heavily by the extent of resection. The traditional measurement of the extent of resection is evaluated by the Simpson grade [28], defined both by postoperative imaging and by the neurosurgeon's assessment *(Figure 6).* Five-year recurrence rates after GTR in grade 1 meningiomas are reported as 7–23%, in grade 2 they are 50–55% and in grade 3 72–78%. [25]

ii. Radiotherapy

After surgery, fractionated external beam radiotherapy (RT) remains an important component in the therapeutic armamentarium for the management of meningiomas, as an "adjuvant" treatment or at

recurrence. Both fractionated external beam RT (EBRT) and single-fraction stereotactic radiation (SRS) are employed. [25]

EANO guidelines recommend RT according to grade. WHO grade 1 meningioma may not require immediate postsurgical RT, especially if asymptomatic; for WHO grade 2 with Simpson grade IV-V resection, RT is recommended; for WHO grade 3 postsurgical RT is always indicated. [1]

Often surgery and radiotherapy, when indicated, are not enough to guarantee an adequate control of disease. In fact, after standard treatment, atypical meningiomas have a recurrence risk of 29-52% and anaplastic meningiomas have a recurrence risk of 50-94%. [29]

iii. Systemic treatment

The role of pharmacotherapy in meningioma still represents an unmet need in neuro-oncology. No positive controlled clinical trials to define recommendation guidelines are available. [1]

For all meningiomas, especially grade 3 ones, there exists a long-term risk of multiple recurrence, and it is necessary to identify options to manage of patients who have exhausted surgical and radiotherapeutic options. [4]

According to National Comprehensive Cancer Network (NCCN) guidelines no preferred systemic regime is determined. Among preferred regimens the following are cited: bevacizumab, bevacizumab+everolimus, sunitinib, somatostatine analogues, somatostatine analogues+everolimus. [30]

Clinical trials for intracranial meningioma are uncommon; those available, have largely explored treatment options for patients with high-grade, recurrent, and progressive disease. [4] In addition to this, other obstacles are present: recruitment of heavily pretreated patients with heterogeneous pathology, treatment-resistant tumor cells, and limited knowledge of the disease biology. [4]

Prior attempts to explore systemic therapies, including cytotoxic chemotherapy (temozolomide, hydroxyurea, irinotecan, cyclophosphamide/doxorubicin/vincristine), tyrosine kinase inhibitors (sunitinib, vatalanib), antiangiogenic agents (bevacizumab), molecular target therapy (everolimus, erlotinib), hormone therapy (mifepristone, somatostatin analogs), immunotherapy (nivolumab, pembrolizumab) have had minimal success but largely limited by underpowered studies and lack of control arms in the trials. [7,31,32] Trabectedin, an alkylating agent, failed to show PFS and OS benefits in the first prospective randomized trial performed in recurrent grade II/III meningiomas [23].

Evidence shows probable activity for bevacizumab, bevacizumab combined with everolimus and sunitinib; uncertain activity has been detected with octreotide/pasireotide and hydroxyurea. [18]

Hydroxycarbamide/hydroxyurea is a ribonucleotide reductase inhibitor that has shown some activity in meningioma. The drug can be used in Italy in the recurrent/unresectable meningioma setting

according to the law n° 648. It is one of the first drugs historically used in the treatment of meningiomas, and in vitro studies on meningioma cells were promising; many studies have been performed but study populations are very heterogeneous. [18]

According to available literature, hydroxyurea in recurrent meningioma achieved a mPFS ranging from 2.4 to 4 months and a mOS ranging from 7.4 to 8 months (*Table 1*).

Bevacizumab is a vascular endothelial growth factor (VEGF-A) targeting monoclonal antibody. It binds VEGF-A preventing the interaction with vascular endothelial growth factor receptors VEGFR and thereby inhibiting the activation of VEGF signaling pathways that promote neovascularization. [13,33]

Due to their high metabolic demands, growing solid tumors depend on vascularization for nutrients and oxygen and disposal of metabolic waste products. Vascularization can be promoted by angiogenesis, i.e. the generation of new blood vessels by sprouting from existing blood vessels. This process is predominantly mediated by VEGF. [13,33]

Among tumor-secreted pro-angiogenic factors, VEGFs and in particular VEGF-A, have been identified as key factors for inducing tumor angiogenesis. VEGFs activate VEGF signaling in endothelial cells by binding to VEGF receptor tyrosine kinases (VEGFR1-3). By these means, VEGF can stimulate the proliferation and survival of endothelial cells and increase the permeability of vessels, thereby supporting the metabolic demands of the growing tumor. Given the importance of angiogenesis in tumor biology, drug development efforts in the past decades have been dedicated to targeting angiogenesis, with VEGF-A as a therapeutic target for inhibition of angiogenesis and normalization of tumor vasculature. [33]

Bevacizumab has demonstrated a role in controlling progression of disease in recurrent glioblastoma, another aggressive CNS tumor. [13,34]

Data supporting bevacizumab in the setting of systemic treatment in recurrent meningioma derive from retrospective trials mainly; some data can be extrapolated from clinical trials exploring the role of other drugs (*Table 2).*

The EORTC-1320-BTG trial is a randomized phase II, multicenter, open label trial investigating the role of trabectedine in recurrent WHO grade 2 and grade 3 meningioma. Patients were 2:1 randomly assigned to intravenous trabectedin (1.5 mg/m2 every three weeks) or local standard of care (LOC). The trial enrolled 55 patients with meningioma grade 2 and 35 patients with meningioma grade 3. The trial did not report any significant difference in PFS or OS between the trabectedin and LOC cohort. The LOC therapies included hydroxyurea, bevacizumab, vincristine, cyclophosphamide, doxorubicin, somatostatin analogs. In a post-hoc analysis, hydroxyurea was associated with a

median PFS of 2.4 months and a PFS-6 rate of 8.8%. Bevacizumab achieved median PFS of 6 months and PFS-6 of 44.4%. [23]

Nayak et al. led a retrospective analysis of 15 patients with grade II or III meningioma, who had previously failed surgery and radiation therapy and were subsequently treated with bevacizumab. Atypical meningiomas were 6/15 and anaplastic were 9/15. All patients received bevacizumab intravenously at a dose of 10 mg/Kg every 2 weeks. For all patients mPFS was 26 weeks (95% CI, 10-29 weeks); the median OS after starting treatment was 15 months (95% CI, 10-22 months); the 6 months PFS rate was 43.8% (95% CI, 15.7-69.1%). [29]

Kumthekar et al. investigated retrospectively 50 patients affected by recurrent grade 1-2-3 meningioma, vestibular schwannoma and hemangiopericytoma, treated with bevacizumab 10 mg/Kg every 2 weeks for the first 6 months; consequently they were allowed to switch to 15 mg/Kg every 3 weeks. mPFS for grade 1-2-3 meningioma were, respectively 22 months, 18 months, 8 months; mOS for grade 1-2-3 meningioma were, respectively 35 months, 27 months, 12 months. [35]

According to available literature, bevacizumab treatment in recurrent meningioma has achieved a mPFS ranging from 6 to 22 months and a mOS ranging from 13.5 to 35 months (*Table 2*).

2. Aim of the study

This retrospective study aims at investigating the role of bevacizumab therapy compared to a historical standard of care (hydroxyurea), in a multicentric recurrent aggressive meningioma cohort.

The primary endpoint of the study was progression free survival (PFS) from the beginning of the treatment.

The secondary endpoints were overall survival (OS), disease control rate (DCR) and safety.

The study also aims at investigating NGS and methylation profile data in order to identify possible biomarkers of response to treatments.

3. Materials and methods

a. Statistical analysis

Patients affected by recurrent meningioma, any grade, previously treated with surgery and radiotherapy, were retrospectively analyzed. Patients at progression were not eligible for further surgical or radiotherapeutic treatment and received systemic treatment. Patients had been treated at different centres (Veneto Institute of Oncology, Istituto Neurologico Carlo Besta Milano, Istituto Nazionale Tumori Regina Elena Roma, Istituto Romagnolo per lo Studio dei Tumori Dino Amadori Meldola, Città della Salute e della Scienza Torino, Humanitas Rozzano, San Raffaele Milano) (*Figure 7*) with bevacizumab or hydroxyurea between June 2003 and September 2025. Clinical data were collected from the electronic medical records, retrospectively. Meningioma grading is based on WHO 2021 classification. Radiologic response was evaluated through Radiologic Assessment in Neuro-Oncology (RANO) criteria. Performance status was graded according to the Eastern Cooperative Oncology Group (ECOG) scale. Toxicity was graded according to the Common Terminology Criteria for Adverse Events (CTCAE) v5.0.

Statistical analysis was carried out using R 4.4 (R Foundation for Statistical Computing, Vienna, Austria) [36]. Data were summarized as median and interquartile range (IQR) (continuous data) and number and percentage (categorical data). Comparisons between the two treatment groups (bevacizumab vs. hydroxyurea) were performed using the Mann-Whitney test (continuous data) and the Chi-square test or the Fisher's exact test (categorical data). Progression-free survival (PFS) was calculated from the date of treatment start to the date of disease progression, death or last follow-up visit. Overall survival (OS) was calculated from the date of start of treatment start to the date of death or last follow-up visit. Survival curves were estimated using the Kaplan-Meier method and compared between the two treatment groups using the log-rank test. Cox regression models were estimated to assess the effect of bevacizumab vs. hydroxyurea on PFS and OS, adjusting for unbalanced characteristics at baseline. Survival curves were also stratified and compared according to gene alterations with exploratory purpose, in strata with at least 10 patients. All tests were 2-sided and a p-value of less than 0.05 was considered statistically significant.

b. Molecular analysis

In case of available tissue, NGS analyses were performed. The assays used are FoundationOne and TruSight Oncology 500 High (TSO500).

For TSO500, nucleic acids extraction from meningioma samples, four 10-µm sections of formalin-fixed, paraffin-embedded (FFPE) tissue were carefully examined by an experienced pathologist to delineate and confirm the tumor-rich regions. These selected sections were then processed using the column-based AllPrep DNA/RNA FFPE Kit (Qiagen), following the manufacturer's

recommendations. The concentration of the extracted nucleic acids was determined using the Qubit 4 Fluorometer with the High Sensitivity DNA or RNA Assay kits (Thermo Fisher Scientific).

Samples were then evaluated for suitability in downstream next generation sequencing (NGS) applications. DNA quality was assessed using the Illumina Infinium FFPE QC Kit, according to the manufacturer's protocol. Only DNA samples with a ΔCq value ≤ 5, indicative of sufficient integrity, were retained for further analysis. RNA quality was assessed on the Agilent TapeStation system with the RNA ScreenTape. The percentage of RNA fragments longer than 200 nucleotides (DV200) was used as quality metric, and samples with DV200 values above 30% were considered adequate for library preparation.

Library preparation was carried out using the TruSight Oncology 500 High Throughput (TSO 500 HT) kit (Illumina), according to the manufacturer's guidelines. This hybrid capture based assay requires a minimum input of 40 ng DNA and 80 ng RNA and targets 523 cancer associated genes. The panel covers a wide range of oncogenic alterations, including single nucleotide variants (SNVs), insertions and deletions (in-dels), copy number variations (CNVs), gene fusions, and clinically relevant biomarkers such as microsatellite instability (MSI) and tumor mutational burden (TMB).

In brief, genomic DNA was sheared using the Covaris ultrasonicator according to the manufacturer's instructions, while RNA was reverse transcribed into complementary DNA (cDNA). Both DNA and cDNA fragments underwent an end-repair and A-tailing processes. Subsequently, SUA1 adapters were ligated to cDNA and UMI1 adapters were attached to gDNA fragments, followed by a clean-up step to remove excess adapters and by-products. In the next stage, adapter-ligated fragments were then amplified by an index PCR, during which unique barcodes were incorporated to enable multiplexing. Two successive rounds of hybridization and target capture were carried out to selectively enrich the genomic regions of interest and reduce non-specific sequences. The enriched libraries were further amplified, normalized to achieve uniform concentrations, pooled, and prepared for sequencing.

Sequencing was carried out on the Illumina NovaSeq 6000 platform at the Bellaria laboratories, using a paired-end sequencing strategy (2 × 101 bp reads), in accordance with the manufacturer's protocol.

Sequencing data generated from FFPE meningioma samples using the TSO 500 panel were processed and analyzed using Illumina cloud platforms Illumina Connected Analytics (ICA) and Illumina Connected Insights (ICI). Raw sequencing reads underwent primary and secondary analysis, which included adapter trimming, alignment to the human reference genome (GRCh37/hg19), quality control and variant calling. This workflow produced outputs covering multiple classes of genomic alterations, including single nucleotide variants SNVs), insertions and deletions

(in-dels), copy number variations (CNVs), gene fusions, splice variants, microsatellite instability (MSI) status, and tumor mutational burden (TMB) and key quality control metrics.

For tertiary analysis and variant interpretation, detected variants were annotated against several databases, including COSMIC, ClinVar, gnomAD, and OncoKB. Variants were then manually reviewed, filtered and classified one by one according to their clinical significance (pathogenic, likely pathogenic, variant of uncertain significance-VUS, benign), with specific consideration of their relevance to brain tumor biology.

c. Methylation analysis

As for the methylation profile, DNA methylation profiling on meningiomas was available for 8 patients at the time of this thesis drafting. The DNA extracted from Formalin-fixed paraffin-embedded (FFPE), was processed, loaded onto the Infinium Methylation EPIC array k, and scanned using the Illumina IScan system. Raw IDAT files were processed through the the CNS tumor classifier developed by the Molecular Neuropathology group at the German Cancer Research Center (DKFZ).

4. Results

a. Patients

The analysis included 98 patients who were treated for relapsed meningioma with bevacizumab (n=49) or hydroxyurea (n=49) between June 2003 and April 2025. Patient characteristics at baseline are summarized in *Table 3*.

In the bevacizumab group, median age at diagnosis was 63 years old; 21 (43%) patients were female, 28 (57%) patients were males. Only 1 (2%) patient had a confirmed diagnosis of neurofibromatosis type 2. In terms of meningioma grading, 5 (10%) patients were grade 1, 23 (47%) patients were grade 2, 21 (43%) patients were grade 3. In this group, 23 (47%) patients received maximum 2 surgeries before systemic treatment and 38 (78%) patients received maximum 2 radiotherapy courses before systemic treatment. In terms of ECOG PS 12 (24%) patients were ECOG 0, 17 (35%) patients were ECOG 1, 20 (41%) patients were ECOG 2, and no patient was ECOG 3 or higher. At progression 17 (35%) patients received further treatment, of which 3 patients were treated with hydroxyurea (6% of the whole cohort).

In the hydroxyurea group, median age at diagnosis was 62 years old; 28 (57%) patients were female, 21 (43%) patients were males. A known diagnosis of neurofibromatosis type 2 was present in 2 (4%) patients. In terms of meningioma grading, 7 (14%) patients were grade 1, 30 (61%) patients were grade 2, 12 (25%) patients were grade 3. In this group, 37 (76%) patients received maximum 2 surgeries before systemic treatment and 41 (84%) patients received maximum 2 radiotherapy courses before systemic treatment. In terms of ECOG PS 9 (19%) patients were ECOG 0, 30 (61%) patients were ECOG 1, 8 (16%) patients were ECOG 2, and 2 (4%) patients was ECOG 3. At

progression 34 (69%) patients received further treatment, of which 5 with bevacizumab (10% of the whole cohort).

Patients treated with bevacizumab more likely received more than 2 surgical procedures before treatment (53% vs 24%, p-value 0.007), had more likely PS ECOG 2-3 (41% vs. 20%, p=0.008) and received less frequently another treatment at progression (35% vs. 69%, p=0.001) compared to patients treated with hydroxyurea.

b. Radiological response and survival

Response to treatment was evaluated in terms of disease control rate (DCR) and objective response rate (ORR). DCR was higher in patients treated with bevacizumab compared to those treated with hydroxyurea (82% vs. 57%, p=0.02), while ORR was not statistically different between the two groups (8% vs. 2%, p=0.36) *(Table 4).*

Median follow-up from the diagnosis was 17 months (IQR 9-32). At the analysis, 71 patients (72%) had experienced a disease progression and 63 (64%) were dead.

Median PFS was 11 months (95% CI 8 to 24) in patients treated with bevacizumab and 5 months (95% CI 3 to 9) in patients treated with hydroxyurea. (*Figure 8*)

PFS at 6-12-24-36 months was 71-48-35-7% in patients treated with bevacizumab and 46-23-9-2% in patients treated with hydroxyurea (p=0.003) *(Table 5).*

Median OS was 25 months (95% CI 18 to 42) in patients treated with bevacizumab and 23 months (95% CI 14 to 36) in patients treated with hydroxyurea. (*Figure 9*)

OS at 6-12-24-36 months was 83-73-56-33% in patients treated with bevacizumab and 91-76-49.35% in patients treated with hydroxyurea (p=0.80) *(Table 6).*

After adjusting for unbalanced characteristics at baseline, bevacizumab was associated with improved PFS (HR 0.44, 95% CI 0.26 to 0.75, p=0.003) but was not associated with improved OS (HR 0.77, 95% CI 0.42 to 1.38, p=0.38).

c. Toxicity

Toxicity of any grade was experienced in 17 (35%) patients treated with bevacizumab, and 12 (25%) patients treated with hydroxyurea (p-value 0.38). *(Figure 10)*

Toxicity in the bevacizumab group included proteinuria (n=4), arterial hypertension (n=13), brain haemorrhage (n=1), while toxicity in the hydroxyurea group included anemia (n=1), fatigue (n=1), lymphopenia (n=1), (n=1), nausea (n=3), neutropenia (n=2), neutropenia and thrombocytopenia (n=1), thrombocytopenia (n=2).

Toxicity of grade ≥ 3 was experienced by 5 (10%) patients treated with bevacizumab and no patient treated with hydroxyurea; specifically in the bevacizumab group there were 4 cases of grade 3 arterial hypertension and 1 case of grade 3 brain hemorrhage.

d. Post-progression treatment

After progression to first line bevacizumab or hydroxyurea, 17 (35%) patients in the bevacizumab group and 34 (69%) patients in the hydroxyurea group received further treatment (systemic and/or local).

Treatments at progression are summarized in *Table 7*.

Most commonly used treatments at progression were somatostatin analogs, as monotherapy or combined/sequenced with other systemic/local treatments (21 patients); everolimus as monotherapy or combined/sequenced with other systemic/local treatments (16 patients); radiotherapy (5 patients); surgery (5 patients); radiometabolic therapy (2 patients).

e. Molecular alterations

Molecular alterations were studied in 37/49 (76%) patients treated with bevacizumab, while the information on gene alteration was not available in 12 patients. Genes altered in at least 2 cases were: *NF2* (26 cases); *ARID1A* (17 cases); *CDKN2A/2B* (12 cases); *LZTR1* (10 cases); *ARID1B, FUBP1, PTEN* (each 9 cases); *NFKBIA* (8 cases); *TP53, MSH6, SUFU* (each 7 cases); *SMARCB1, SETD2* (each 6 cases); *DICER, CDKN2C, FBXWT* (5 cases); *MSH2, MTAP* loss, *EPCAM, BAP1* (each 4 cases); *SMARCA4, PIK3CA, ROS1, POLE, MLH1, TRAF7, NF1* (each 3 cases); *NTRK2, ALK, CIC* (each 2 cases). (*Table 8*)

The impact of molecular alterations on survival was assessed only with molecular alterations with a frequency ≥ 10 cases. (*Table 9*)

NF2 alterations showed a detrimental effect of PFS (p-value 0.01) and OS (p-value 0.02). Survival curves for PFS (*Figure 11*) and OS (*Figure 12*) confirm such result.

No other alterations showed an impact on survival.

f. Methylation analysis

During the drafting of this thesis, methylation data were still immature. Exploratory methylation class analysis is available for 8/49 patients treated with bevacizumab.

Results so far obtained identified: 1 case of meningioma compatible with a benign methylation class; 2 cases compatible with intermediate A methylation class; 2 cases of intermediate B methylation class. Three cases did not correspond to meningioma histology.

5. Discussion

A standardized systemic treatment for relapsed aggressive meningioma is still an unmet need in neuro-oncology. The present study aims at retrospectively comparing the outcomes of meningioma patients treated either with bevacizumab or hydroxyurea, exploring possible tissutal biomarkers of response.

In this retrospective study, baseline population characteristics differ in terms of PS ECOG, number of surgeries before the beginning of treatment and treatment at progression; patients in the bevacizumab group more likely received more surgical procedures before treatment, had more likely worse PS ECOG and less likely received treatments at progression.

In terms of survival, data on PFS and OS in this cohort are consistent with those available in previous literature (*Table 1* and *Table 2*).

Considering data coming from the only randomized phase II trial (EORTC-1320-BTG) for systematic treatment in meningioma, bevacizumab, one among the local standards of care, achieved a median PFS of 6 months and PFS-6 of 44.4%, while hydroxyurea achieved a PFS of 2.4 months, and a PFS-6 rate of 8.8%. [23] Numerosity was very limited though, inferior to the one in this cohort, with 9 patients treated with bevacizumab and 13 with hydroxyurea, therefore data cannot be directly compared. [23] In this study, bevacizumab achieved a 6 months PFS rate of 71% vs 46% for hydroxyurea.

Studies investigating systemic treatments in meningioma are rare, even rarer are randomized ones. Therefore comparisons among different studies can be difficult to achieve, because of the lack of standardized agreement on outcome measures. [4]

A previous review by the RANO group analyzing outcome data of 47 clinical studies on medical therapies of surgery and radiation-refractory meningiomas concluded that PFS-6 rates were the only endpoint reported consistently enough to be useful as historical benchmark. For patients with recurrent WHO grade 2 and 3 meningiomas, PFS-6 rates of 0%-64% have been reported and a PFS-6 rate of 30% was suggested as control threshold based on a pooling of all available data. [23]

Besides, Millward et al. and Kaley et al. published a systematic review aimed at summarizing the outcomes measured and reported in meningioma clinical trials. [4]
Kaley et al.'s work demonstrated heterogeneity in both the definition of response criteria, and the reported survival outcomes; for instance, some studies reported median overall survival while others reported median PFS. In fact, only PFS at 6 months (PFS-6) was found to be common to all but 1 study in Millward's review; the unique outcome "PFS" was reported most frequently. To that end, the importance of reporting PFS-6 was emphasized and recommended as an outcome for future studies evaluating interventions for those who have progressed after local therapies (with benchmarks for both WHO grade 1 and WHO grade 2/3 provided), to allow comparative analysis of trial results. [4]

A further effort at harmonization, aiming at defining a core outcome set (COS) will be achieved within The COSMIC Project (interventional and observational), an international effort to develop 2 COS for meningioma. [4]

Information available in literature therefore justifies choosing PFS as the primary endpoint in this study.

As for OS, considering the lack of validated radiological response criteria in meningiomas, trial designs using a median OS of 10.6 months, an OS-6 rate of 74.5%, or an OS-12 rate of 44.7% as benchmark may provide more robust results than trial designs based on PFS data. [23]

In the current study no statistically significant difference was seen in terms of OS. OS was comparable between the two groups and much longer than data deriving from EORTC-1320-BTG, which demonstrated for hydroxyurea median OS of 7.4 months, and an OS-6 rate of 55.9%, while for bevacizumab a median OS of 13.5 months, and an OS-6 rate of 88.9%. This effect on OS could derive from the fraction of patients who received treatments at progression (especially in the hydroxyurea group), the confounding effect of crossover and, last but not least, from the limited numerosity of the sample. No data on treatment at progression was available in EORTC-1320-BTG.[23]

As for the molecular alterations and their impact on survival, *NF2* alterations are confirmed to have a detrimental effect on progression free and overall survival. No conclusion can be extrapolated for the other alterations because of limited numerosity, no matter the presence of non significant p-values. The frequency of *NF2* alteration cases in this study is consistent with the frequency present in the EORTC-1320-BTG trial, where 78.9% of patients were sequenced and *NF2* mutation in was present in 33/71 (46.5%) patients.

Only 3 cases of mutations related to the TRAKL group were detected in this study, confirming that those are usually related to more benign forms of meningioma. [15]

The detrimental effect of *NF2* alteration is consistent with data available in literature, specifically relative to methylation class data, which tend to assign *NF2* mutated meningiomas to more aggressive methylation classes. [16]

As for toxicity, treatment with bevacizumab in this study resulted more toxic than hydroxyurea. Probably attention should be paid at adequately choosing patients, especially those with comorbidities that may suffer worsening during bevaciuamb treatment.

Recently, due to the lack of therapeutic options and the new knowledge deriving from NGS analysis, new efforts have been made to provide targeted trials for meningiomas.

An Alliance National Cancer Institute sponsored cooperative group trial began to enroll patients based on the genetic alteration of their meningiomas, in the trial "Vismodegib, FAK Inhibitor GSK2256098, Capivasertib, and Abemaciclib in Treating Patients With Progressive Meningiomas".[37] This phase II biomarker-driver trial studies how well vismodegib, focal adhesion kinase (FAK) inhibitor GSK2256098, and capivasertib work in treating patients with progressive meningioma. Patients are assigned to 1 among 4 treatment arms based on their mutation status. Arm A (*SMO/PTCH1* mutation) receive vismodegib; arm B (*NF2* mutation) receive *FAK* inhibitor GSK2256098; arm C (*AKT1, PIK3CA*, or *PTEN* mutation) receive capivasertib; arm D (*CDK4, CDK6, CDKN2A, CCND1, CCND2, CCND3, CCNE1* alterations) receive abemaciclib. [37]

Streategies to target *NF2* and its co-dependent pathways are attractive since it is the most common molecular alteration in meningiomas.

A phase II study of *mTORC1/mTORC2* inhibitor Vistusertib showed promising preliminary results, with 51.5% PFS in 6 months, exceeding the RANO target of 35% for recurrent high-grade meningiomas. Vistusertib was also studied in *NF2* patients with progressive or symptomatic meningiomas (NCT02831257). [38]

Selumetinib, a *MEK* inhibitor, is tested in patients with *NF2*-related tumors, including meningiomas (NCT03095248). [38]

Targeting *ALK* fusion oncogene using brigatinib, upstream of *MAPK* pathway, is also under investigation in *NF2*-related meningiomas (NCT04374305). Evaluating concurrent *MEK* inhibitor trametinib and *PI3K* inhibitor alpelisib in patients with progressive refractory meningiomas (NCT03631953) is also ongoing. Focal adhesion kinase (*FAK*) is an essential molecule affecting cell migration and invasion, and its phosphorylation can be suppressed by overexpression of *NF2*. Excitingly, inhibiting FAK in the Alliance genomically-guided trial (NCT02523014) resulted in an improved 6-month PFS of 83% in grade I NF2-m. [37]

Targeting the hedgehog pathway using smoothened inhibitors in Smoothened (*SMO*)-mutated meningiomas is promising, given the existence of two FDA-approved *SMO* inhibitors vismodegib and sonidegib. If *SMO* inhibitors were proven to be efficacious, they could potentially spare patients from the morbidities of high-risk surgeries since *SMO*-mutated meningiomas are frequently located near many critical structures in the anterior skull base.

Currently, there are several clinical trials using *CDK4/6* inhibitors in treating meningiomas, including ribociclib (NCT02933736), palbociclib (NCT02255461), and abemaciclib (NCT03220646; NCT02523014); these drugs inhibit *CDK4/6*, whose activity is increased in meningiomas with *CDKN2A/CDKN2B* loss. [7]

Merlin regulation of glucocorticoid signaling drives meningioma apoptosis, and Merlin-intact meningiomas have the best clinical outcomes with current therapies. In contrast, Immune-enriched and Hypermitotic meningiomas have adverse outcomes and elevated cell proliferation, and are resistant to cytotoxic therapies due to loss of Merlin or misactivation of *FOXM1*. The convergence of mechanisms driving cytotoxic resistance and cell cycle misactivation in Immune-enriched and Hypermitotic meningiomas suggests cytostatic cell cycle inhibitors may be effective treatments for meningiomas from these DNA methylation groups. To test this, the *CDK4/6* inhibitors abemaciclib, palbociclib, and ribociclib were studied in cell culture, organoids, and xenografts. [17] In support of these preclinical investigations, we have observed encouraging early results with compassionate use of *CDK4/6* inhibitors in patients with Hypermitotic meningiomas that are resistant to surgery and radiotherapy.[17]

Currently open phase II or beyond trials with systemic treatments aim at targeting known biomarkers in meningioma with the following therapeutic options: abemaciclib, 177Lu-DOTATATE + everolimus, somatostatin antagonist 177Lu-satoreotide, regorafenib. [38,39]

6. Conclusions

According to this retrospective study bevacizumab offers a benefit in PFS to relapsed meningioma patients. Even though the benefit cannot be confirmed in terms of OS, available data seem a good base to consider bevacizumab the most effective systemic therapy option in this setting and might also be considered a useful comparison arm for trials and studies in general, instead of historical hydroxyurea.

The role of *NF2* mutations has been confirmed as the most common molecular alteration in meningioma, with a detrimental effect on survival; the limited number of patients did not allow for definitive conclusions regarding other genetic or epigenetic events.

7. Limitations and Future Perspectives

The current study presents some limitations, mainly deriving from the retrospective nature of the study and the limited size of the sample size of the two cohorts, consequence of the rarity of the condition. Therefore results cannot be completely generalized.

In particular, the absence of information on treatment sequences and the presence of, even if not massive, crossover between hydroxyurea and bevacizumab at progression may have confounded overall survival estimates, making progression-free survival a more reliable endpoint in this setting. Furthermore, the imbalance of baseline characteristics between the two groups (e.g., ECOG performance status, number of previous surgeries) could have influenced outcomes. Although multivariate Cox regression was applied to mitigate this bias, a more solid answer might derive from randomized trials more apt to enrolling balanced and highly selected populations.

Another limit is the unavailability of molecular data and methylation regarding the hydroxyurea cohort, thereby preventing a comparison between the two cohorts in terms of unfavourable molecular alterations. Future studies should systematically integrate next-generation sequencing and methylation profiling into clinical trials to validate molecular predictors of response and refine patient stratification.

In the future, integrating molecular classification into trial design will be crucial to overcome disease heterogeneity and to pave the way towards personalized treatment approaches in aggressive and recurrent meningiomas and to targeted treatments trials.

8. Figures

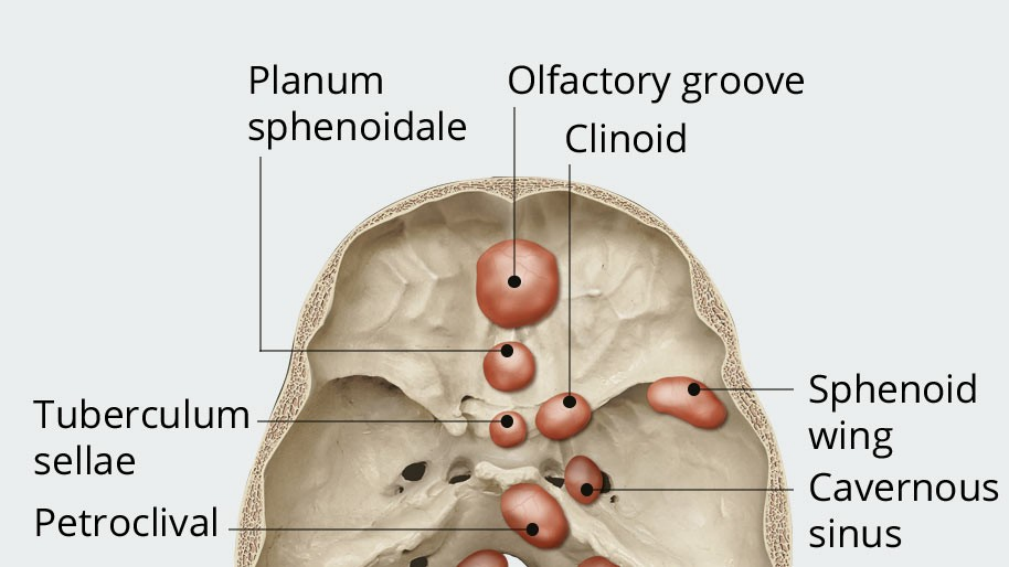

Figure 1: intracranial location of meningioma [24]

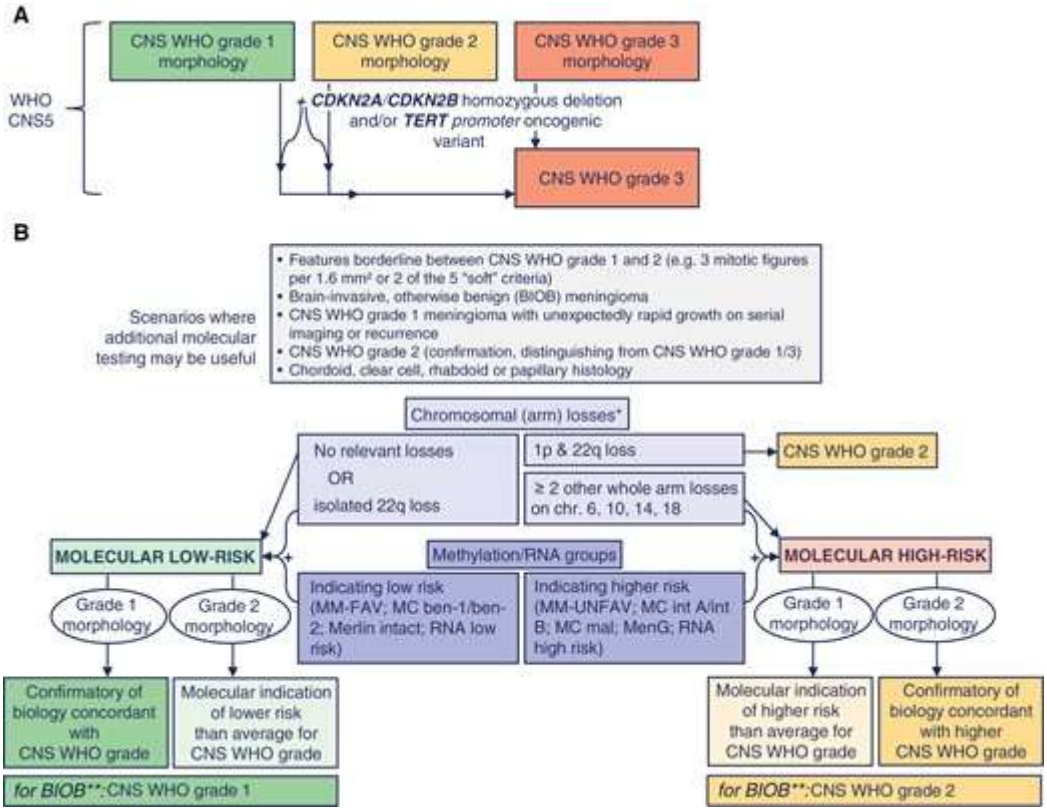

Figure 2: CNS 5 grading scheme according to cIMPACT-NOW 8 [15]

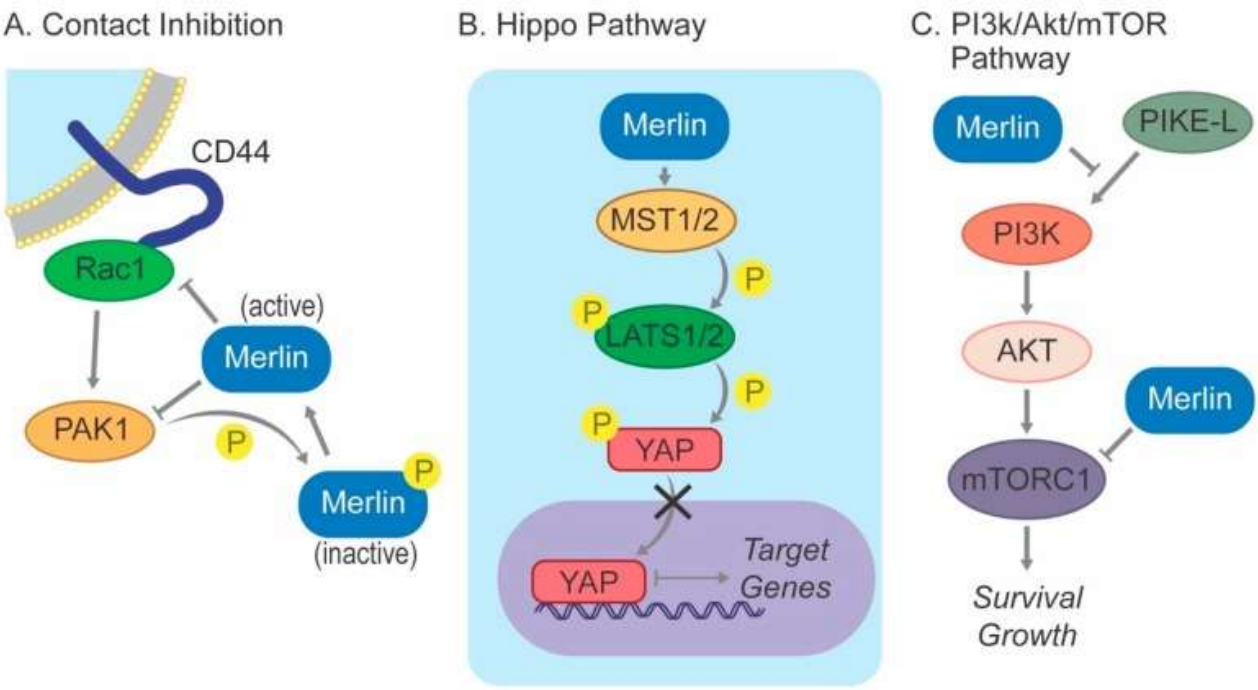

Figure 3: molecular pathways related to Merlin [20]

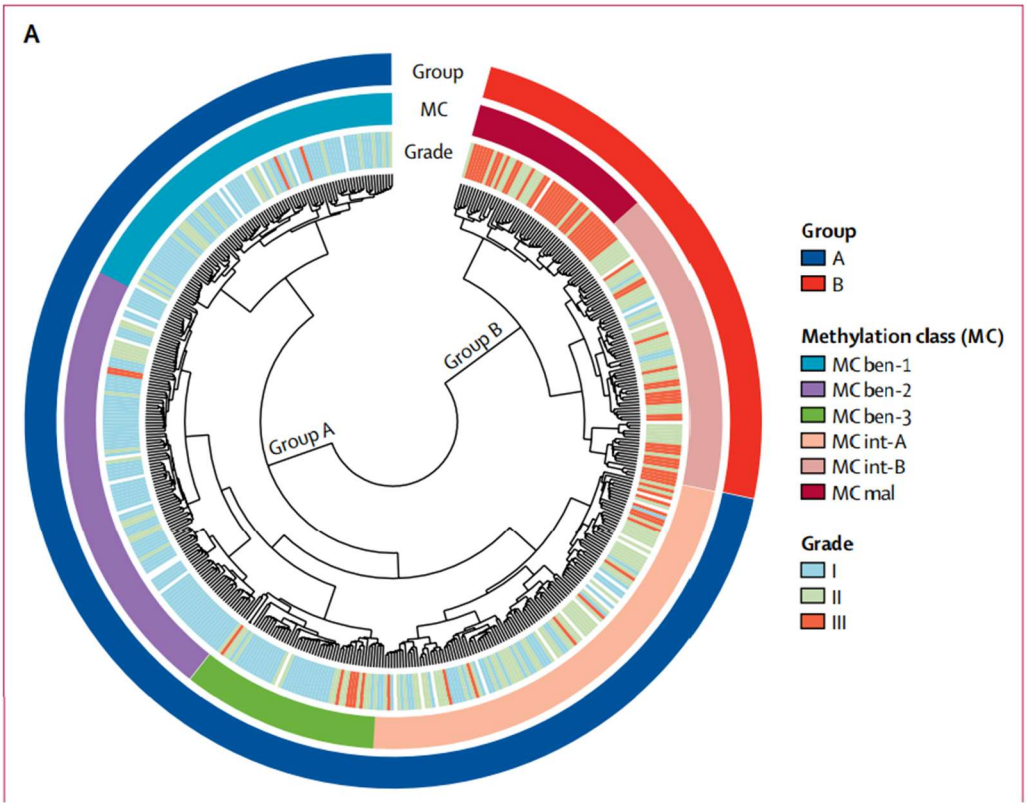

Figure 4: Sahm's methylation classes compared to WHO grading [16]

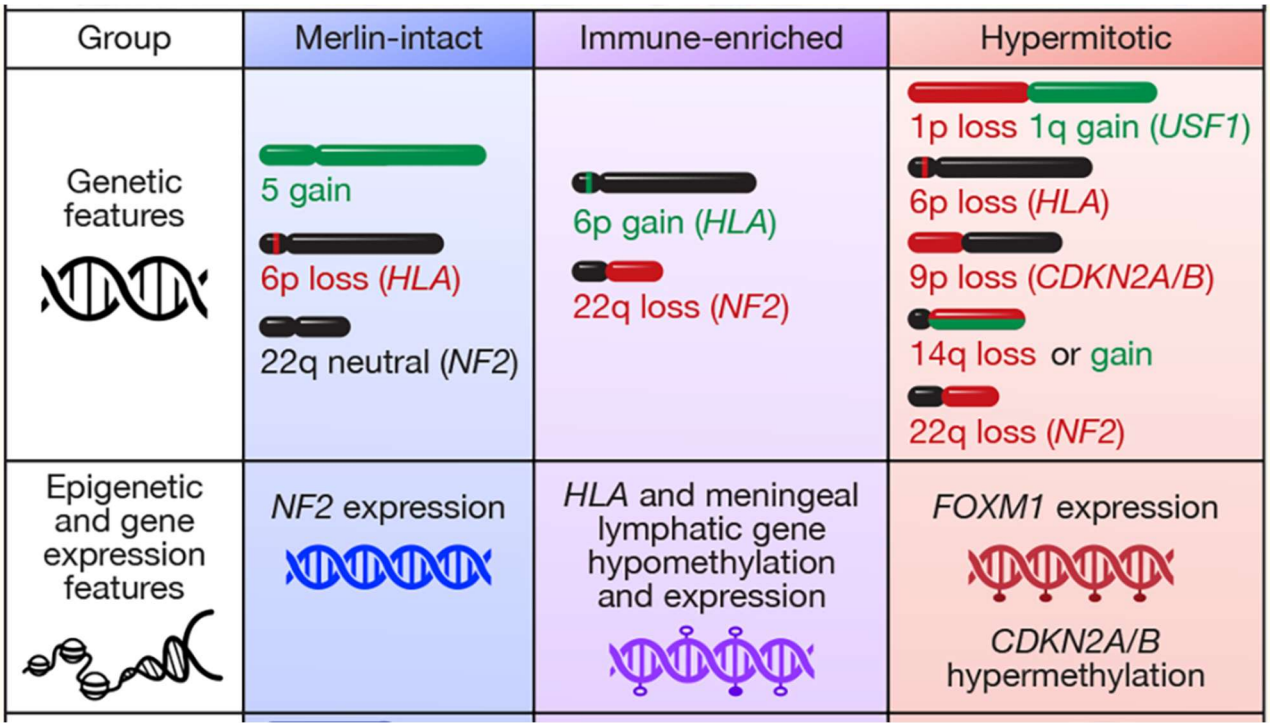

Figure 5: Choudhury's methylation classes [17]

Simpson Grade	Description
Grade 0	Complete tumor removal, plus removal of an additional 2–3 cm from the tumor insertion site
Grade I	Complete tumor removal, including any dural attachments or abnormal bone
Grade II	Complete tumor removal with coagulation of dural attachment
Grade III	Complete tumor removal without resection or coagulation of its dural attachment
Grade IV	Partial tumor removal
Grade V	Biopsy only

Figure 6: surgical extent of resection according to Simpson's grade [28]

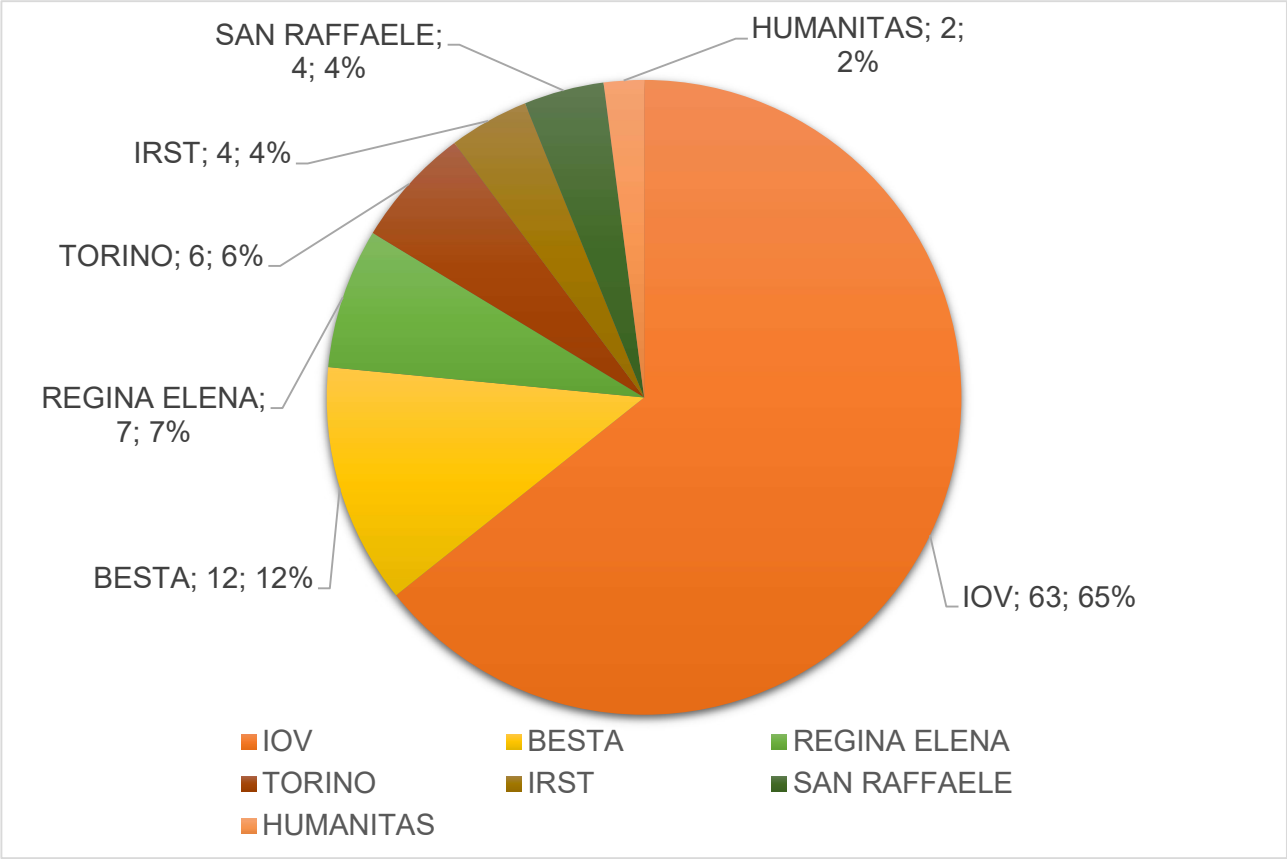

Figure 7: patients' distribution across italian neuro-oncology centres

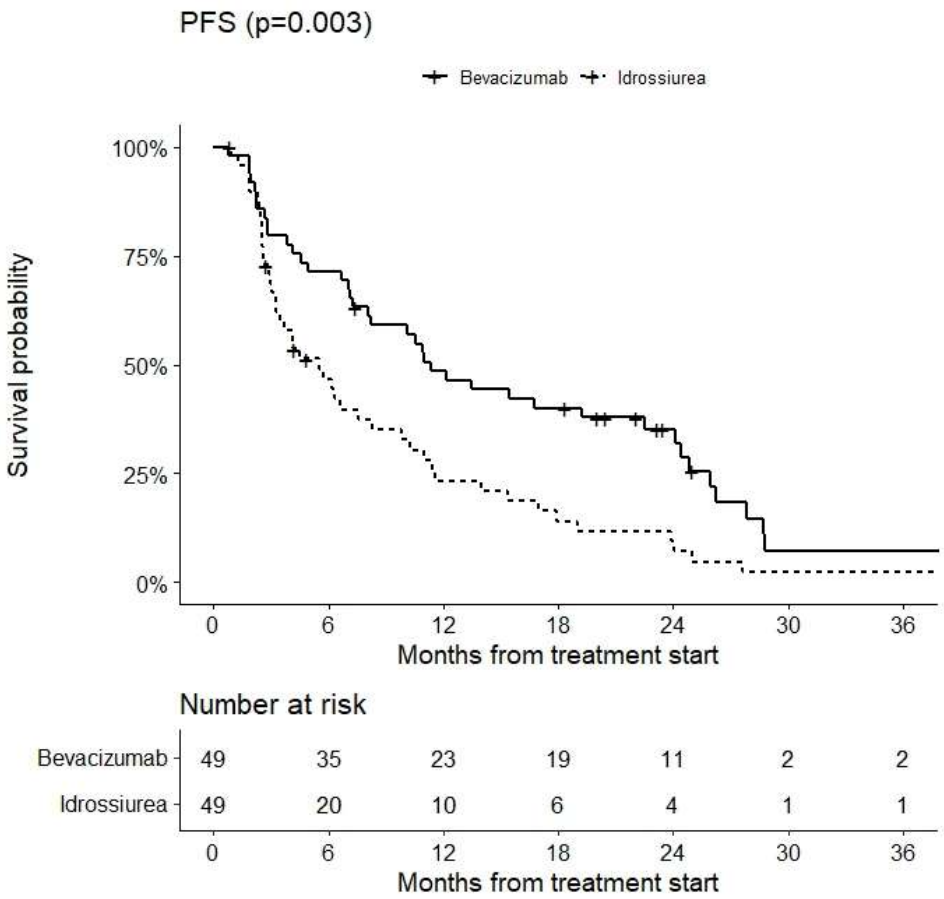

Figure 8: progression-free survival (PFS) in patients who were treated for meningioma with bevacizumab or hydroxyurea

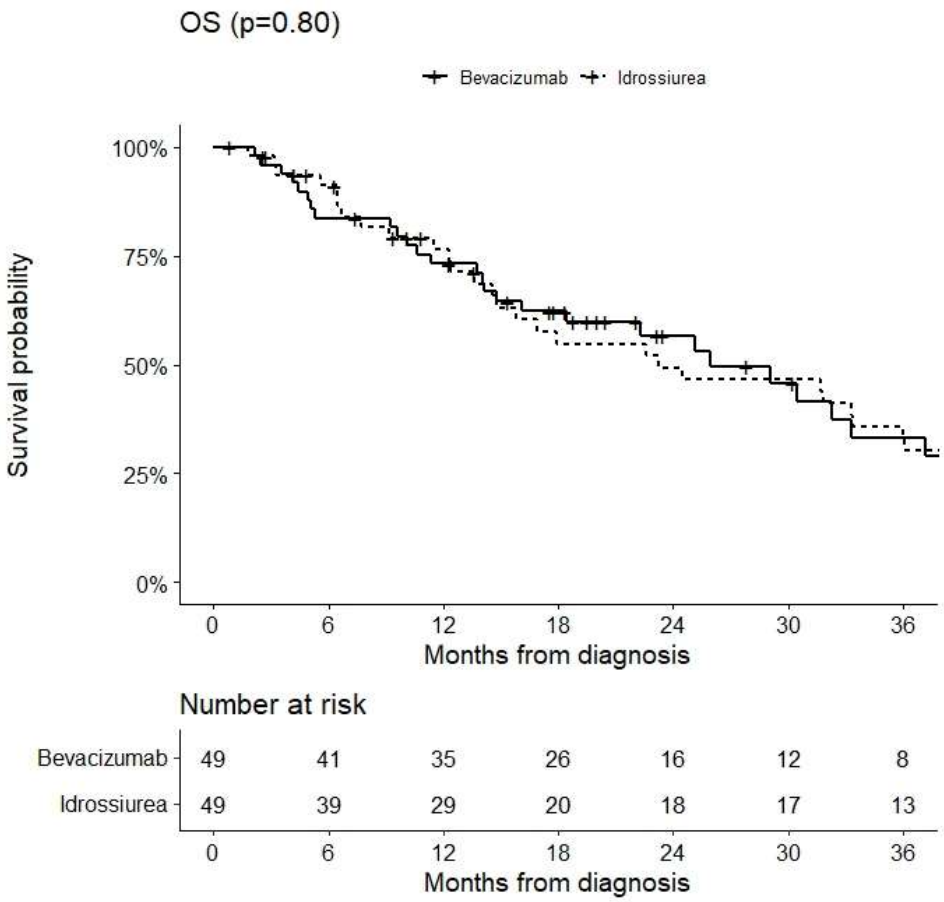

Figure 9: overall survival (OS) in patients who were treated for meningioma with bevacizumab or hydroxyurea

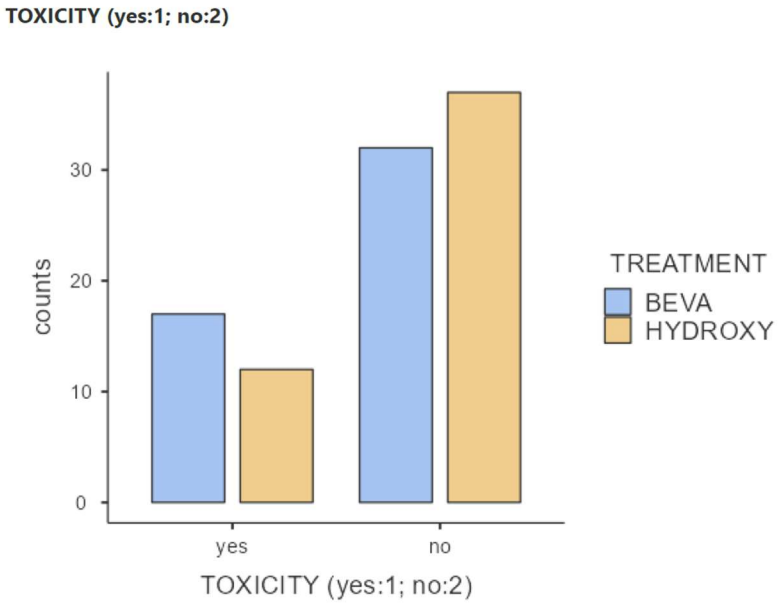

Figure 10: histograms for toxicity any grade, divided by treatment

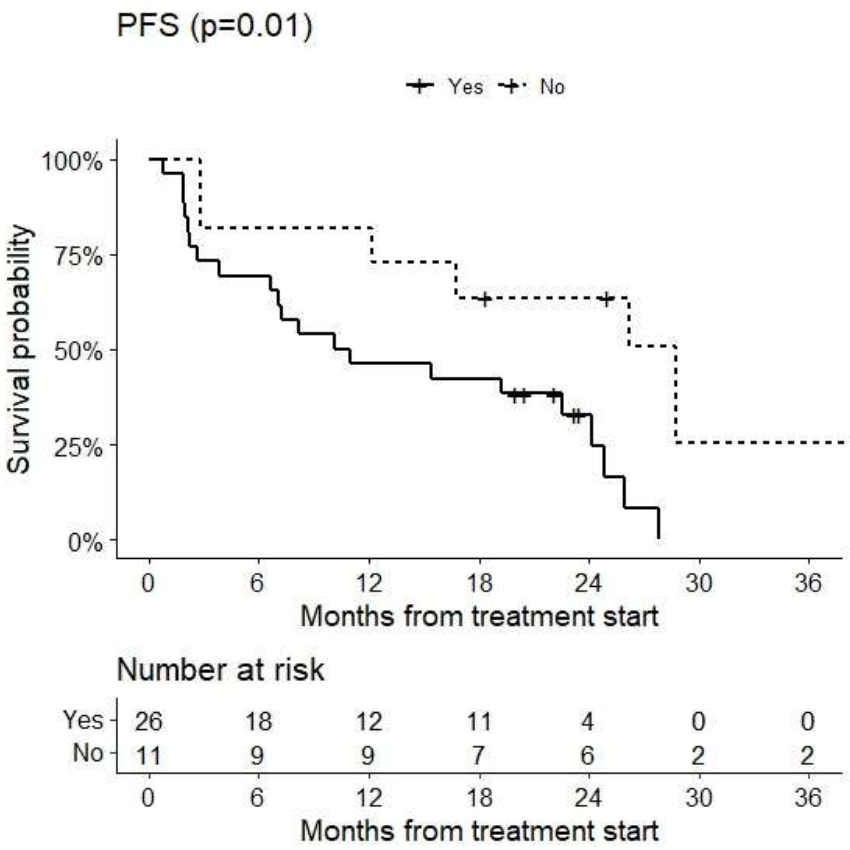

Figure 11: progression-free survival (PFS) in patients who were treated for meningioma with bevacizumab with/without NF2 alterations

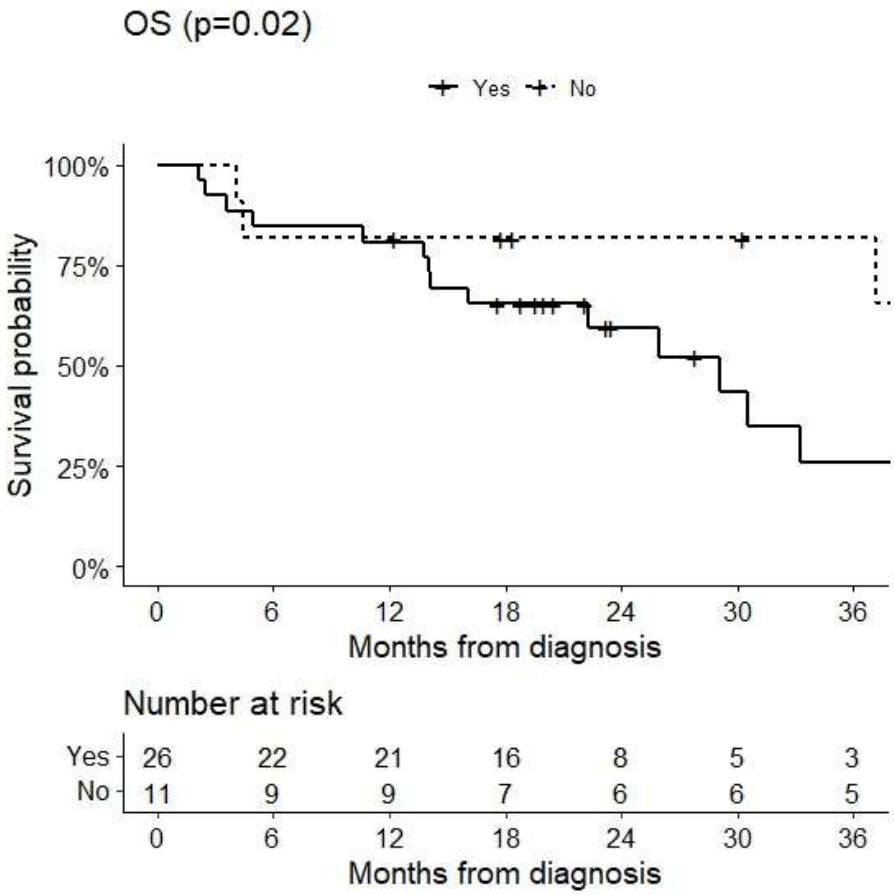

Figure 12: progression-free survival (PFS) in patients who were treated for meningioma with bevacizumab with/without NF2 alterations

9. Tables

Authors	Type of study	N° and type of patients	Previous treatments	Treatment	mPFS/response	mOS	Toxicity
Schrell UM et al, 1997 [40]	Retrospective	4 (3 grade 1, 1 grade 3)	Surgery, RT (except for 1)	Hydroxyurea 20 mg/Kg (1250-1500 mg/day) per day orally	3 PR (grade 1), 1 PD (grade 3) DCR 75%	not available	no toxicity reported
Newton HB et al., 2000 [41]	Prospective	17 unresectable/residual meningioma (unspecified grade)	unspecified	Hydroxyurea 20 mg/Kg (1250-1500 mg/day) per day orally	Not available 14/16 patients (88%) SD; no CR or PR. DCR 82,35%	not availabile	Grade ≥ 3-4, 6/17 (..) 9/17 (53%) required dose reductions of 250-500 mg/day
Mason WP, 2002 [42]	Retrospective	20 recurrent/unresectable meningiomas (16 benign, 3 atypical, 1 malignant)	8 patients had had previous RT	Hydroxyurea 20 mg/Kg per day orally	estimated 1- and 2-year freedom from progression rates of 0.93 (SE 0.07) and 0.77 (SE 0.12) respectively	not available	3 pts with grade 3 haematological toxicity
Rosenthal MA et al., 2002 [43]	Retrospective	15 recurrent/high-risk meningioma (10 benign, 5 atypical)	15 prior surgery, 1 prior RT	Hydroxyurea 20 mg/Kg per day orally	3 PD, 11 SD, 1 NE DCR 73,33%	not available	2 interrupted treatment for grade 2 and 3 rash 1 grade 3 haematological toxicity

Fuentes S et al., 2004 [44]	Retrospective	43 unresectable meningioma with confirmed PD; 20/43 available hystology (13 “benign”, 7 atypical)	4 patients had had previous RT	Hydroxyurea 20 mg/Kg per day orally	2 pts (4,6%) response; 15 (35%) SD; 26 (60.5%) PD DCR 39,5%	not available	12/43 had grade 1-2 haematological toxicity
Loven D et al., 2004 [45]	Phase II	12 patients, recurrent “non malignant” meningioma	surgery	Hydroxyurea 20 mg/Kg/day orally for a maximum of 24 months	9 PD, 1 PR, 2 NE DCR 8,33%	not available	2 patients had grade ≥ 3 haematological toxicity
Weston GJ et al., 2006 [46]	Case series	6 (5 grade 1) + 1 radiological diagnosis	surgery	Hydroxyurea 15 mg/Kg per day orally for a maximum of 12 months	1 PD, 2 SD, 3 NE DCR 33,33%	not available	1 interrupted treatment for myelosuppression
Chamberlain et al., 2011 [47]	Retrospective case series	60, recurrent grade 1	surgery (49% more than 1), RT (all)	Hydroxyurea 1000 mg/m2/day orally divided twice per day	0 CR, 0 PR, 21 SD mPFS 4 months (3-12) DCR 35% PFS-6 10% and PFS-12 0%	not available	6 (10%) had grade 3 toxicity (anemia and fatigue)

Chamberlain et al, 2012 [48]	Retrospective	35 (22 grade 2, 13 grade 3), recurrent	surgery (60% more than 1), RT (all)	Hydroxyurea 1000 mg/m^2/single daily dose orally	0 CR, 0 PR, 21 SD, 14 PD PFS-6 and PFS-12 was 3.0 and 0% DCR 60%	8 months (10 for grade 2, 6 for grade 3)	3 (8,5%) had grade 3 toxicity (anemia and fatigue)
Preusser et al., 2022 [23]	Phase II, randomized	90 (55 grade 2, 35 grade 3) – only 13 treated with hydroxyurea	Surgery and radiotherapy, no systemic treatment was allowed	Trabectedine vs local standard of care (LOC)	mPFS of 2.4 (95% CI, 1.4-4.2) months PFS-6 rate of 8.8% (95% CI, 0.5-32.3),	mOS of 7.4 (95% CI, 3.1-19.9) months, OS-6 rate of 55.9% (95% CI, 24.0-79.0).	Not available

Table 1: literature review of hydroxyurea treatment in recurrent meningioma

Authors	**Type of study**	**N° and type of patients**	**Previous treatments**	**Treatment**	**mPFS**	**mOS**	**Toxicity**
Nayak et al., 2012 [29]	Retrospective, multi-institutional	15 (6 grade 2, 9 grade 3)	Median of 3 previous surgeries, multiple radiation treatment; 7 had received previous CT	Bevacizumab (10 mg/Kg every 2 weeks)	mPFS 26 weeks (95% CI, 10-29 weeks) 6 months PFS rate 43,8% (95% CI, 15.7-69.1%)	mOS (after starting bevacizumab) 15 months (95% CI, 10-22 months)	4 patients developed grade I-II intratumoral haemorrhage

Kumthekar et al., 2022 [35]	Phase II, single-arm	50 patients (10 grade 1, 18 grade 2, 10 grade 3) + 4 vestibular schwannoma, 4 hemangioperycitoma	2 median surgeries, median 1 RT, median 4 chemotherapy	Bevacizumab 10 mg/Kg every 2 weeks for 6 months, then 15 mg/Kg every 3 weeks	PFS 6 months (93% grade 1, 85% grade 2, 51% grade 3) mPFS 22 months grad e1, 18 months grade 2, 8 months grade 3	mOS 35 months grade 1, 27 months grade 2, 12 months grade 3	nd
Hawasli AH et al., 2013 [49]	Retrospective	9 meningioma patients (6 affected by NF2)	5 with previous surgery, 5 with previous RT, 2 with previous pharmacological treatment	Bevacizumab (dosages ranged from 5 to 10 mg/kg every 2 to 3 weeks)	2 PR, 5 SD, 2 PD DCR 77,7%	Not available	No grade 3 or 4 toxicities were observed
Nunes FP et al., 2013 [50]	Retrospective	48 meningiomas in 16 patients (all affected by NF2)		Bevacizumab 5 mg/Kg q2w ev	14 PR, 29 PD mPFS 20 months DCR 29,16% PFS-6 93% PFS-12 79%	Not available	Not available
Franke AJ et al., 2018 [51]	Review	92 patients (11 articles) – 13 grade 1, 19 grade 2, 21 grade 3, 39 unknown grade	69 had had previous surgery, 51 had had previous RT, 22 had received	Bevacizumab 5 mg/Kg q2w (4 studies), 10 mg/Kg (3 studies), 1 study both regimens, 1 study 10 mg/Kg	mPFS 16.8 months (6.5-22 months) and PFS-6 73% (range: 44%-93%).	Not available	Toxicity grade 3 in 9.8%, grade 4 4.3%, grade 5 in 2.1%. No difference in toxicity between different dosages

			previous systemic treatment	q2w for 6 months, then 15 mg/Kg q3w			
Lou et al., 2012 [13]	Retrospective	14 (5 grade 1, 5 grade 2, 3 grade 3)	71% had 2 or more previous surgeries, 77% had had previous RT, 50% had had previous radiosurgery, 9 patients had had previous CT	Bevacizumab with/withou chemotherapy (4 single agent, 10 combination therapy)	mPFS 12.2 months grade 1 (95% CI, 1.1-27.2); 15.8 months grade 2/3 (95% CI, 5.5-17.9) PFS-6 80% (95% CI, 20.4-96.9) grade 1, 87,5% (38.7-98.1) grade 2/3	mOS not reached	Proteinuria grade ½ 6 cases, hypertension grade ½ 4 cases, haemorrhage grade 1 5 cases (1 case of cerebral haemorrhage), intestinal perforation grade 4 1 case
Preusser et al., 2022 [23]	Phase II, randomized	90 (55 grade 2, 35 grade 3) – only 9 treated with bevacizumab	Surgery and radiotherapy, no systemic treatment was allowed	Trabectedine vs local standard of care (LOC)	mPFS 6.0 (95% CI, 2.1-18.6) months PFS-6 rate of 44.4% (95% CI, 13.6-71.9) (95% CI, 43.3-98.4	median OS of 13.5 (95% CI, 5.42-not reached) months, and an OS-6 rate of 88.9%	Not available

Table 2: literature review of bevacizumab treatment in recurrent meningioma

Baseline characteristics	Patients treated with bevacizumab (n=49)	Patients treated with hydroxyurea (n=49)	p-value
Age at diagnosis, years	63 (54-72)	62 (52-71)	0.86
Age at diagnosis ≥ 65 years	23 (47%)	21 (43%)	0.84
Females	21 (43%)	28 (57%)	0.22
Histotype: *Grade 1* *Grade 2* *Grade 3*	 5 (10%) 23 (47%) 21 (43%)	 7 (14%) 30 (61%) 12 (25%)	0.16
Neurofibromatosis type 2	1 (2%)	2 (4%)	0.99
n° surgeries before systemic treatment	3 (2-4)	2 (1-2)	**0.01**
≤2 surgeries before systemic treatment	23 (47%)	37 (76%)	**0.007**
n° radiotherapy before systemic treatment	2 (1-2)	2 (1-2)	0.22
≤2 radiotherapy before systemic treatment	38 (78%)	41 (84%)	0.61
PS ECOG: *0* *1* *2* *3*	 12 (24%) 17 (35%) 20 (41%) 0 (0%)	 9 (19%) 30 (61%) 8 (16%) 2 (4%)	**0.008**
Toxicity	17 (35%)	12 (25%)	0.38
Therapy post progression	17 (35%)	34 (69%)	**0.001**
Crossover therapy	3 (6%)	5 (10%)	

Table 3: baseline characteristics of patients who were treated for meningioma with bevacizumab or hydroxyurea; data summarized as n (%) or median (IQR)

Response	Patients treated with bevacizumab (n=49)	Patients treated with hydroxyurea (n=49)	p-value
DCR (CR+PR+SD)	40 (82%)	28 (57%)	**0.02**
ORR (CR+PR)	4 (8%)	1 (2%)	0.36

Table 4: response to treatment

PFS% months	Bevacizumab	Hydroxyurea	p-value
6 months	35%	46%	**0.003**
12 months	48%	23%	
24 months	35%	9%	

Table 5: PFS rate at 6-12-24 months

OS% months	Bevacizumab	Hydroxyurea	p-value
6 months	83%	91%	0.80
12 months	73%	76%	
24 months	56%	49%	

Table 6: OS rate at 6-12-24 months

Post progression therapy:	Number of patients
alectinib, beva+everolimus	1
analogo	14
analogo + everolimus	1
analogo + TMZ	1
analogo, RT	1
beva+everolimus	8
bevacizumab	4
chirurgia, beva+everolimus	1
everolimus	3
hydroxyurea	1
hydroxyurea + analogo	2
hydroxyurea, analogo	1
hydroxyurea, beva+everolimus, chirurgia, RT, analogo	1
NCH	4
radiochirurgia	1
radiometabolica	1
radiometabolica, beva+everolimus	1
RT	2
TMZ	2
vismodegib + bevacizumab	1

Table 7: post progression therapies

Gene	Frequency (n° of cases)
NF2	26
ARID1A	17
LZTR1, FUBP1	10
ARID1B, PTEN	9 each
NFKBIA	8
TP53, MSH6, SUFU	7 each
SMARCB1, SETD2	6 each
DICER, CDKN2C, FBXWT	5 each
MSH2, MTAP loss, EPCAM, BAP1	4 each
SMARCA4, PIK3CA, ROS1, POLE, MLH1, TRAF7, NF1	3 each
NTRK2, ALK, CIC	2 each

Table 8: gene alterations present in at least 2 cases in patients treated with bevacizumab and its relative frequencies

Gene	Alteration	n° pts	PFS		OS	
			PFS% 6-12-24-36 months	**p-value**	**OS% 6-12-24-36 months**	**p-value**
NF2	Yes No	26 11	69-46-32-0% 81-81-63-25%	**0.01**	84-80-59-26% 81-81-81-81%	**0.02**
LZTR1	Yes No	10 27	60-40-0-0% 77-63-51-11%	0.06	80-80-60-0% 85-81-69-51%	0.20
ARID1A	Yes No	18 19	66-55-35-0% 78-57-47-13%	0.20	77-77-65-43% 89-84-67-45%	0.70
CDKN2A.2B	Yes No	13 24	61-46-30-0% 79-62-49-20%	0.09	76-76-53-32% 87-83-71-52%	0.20
FUBP1	Yes No	10 27	90-70-45-0% 67-51-40-11%	0.10	90-90-90-60% 81-77-57-39%	0.20

Table 9: Association between gene alterations and survival in patients treated with bevacizumab. The information was not available for 12 patients.

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