Treatment response of CNS high-grade neuroepithelial tumors with **MN1** alteration

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This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1002/pbc.28627](#).

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Funding: Meagan's Walk Foundation Fellowship in Pediatric Neuro-Oncology and The Terry Fox Foundation International Fellowship to LB. Canadian Institutes for Health Research, Garron Family Cancer Centre, Meagan's Walk and brain child to VR.

Conflict of interest: the authors declare no conflicts of interest.

Short Running Title: CNS high-grade neuroepithelial tumors MN1 altered

Number of Word: Abstract: 95. Main text: 1212

Number of tables: 2

Number of supplementary Figure: 1

<i>CNS HGNET-MN1</i>	CNS high-grade neuroepithelial tumor with MN1 alteration
CNS-PNET	Central nervous system primitive neuroectodermal tumor

PNET	<i>peripheral primitive neuroectodermal tumors</i>
GTR	Gross Total Resection
STR	Subtotal Resection
MRI	Magnetic resonance imaging

Abstract

CNS high-grade neuroepithelial tumor with MN1 alteration (CNS HGNET-MN1) is a rare recently described entity. Fourteen CNS HGNET-MN1 patients were identified using genome wide methylation arrays/RT-PCR across 7 institutions. All patients had surgery (GTR:10/STR:4) as initial management followed by observation alone in 3 patients, followed by radiotherapy in 8 patients (focal:5/craniospinal:2/CyberKnife:1) and systemic chemotherapy in 3 patients. Seven patients relapsed; 5 local and 2 metastatic, despite adjuvant radiotherapy, of which three died. Treatment of CNS HGNET-MN1 remains a major treatment challenge despite aggressive surgical resections and upfront radiotherapy, warranting new approaches to this rare malignancy.

Keywords: CNS HGNET-MN1, MN1, Astroblastoma, Ependymoma, neuroepithelial tumor

Introduction

High-grade neuroepithelial tumor with MN1 alteration (CNS HGNET-MN1) is a rare entity recently described as a high-grade tumor containing a mixture of solid and pseudopapillary

patterns *with* a distinct methylation profile, which were formally classified as a heterogeneous group of tumors. This entity was described through a large scale study of genome wide methylation analysis of pediatric brain tumors, including those previously reported as central nervous system primitive neuroectodermal tumor (CNS-PNET), and reported as a new distinct entity representing a diverse morphological spectrum ⁽¹⁾. Specifically, many of the tumors clustering with the CNS-HGNET-MN1 group were initially described morphologically as either astroblastoma or ependymoma within the current WHO classification ^(2, 3). This new group is specifically associated with gene fusions involving *MN1* (22q12.3), often paired with *BEND2* (Xp22.13), but also the alternative partner *CXXC5* (5q31.2), and constituted most cases diagnosed as astroblastoma in the pre molecular era ⁽⁴⁾.

CNS HGNET-MN1 *is a* poorly defined entity with an indeterminant biological and clinical behavior rendering it very difficult to ascribe treatment recommendations. Although there have been only individual cases or small series ⁽⁵⁻⁸⁾, there is a paucity of data to propose a standard therapy, primarily due to the rarity of the condition resulting in any individual center having a limited clinical experience. Herein, we describe our experience *with the radiologic features, treatment and outcome of an international cohort of CNS HGNET-MN1 tumors.*

Methods

CNS HGNET-MN1 patients were identified across 7 international institutions (the Hospital JP Garrahan, Hospital for Sick Children, the University Hospital Motol, Royal Children's Hospital Melbourne, Hospital Sant Joan de Deu, Christchurch Hospital and University Medical Center, Hamburg-Eppendorf) and were correlated with treatment and outcome. Patients at these centres were identified as part of pathological workup's for a variety of

entities where application of existing molecular methods did not yield a diagnosis. Samples were identified using the Heidelberg brain tumor classifier (<https://www.molecularneuropathology.org/mnp>) and tumors were considered CNS HGNET-MN1 only if the calibrated score was over 0.9. Two patients were diagnosed by RT-PCR where a MN1ex1-BEND2ex7 transcription fusion detected. All data collection was performed in accordance to local institutional review board approval and guidelines. Central imaging review with radiological features analysis was performed in Hospital JP Garrahan.

Results

Cohort Demographics

A total of 14 patients harboring CNS HGNET-MN1 tumors were identified across 7 institutions. Eleven tumors were supratentorial, either in the parietal (6), parieto-occipital (2), frontal (1), fronto-parietal (2) locations, and three in the cervico-dorsal spinal cord. Four of 10 supratentorial lesions were initially diagnosed morphologically as anaplastic ependymoma. Others diagnoses included peripheral primitive neuroectodermal tumors (PNET) (1), embryonal tumor NOS (1), atypical glial neoplasia (2), malignant tumor with rhabdoid characteristics (1) and astroblastoma (2). All three spinal tumors were morphologically consistent with ependymoma. Median age was 7.5 (range 3.6-36) with a clear female predominance (12 female: 2 male). Thirteen of 14 cases were non-metastatic at diagnosis, with one patient harboring metastatic deposits in the dorsal spinal cord.

Demographics of the cohort are summarized in table 1.

Magnetic resonance imaging (MRI) findings

MR imaging at diagnosis was available in 9/14 cases; 7 supratentorial and 2 in the spinal cord. Supratentorial tumors revealed a well demarcated tumor with solid and micro-cystic components in all patients and little to no edema in 4 supratentorial cases. The lesions were predominantly iso/hypointense on T1-weighted and iso/hyperintense on T2-weighted images. Post-gadolinium imaging showed walled cystic enhancement in 5 supratentorial lesions and a homogeneous solid component enhancement in all supratentorial tumors. Bone remodeling was seen in 3 supratentorial cases.

The 2 patients who had spinal cord lesions were iso/hypointense on T1-weighted and hyperintense on T2 weighted images with homogeneous solid component enhancement. Both cases had severe cord edema (Table 2 and Supplemental Figure 1).

Treatment and outcome

All patients had surgery, 10 Gross total resection (GTR) and 4 Subtotal resection (STR), as initial management followed by radiotherapy (5 focal/2 craniospinal/1 focal radiosurgery) and systemic chemotherapy in 3 patients. The single patient with upfront metastatic dissemination received craniospinal radiation after partial resection as initial management. Five of the 14 patients relapsed within 3 years post diagnosis; 3 local and 2 metastatic failures despite initial surgical resections (GTR:2/STR:3) followed by radiotherapy in 3 of them. Two patients presented a very late relapse after 5 years from diagnosis. Three patients died due to tumor relapse after 24 months despite upfront radiotherapy.

Discussion

Our study adds to the emerging spectrum of children with CNS HGNET-MN1 tumors, in particular the relationship of initial treatment and response. This study provides an additional and important experience with this distinctive entity where the optimal management is still unknown.

Although previous observations hypothesized that MN1 rearranged tumors could be associated with a favorable prognosis and indolent course, our experience suggests a more aggressive clinical course ^(6, 9). The majority of CNS-HGNET-MN1 tumors were initially classified as astroblastoma or ependymoma, however some were even diagnosed as PNET, suggesting initial therapies were highly heterogeneous. This also suggests that supratentorial tumors that do not harbor rearrangements on chromosome 11, a hallmark of supratentorial ependymoma, all morphologically diagnosed astroblastoma's, and CNS embryonal tumors require additional work-up including methylation profiling irrespective of initial morphological diagnosis. Recently, a multi-institutional analysis revealed a favorable outcome with 88.0% overall survival, although 48.0% presented with tumor progression ⁽¹⁰⁾. However, those results should be interpreted with caution due to paucity of data pertaining to the adjuvant treatment and extent of surgical resection. Specifically, our study provides therapy details at diagnosis and relapse, suggesting that complete surgery and upfront focal radiotherapy is inadequate in a substantial subset of patients with CNS-HGNET-MN1 tumors.

Although astroblastoma was included as an initial histopathological diagnosis, ependymoma was the most frequent pathological diagnosis we observed. Indeed, a recent study shows that the diagnosis of supratentorial ependymoma by morphology alone is fraught with challenges where upwards of forty percent did not match to the epigenetic profile including a subset which were in fact HGNET with MN1 alteration ⁽¹¹⁾. Methylation profiling was consistent with a CNS-HGNET-MN1 in 12 of our patients, however routine clinical application of genome wide methylation profiling is challenging, and considering the prevalence of this entity, methods to diagnosed CNS-HGNET-MN1 tumors routinely in CLIA certified settings are required. RT-PCR using primers designed against *MN1* and *BEND2* and FISH using probes directed against *MN1* and *BEND2*, detecting fusion signals, are two new promising techniques options that can be considered ⁽⁴⁾.

Although our study has several limitations inherent to its retrospective observational design, the prognosis is not consistent with a "lower grade" tumor. Moreover, our study is likely an underrepresentation of cases, and it is anticipated additional cases will be identified with the routine application of genome wide methylation profiling and next generation sequencing.

Further *multicenter and international prospective studies are required to determine the optimal treatment strategy for this group of tumors. Genome wide DNA-methylation* profiling represent an essential instrument for diagnosis in the molecular era and should be included in treatment stratification to rule out the major differential diagnosis, in particular in future prospective studies. The rarity of this entity will make prospective interventional trials challenging, and registry based approaches may be of benefit as more cases emerge with additional screening. Future studies to address the clinical behavior of this tumor entity will

require global cooperation and widespread application of genome-wide methylation profiling, with the ultimate goal to identify appropriate treatment approaches and regimens for this novel group.

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Supplemental Figure 1: Magnetic resonance images of a supratentorial HGNET-MN1 tumor at diagnosis in (A) T1-weighted axial image, (B) T2-weighted axial image, (C) FLAIR axial image, and (D) Gadolinium enhancement T1-weighted axial image. There is a cortico-subcortical parietal expansile lesion that remodels the inner table of the skull. The process is predominantly isointense on T1 and T2-weighted images, contains a small cystic area, it markedly enhances with gadolinium and it is surrounded by vasogenic edema.

Magnetic resonance image of a cervico-dorsal HGNET-MN1 tumor at diagnosis in (F) T2-weighted sagittal image, (G) Gadolinium enhancement T1-weighted sagittal image and (H) Dixon technique sagittal images. At C5-T4 level there is an expansile spinal cord lesion with small cystic areas and predominantly iso-hyperintense signal intensity on T2. The tumor has a small hypointense spot on T2-weighted image at its cranial pole compatible with hemosiderin. It avidly enhances with gadolinium and it is associated with supra and infrasegmental vasogenic edema.

ID	A (years), G	Location	Surgery ₁	RT ₁ , dose (Gy)	Chemo ₁	EFS ₁ (months)	Relapse location	Surgery ₂	RT ₂ , dose (Gy)	Chemo ₂	OS (months)	Status
1	4.8, F	ST	GTR	Focal, 59.4	None	11	None	None	None	None	11	Alive
2	5.8, F	ST	GTR	None	None	24	None	None	None	None	24	Alive
3	8.9, F	ST	GTR	Focal, 50	VAC/VAdriaC	115	None	None	None	None	115	Alive
4	5, F	Spine	STR	None	None	15	Local	STR	Focal, 45	None	18	Alive
5	4.5, F	ST	GTR	Focal, 54	None	26.4	Local	GTR	None	CP/celecoxib	35.4	Dead
6	7, F	ST	GTR	Focal, 59.4	None	36	Metastatic	GTR	CSI, 36+ boost	None	72	Dead
7	3.6, F	ST	STR	None	None	6	Local	GTR	Focal, 54	VCR/VP/CP	197	Alive
8	6.7, F	ST with spinal metastasis	GTR	CSI, 36+ boost	SJMB12	25	None	None	None	None	25	Alive

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9	14.6, M	Spine	STR	Focal, 45	None	23	Metastatic	None	CSI, 36+boost	Oral VP	88	Dead
10	13, F	ST	GTR	None	None	24	None	None	None	None	24	Alive
11	36, M	Spine	STR	CYKE	None	60	Local	STR	None	None	132	Alive
12	10, F	ST	GTR	None	None	100	None	None	None	None	100	Alive
13	8, F	ST	GTR	CSI, 23.5 + boost	SJMB-96	63	Local	GTR	Focal, 50.4	Metronomic (5D)	90	Alive
14	14, F	ST	GTR	None	None	64	None	None	None	None	64	Alive

TABLE 1: Demographic characteristics - High grade neuroepithelial tumor with MN1 alteration

Abbreviation: A: age, G: gender, F: female, M: male, ST: supratentorial, GTR: gross total resection, PR: partial resection, STR: subtotal resection, RT₁: Radiation at diagnosis, RT₂: radiation at recurrence, Chemo₁: chemotherapy at diagnosis, Chemo₂: chemotherapy at relapse, surgery₁: surgery at diagnosis, Surgery₂: surgery at relapse, CSI: craniospinal irradiation, EFS: event free survival; OS: overall survival, SJMB12: St. Jude Medulloblastoma Regimen, SJMB-96: St. Jude Medulloblastoma-96 Regimen, VAC: vincristine-actinomycin D-cyclophosphamide, VAdriaC: Vincristine-Adriamycin-Cyclophosphamide, VCR: Vincristine, VP: Etoposide, CP: Cyclophosphamide, CYKE: Cyber Knife, 5D: five Drugs

TABLE 2: Radiological characteristic of High grade neuroepithelial tumor with MN1 alteration

Location	Solid/cystic component	T1	T2	Enhancement (solid component)	Enhancement (cystic component wall)	Edema percentage (%)	Dissemination	Bone remodeling
Right parietal	intracystic	Iso	Hypo	Homogeneous*	yes	<25	No	Yes
Left parietal	Micro/intracystic	Iso	Iso	Homogeneous*	no	≥25 - ≤50	No	Yes
Left parietal	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Cervico-dorsal spine	Polar micro cystic	Hypo	Hyper	Homogeneous*	yes	> 50	No	Yes
Right frontal	Solid with micro cystic	Iso	Iso	Homogeneous*	no	None	No	No
Left parieto- occipital	Solid with micro cystic	N/A	Iso	Homogeneous*	yes	<25	No	No
Left parieto- occipital	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Right parietal	Solid with micro cystic	N/A	Iso	Homogeneous*	yes	<25	Yes	Yes
Cervico-dorsal spine	solid	Iso	Iso/hyper	Homogeneous*	N/A	> 50	No	No
Right fronto- parietal	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Spine	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Left fronto-	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

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parietal								
Left parietal	Solid/intracystic	Iso/Hypo	Iso	Homogeneous*	yes	≥25 - ≤50	No	No
Right Parietal	Solid/micro and macro cystic	Hypo	Iso/Hyper	Homogeneous*	yes	> 50	No	No

Abbreviation: Hypo: Hypointense; Iso: isointense; Hyper: Hyperintense; #: cystic component is defined as ≥2 cystic lesion in the tumor area; *: enhancement more than 50%; N/A: not available.

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Title:

TREATMENT RESPONSE OF CNS HIGH-GRADE NEUROEPITHELIAL TUMORS WITH MN1 ALTERATION

Date:

2020-12-01

Citation:

Baroni, L. V., Rugilo, C., Lubieniecki, F., Sampor, C., Freytes, C., Nobre, L., Hansford, J. R., Malalasekera, V. S., Zapotocky, M., Dodgshun, A., Ramaswamy, V. & Alderete, D. (2020). TREATMENT RESPONSE OF CNS HIGH-GRADE NEUROEPITHELIAL TUMORS WITH MN1 ALTERATION. NEURO-ONCOLOGY, 22 (12), pp.453-453. <https://doi.org/10.1002/pbc.28627>.

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