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Pediatric high-grade diffuse midline gliomas in Neurofibromatosis Type 1 in comparison with non-syndromic subjects: a single center experience

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Abstract:	Background Neurofibromatosis type 1 (NF1) pediatric patients can rarely develop aggressive central nervous system tumors. Among high-grade gliomas, diffuse midline gliomas (DMG) have exceptionally been reported. The aim of this retrospective single center study was to evaluate clinical and imaging features of high-grade diffuse midline gliomas (DMG) in pediatric NF1 patients and to compare their clinical behavior with high-grade DMG in non-syndromic patients.Methods We conducted a retrospective review of all pediatric patients with cerebral high-grade DMG H3K27M-mutant or wild-type with NF1 or sporadic followed at our Institution between 2010 and 2018. Progression free survival (PFS) and overall survival (OS) were evaluated.Results Two patients were identified with clinical and genetic diagnosis of NF1 and high-grade DMG (one DMG H3K27M-mutant and one wild-type). During the same time period, 16 non-syndromic patients with high-grade DMG (11 subjects with DMG-H3K27M-mutant and 5 with DMG-H3K27M wild-type) were diagnosed and treated at our Institution. The two pediatric patients with NF1 and high-grade DMG presented a PFS of 3 months and an OS of 5 and 7 months. Median PFS and OS of children without NF1 were respectively 6 and 9 months in DMG H3K27M-mutant, and 6 and 11 months in DMG H3K27M wild-type. Seventy-five percent of subjects with sporadic high-grade DMG presented a PFS greater than 4 months compared to 0% in NF1 patients. The eight months overall survival of patients with sporadic high-grade DMG was 78% compared to 0% in NF1 patients.Conclusions High-grade DMG can rarely occur in pediatric patients with NF1 and may present an extremely poor prognosis, even worse than sporadic high-grade DMG, independently of the presence or absence of H3K27M mutation. In pediatric NF1 patients, lesions in evocative regions and with features of increased biological activity on advanced magnetic resonance or molecular amino-acid positron emission tomography imaging may alert clinicians, suggesting prompt neuropathological and molecular investigations.
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Title page

Pediatric high-grade diffuse midline gliomas in Neurofibromatosis Type 1 in comparison with non-syndromic subjects: a single center experience

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Abstract

Background

Neurofibromatosis type 1 (NF1) pediatric patients can rarely develop aggressive central nervous system tumors. Among high-grade gliomas, diffuse midline gliomas (DMG) have exceptionally been reported. The aim of this retrospective single center study was to evaluate clinical and imaging features of high-grade diffuse midline gliomas (DMG) in pediatric NF1 patients and to compare their clinical behavior with high-grade DMG in non-syndromic patients.

Methods

We conducted a retrospective review of all pediatric patients with cerebral high-grade DMG H3K27M-mutant or wild-type with NF1 or sporadic followed at our Institution between 2010 and 2018. Progression free survival (PFS) and overall survival (OS) were evaluated.

Results

Two patients were identified with clinical and genetic diagnosis of NF1 and high-grade DMG (one DMG H3K27M-mutant and one wild-type). During the same time period, 16 non-syndromic patients with high-grade DMG (11 subjects with DMG-H3K27M-mutant and 5 with DMG-H3K27M wild-type) were diagnosed and treated at our Institution. The two pediatric patients with NF1 and high-grade DMG presented a PFS of 3 months and an OS of 5 and 7 months. Median PFS and OS of children without NF1 were respectively 6 and 9 months in DMG H3K27M-mutant, and 6 and 11 months in DMG H3K27M wild-type. Seventy-five percent of subjects with sporadic high-grade DMG presented a PFS greater than 4 months compared to 0% in NF1 patients. The eight months overall survival of patients with sporadic high-grade DMG was 78% compared to 0% in NF1 patients.

Conclusions

High-grade DMG can rarely occur in pediatric patients with NF1 and may present an extremely poor prognosis, even worse than sporadic high-grade DMG, independently of the presence or absence of H3K27M mutation. In pediatric NF1 patients, lesions in evocative regions and with

features of increased biological activity on advanced magnetic resonance or molecular amino-acid positron emission tomography imaging may alert clinicians, suggesting prompt neuropathological and molecular investigations.

Key words: H3K27M; Pediatric; Brain Tumor; Diffuse Midline Glioma; NF1

Background

Neurofibromatosis type 1 (NF1) is the most common tumor predisposition syndrome caused by germline mutations in the NF1 gene which encodes for the protein Neurofibromin, a GTPase activating protein that negatively regulates signals transduced by RAS oncoproteins [1,2]. Dysregulation of RAS is predicted to contribute to increased cell proliferation and tumorigenesis. Indeed, patients with NF1 have an increased risk of developing both benign and malignant central and peripheral nervous system tumors [3-5]. The most common central nervous system (CNS) tumor in NF1 is optic pathway glioma, which occurs in approximately 15-20% of pediatric patients [4,6]. These tumors are often asymptomatic, very slowly progressive and only rarely require specific treatment [4,5], with possible spontaneous regression [7]. Much rarely, pediatric patients with NF1 may develop more aggressive central nervous system tumors, including high-grade gliomas (HGG) [8]. Of note, the overall survival in NF1 patients with HGG has been reported to be higher than their sporadic counterparts [9].

In the revised 2016 World Health Organization (WHO) classification of tumors of the central nervous system, the diffuse midline glioma (DMG) H3K27M-mutant has been introduced as a completely new entity. Diffuse midline gliomas H3K27M-mutant grow in all midline central nervous system compartments with the most common locations being the brain stem, thalamus, and spinal cord. The detection of H3K27M mutation in infiltrating midline gliomas determines an assignment to WHO grade IV [10].

The objective of this retrospective single center study was to report and compare the clinical behavior of cerebral high-grade DMG H3K27M-mutant or wild-type in pediatric patients with NF1 versus non-syndromic subjects, focusing on imaging and clinical features of NF1 high-grade DMG.

Methods

After approval from the Institutional Review Board (Regional Ethics committee of Liguria, Genoa, Italy), we performed a retrospective review of the electronic database of our Institution to identify

potential patients admitted between 2010 and 2018 using the key terms “NF1” or “neurofibromatosis type 1” and “high-grade DMG”. Patients were included for analysis only if they met pathologic and neuroimaging criteria for cerebral high-grade DMG, if they underwent molecular analysis for H3K27M, if they met the clinical criteria for NF1, established by the National Institutes of Health, and genetical NF1 analysis. An additional search was performed to identify sporadic patients with cerebral high-grade DMG H3K27M-mutant or wild-type who received definitive treatment at our Institution during the same time period.

Patients’ age, diagnosis, clinical course, treatment plan, and follow-up were reviewed. In particular, progression free survival (PFS) and overall survival (OS) (defined as the interval between initial diagnosis and the onset of disease progression and of death from any cause, respectively) were obtained.

Differences in PFS and OS between non-NF1 patients with and without H3K27M mutation were evaluated by the Kaplan-Meier method and compared across groups by the log-rank test. Statistical analysis was performed by using SPSS Statistics for Mac, version 21.0 (IBM, Armonk, NY). A p value of 0.05 was used to define nominal statistical significance.

Results

Two pediatric patients were identified who met criteria for NF1 and had high-grade DMG (1 ponto-mesencephalic glioblastoma H3K27M-mutant and 1 thalamic anaplastic astrocytoma H3K27M-wildtype). During the same time period, 16 sporadic patients with cerebral high-grade DMG were identified (9 glioblastomas with H3K27M mutation in 6 subjects and 7 anaplastic astrocytomas with H3K27M mutation in 5 subjects), treated at our Institution and with survival data available for comparison. All subjects with DMG H3K27M-mutant presented mutations in the histone variant H3.3 (H3F3A). Location, neuropathological and clinical features (treatments and outcome) of sporadic and NF1 patients are reported in Table 1.

The two pediatric patients with NF1 and high-grade DMG presented a PFS of 3 months and an OS

of 5 and 7 months. Median PFS and OS of high-grade DMG in non NF1 patients were respectively 6 and 10 months (PFS range 4-10 months, OS range 6-18 months). In detail, median PFS and OS in non NF1 high-grade DMG were 6 and 9 months in subjects with H3K27M-mutant (PFS range 4-10 months, OS range 6-18 months), and 6 and 11 months in H3K27M-wildtype lesions (PFS range 4-9 months, OS range 7-14 months).

Leptomeningeal dissemination was diagnosed during follow-up in one NF1 high-grade DMG H3K27M-mutant. Among sporadic high-grade DMG, it was revealed in 3 out of 11 subjects with H3K27M-mutant and in 1 out of 5 H3K27M-wildtype lesions. In all subjects, leptomeningeal dissemination was better recognizable and much more prominent in the spinal region; none of the patients presented leptomeningeal dissemination at admission.

No statistically significant differences in terms of PFS and OS emerged between non-NF1 subjects with high-grade DMG H3K27M-mutant or wild-type ($\chi2(2) = 0.114$, $p < 0.736$ and $\chi2(2) = 0.000003$, $p < 0.989$, respectively) (Figure 1). While the small number of patients with NF1 precludes formal statistical analysis, 75% of subjects with sporadic high-grade DMG presented a PFS greater than 4 months compared to 0% in NF1 patients. The eight months overall survival of patients with sporadic high-grade DMG was 78% compared to 0% in NF1 patients. A brief description of each of the two NF1 cases follows.

Case 1

An 11-year-old female presented a few days history of headache, vomiting, difficult writing, dysphagia, dysarthria and right-sided hemiparesis. NF1 had already been diagnosed on a clinical basis and through the identification of the de novo c.6792C>A variant in the neurofibromin gene, determining the substitution of a tyrosine with a stop codon (p.Tyr2264*) resulting in a protein lacking 34 amino acids [11].

Magnetic resonance imaging (MRI) at admission showed in addition to typical unidentified bright objects (UBOs) located in the deep cerebellar white matter and basal ganglia, a mass lesion with an

irregular central necrotic area in the left ponto-mesencephalic region; an additional adjacent expansile lesion without contrast enhancement nor necrotic areas was found in the medulla (Figure 2). The patient underwent biopsy of the ponto-mesencephalic lesion and neuropathology demonstrated a diffuse midline glioma H3K27M-mutant (glioblastoma). She was started with focal radiotherapy in association with medical treatment with vinorelbine and nimotuzumab.

Follow-up MRI performed 3 months later, following first line treatment, revealed brain and spine leptomeningeal dissemination, not present at diagnosis, in keeping with progressive disease (Figure 2). Primary lesion demonstrated an increased extension of the necrotic area with perilesional edema, suggestive of radiation induced changes. Clinically, the patient presented a global deterioration of the neurological status and she underwent a cerebrospinal fluid diversion due to symptomatic hydrocephalus. Subsequently, she was started with a second line chemotherapy course with etoposide and temozolomide.

She was re-evaluated after the first two cycles (5 months since diagnosis) and brain and spinal imaging demonstrated further increase of the degree of secondary dissemination; the primary ponto-mesencephalic lesion presented decreased volume of the necrotic component, supporting the diagnosis of radiation induced changes, and no evidence of local progression.

Clinical conditions worsened and a subsequent brain MRI performed 7 months since diagnosis demonstrated a massive brain and spine leptomeningeal dissemination with diffuse brain edema (Figure 2). The patient died few days later.

Case 2

A 13-year-old female presented with a recent history of headache, episodes of vomiting and left-sided hemiparesis. Clinical examination revealed the presence of multiple café-au-lait macules and axillary and inguinal freckling. She was found to carry the c.5705C>A variant which determines an amino acid substitution threonine with a lysine (p.Thr1902Lys). This variant was transmitted by her affected mother. Brain and spine MRI at admission showed in addition to typical UBOs in the deep

cerebellar white matter, dorsal pons and in the globus pallidus bilaterally, an expansile and infiltrating lesion with epicenter in the right thalamus extending to the contralateral thalamus characterized by irregular contrast-enhancement (Figure 3).

Advanced MRI modalities, such as diffusion weighted imaging (DWI), magnetic resonance spectroscopy (MRS) and perfusion weighted imaging with arterial spin labeling (ASL) technique, demonstrated an aggressive pattern (Figure 3). There was no evidence of leptomeningeal dissemination. The patient also underwent cerebral 18F-dihydroxyphenylalanine (DOPA) PET imaging demonstrating markedly increased uptake of the lesion (Figure 3). She underwent a stereotaxic biopsy and neuropathology demonstrated a midline anaplastic astrocytoma, H3K27M-wild-type. She underwent treatment with radiotherapy and chemotherapy with temozolomide and vinblastine.

Follow-up MRI performed 3 months later, following first line treatment, documented a significant disease progression without leptomeningeal dissemination (Figure 3). Neurological status deteriorated rapidly and the patient died 5 months after diagnosis.

Discussion

Pediatric HGG are a relatively rare group of CNS neoplasms with an aggressive behaviour and poor prognosis [12,13]. About 50% of pediatric cerebral HGG arise in midline structures such as the brain stem and in particular the pons, as diffuse intrinsic pontine gliomas (DIPG), the thalamus and rarely the cerebellum [12]. In the revised 2016 WHO classification of tumors of the central nervous system, and in the updated recommendations of the cIMPACT-NOW (the Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy) Working Committee 3, the DMG H3K27M-mutant, which should only include infiltrating midline gliomas, has been recognized as a new diagnostic entity [10,14].

H3K27M mutation results in substitution of the amino acid lysine to methionine at residue 27, inducing unique gain-of-function mechanisms that lead to global reduction H3 with trimethylated

lysine 27 (H3K27me3). Even though the precise role of H3K27M mutation in tumor initiation remains not clearly defined, functional analysis has highlighted the role of H3K27M as contributing to inhibition of autophagy, abnormal cell-cycle control, and potentially increasing tumor resistance to radiotherapy [15-18]. Genomic analysis of sporadic H3K27M-mutant DMG has revealed a number of cooperating genetic alterations. In particular, these tumors often also have TP53 and ATRX mutations [12,18].

In the setting of NF1, pediatric HGG have been described [4,9,12,19-21], with a prevalence ranging from 0.28% to 5% [19]. NF1 pediatric HGG share with non-syndromic patients, genetic alterations of TP53 and CDKN2A [22]. A recent study [20], demonstrated also that NF1 HGG harbor frequent mutations of ATRX associated with Alternative Lengthening of Telomere, and are enriched in genetic alterations of transcription/chromatin regulation and PI3 kinase pathways. Frequent mutations of ATRX drive aggressiveness in NF1 gliomas. Furthermore, loss of ATRX in high-grade NF1 glioma is unique when considered within the genetic contexts associated with ATRX mutations in sporadic gliomas, in which they are typically associated with pediatric H3K27M-mutant DMG [20].

Of note, due to their rarity, a child with a clinical diagnosis of NF1 and HGG should be investigated for constitutional mismatch-repair deficiency (CMMRD), if an NF1 mutation has not been previously identified [23]. CMMRD frequently display features reminiscent of NF1 [24]. Genetic confirmation of NF1 is therefore mandatory for genetic counselling to families and because alternative therapies are available for CMMRD-associated HGG [23,24].

In our patients, genetic analysis of NF1 was performed identifying in patient 1 a c.6792C>A variant in the NF1 gene determining a p.Tyr2264* premature termination with skipping of exon 37 resulting in a protein lacking 34 amino acids [11], while patient 2 was carrying the c.5705C>A variant that determines an amino acid substitution p.Thr1902Lys. This variant is not present in gnomAD (Genome Aggregation Database, https://gnomad.broadinstitute.org/); it involves a highly conserved amino acid and was transmitted by her affected mother. Eight different prediction tools,

which predicts possible impact of an amino acid substitution on the structure and function of a human protein using straightforward physical and comparative considerations, indicate that the variant has deleterious (D) effect: SIFT, Sorting Intolerant from Tolerant (https://sift.bii.a-star.edu.sg/): D; PolyPhen-2, Polymorphism Phenotyping v2 (http://genetics.bwh.harvard.edu/pph2/): HumDiv = 1.000 - HumVar = 0.999 (probably damaging); CADD, Combined Annotation Dependent Depletion (https://cadd.gs.washington.edu/snv): 34; DANN, omicX, deep neural network (https://omictools.com/dann-tool): 0,992; FATHMM, Functional Analysis through Hidden Markov Models (v2.3) (http://fathmm.biocompute.org.uk/): -4,23; GERP++, omicX (https://omictools.com/gerp-tool): 5,3; Mutation Taster (http://www.mutationtaster.org/): D, D; PROVEAN, Protein Variation Effect Analyzer (http://provean.jcvi.org/): D.

Furthermore this variant is not present in ClinVar; the C>G change (c.5705C>G; p.Thr1902Arg - hiips://www.ncbi.nlm.nih.gov/clinvar/variation/404539) and the C>T change (c.5705C>T; p.Thr1902Met - hiips://www.ncbi.nlm.nih.gov/clinvar/variation/187560) in the same position (rs786203824; chr17: 29657472; GRCh37.p13) have been defined in ClinVar of uncertain significance.

It is well known that neurofibromin has an important function in cancer development and progression. Whilst it is unclear whether the biallelic loss of NF1 is necessary to tumour progression, mouse cells heterozygous for NF1 mutations show abnormal growth and invasion [25,26].

HGG involving midline structures in the setting of NF1, are extremely rare. To the best of our knowledge, only 10 pediatric patients with primary high-grade DMG have been reported so far [4,9,19,21]. In these prior studies, the association of high-grade DMG with NF1 was mainly based on a clinical NF1 diagnosis, thus potentially not excluding a CMMRD.

Of note, one of our NF1 patients presented a DMG H3K27M-mutant and currently represent the first description in the literature where this type of tumor is reported in association with a

genetically confirmed diagnosis of NF1. The rarity of DMG H3K27M-mutant in NF1 patients is underlined by a recent study where genomic profile of 59 gliomas (22 children, 33 adults) was evaluated. Remarkably, H3.3 histone variants were absent in all 59 cases [20].

Regarding our NF1 patients, none of them presented an optic pathway glioma or a mass lesion in another district, nor received prior radiotherapy; typical UBOs were present. In both cases, neoplasms demonstrated an unexpected aggressive behaviour, with no response to therapies and rapid leptomeningeal dissemination in one subject, with a PFS of 3 months and an OS of 7 and 5 months.

Neuroimaging studies can play a pivotal role in suggesting a high-grade glioma, thus recommending biopsy sample in NF1 subjects. Both patients presented areas of restricted diffusivity within the lesions. MRS demonstrated markedly increased Cho/NAA and Cho/Cr ratios. One NF1 subject underwent both MRI perfusion imaging with ASL and molecular imaging with 18F-DOPA PET, demonstrating increased perfusion and markedly increased amino-acid uptake. All these techniques, have been demonstrated to add significant information in discriminating low-grade from high-grade cerebral gliomas, both midline and off-midline, providing non-invasive microstructural, microvascular and metabolic information of these lesions [27-29]. In detail, DWI provides estimation of differences in cell density and tissue structure, and in our NF1 subjects reduced diffusivity of the lesions was suggestive of increased cellularity.

ASL allows quantification of cerebral blood flow correlated with microvascular density and displays a high potential in evaluating pediatric brain gliomas aggressiveness, as demonstrated in case 2. MRS allows non-invasive detection and estimation of normal and abnormal metabolites within brain tissue, indicating loss of neuroaxonal integrity and increased myelin turnover [28]. Both subjects presented an MRS pattern compatible with increased biological activity of the lesions. Among amino-acid PET tracers, 18F-DOPA has demonstrated high potential in defining tumor grade in pediatric infiltrative astrocytomas [28,29]. Increased 18F-DOPA uptake has been shown to correlate with an overexpression of amino-acid transporters within regions of high proliferation

with increased use of amino-acids for energy, protein synthesis, and cell division [29]. Advanced MR imaging studies and/or molecular amino-acid PET imaging are therefore recommended in those NF1 subjects with suspected aggressive lesions on conventional MRI in order to provide additional information and increase diagnostic confidence. Of note, prior studies have reported that thalamic localization, symptoms at diagnosis and diffusion restriction on MRI are elements suggestive of a high-grade tumor in NF1 subjects [19,21], as confirmed in our study.

In the non-NF1 high-grade DMG counterpart, composed by 11 patients with H3K27M-mutant and 5 H3K27M-wildtype lesions, we did not find statistically significant differences in terms of PFS and OS. In the revised 2016 WHO classification of tumors of the central nervous system, the finding of an H3K27M mutation in DMG confers a worse prognosis than that of wildtype cases, as also stated in prior studies [10,30]. However, as reported by more recent researches, not univocal findings are emerging regarding the prognostic role of H3K27M mutation in DMG, in accordance with our results. For instance, recent studies highlighted that H3K27M-wildtype DIPG (approximately 15% of the biopsied population) shared the same unfavorable prognosis as H3K27M-mutant DIPG [31,32], independently of their underlying histological tumor grading. Survival comparison between H3K27M-mutant and wildtype midline gliomas in adults have also demonstrated that survival may be similar or possibly improved if the mutation is present [33,34]. Further and larger prospective studies are therefore recommended to better define the prognostic significance of H3K27M mutation in DMG, as also suggested in a recent meta-analysis [35].

In both NF1 and sporadic high-grade DMG, leptomeningeal dissemination during treatment response evaluation was revealed in five subjects, both H3K27M-mutant and wildtype, with no evidence at admission. This finding was clearly evaluable and much more prominent in the spinal compartment, when compared to the cerebral region. Of note, in the NF1 subject the appearance of leptomeningeal dissemination did not show a concomitant primary lesion progression. Overall, these findings advise whole brain and spine MR imaging studies in DMG, at admission and during follow-up, for a complete evaluation of the disease status.

In the setting of NF1, leptomeningeal dissemination of pediatric gliomas is an extremely rare event. We found only one description of a pediatric patient with an HGG and diagnostic criteria of NF1, where evidence of tumor dissemination is reported [19]. An additional single patient with NF1 and a midline low-grade glioma, a pilocytic astrocytoma, with secondary dissemination to the brain has been previously described [36].

Among the limitations of our study, we are aware of its retrospective nature and of the relatively small sample of patients; however, we included only pediatric patients with high-grade DMG, histologically and molecularly classified, and NF1 subjects with genetical diagnosis, which are extremely rare, particularly for a single center. The limited number of NF1 patients did not allow performing formal statistical analysis, and further multicentre studies with larger samples of patients are needed to extend knowledge in this field.

Conclusion

According to our experience, high-grade DMG occurring in NF1 patients, although rare, may have an aggressive behaviour and an extremely poor prognosis, even worse than sporadic high-grade DMG, independently of the presence or absence of H3K27M mutation. Lesions in evocative regions and with features of increased biological activity on advanced MR or molecular amino-acid PET imaging may alert clinicians, suggesting prompt neuropathological and molecular investigations.

List of abbreviations:

NF1: Neurofibromatosis type 1

CNS: Central nervous system

HGG: High-grade gliomas

WHO: World Health Organization

DMG: Diffuse midline glioma

PFS: Progression free survival

OS: Overall survival

UBOs: Unidentified bright objects

MRI: Magnetic resonance imaging

DWI: Diffusion weighted imaging

MRS: Magnetic resonance spectroscopy

DOPA: Dihydroxyphenylalanine

DIPG: Diffuse intrinsic pontine glioma

CMMRD: Constitutional mismatch-repair deficiency

Declarations

Ethics approval and consent to participate: This retrospective study was approved from the Institutional Review Board (Regional Ethics committee of Liguria, Genoa, Italy),

Consent for publication: Written informed consent was obtained from the patients or their legal guardians, for publication of clinical details and clinical images. Upon request, a copy of the consent form is available for review by the Editor of this journal.

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interest: The authors declare that they have no competing interests.

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Authors' contributions: FG: Conceptualization, Data curation, Writing - original draft; FA: Conceptualization, Investigation, Writing - review & editing; CM: Conceptualization, Data

curation, Writing - original draft; AV: Conceptualization, Data curation; AP: Conceptualization, Formal analysis, Investigation; DT: Conceptualization, Formal analysis, Methodology; GP: Conceptualization, Data curation; MCD: Conceptualization, Data curation, Resources; VC: Conceptualization, Formal analysis, Writing - original draft; MLG: Conceptualization, Data curation, Supervision, Writing - review & editing; GM: Conceptualization, Data curation, Supervision, Writing - review & editing. All authors read and approved the final manuscript

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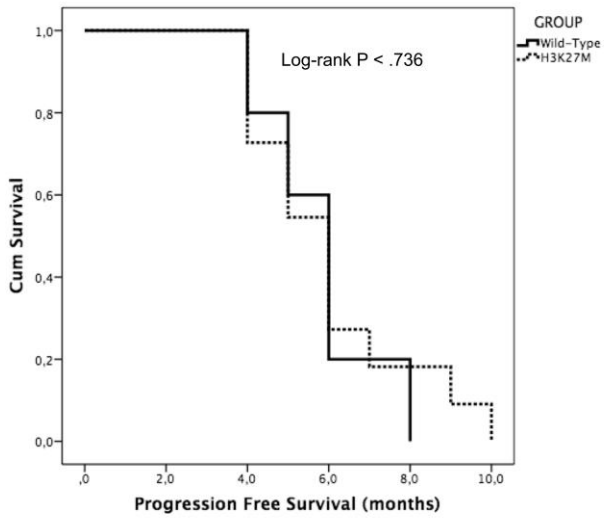
Table 1 Summary of patient characteristics, treatments and outcome

Case	Age at diagnosis	Sex	Histological diagnosis	H3K27M status	Location	WHO Grade	Treatments	Outcome	PFS (months)	OS (months)
SPORADIC CEREBRAL HIGH-GRADE DIFFUSE MIDLINE GLIOMAS										
1	12	M	GB	H3K27M-m	L-Th	IV	PS/RT/VIN+NIM/TEM	PD	6	(10*)
2	3	F	GB	H3K27M-m	R-Th/L-Th	IV	B/RT/VIN+NIM	PD and DOD	9	13
3	10	M	GB	H3K27M-m	Pons	IV	B/RT/VIN+NIM/TEM+ETO	PD and DOD	4	6
4	12	F	GB	H3K27M-m	Medulla	IV	B/ RT/VIN+NIM/DAB+TRA	PD and DOD	5	9
5	6	F	GB	H3K27M-m	R-DMJ	IV	B/RT/TEM+BEV	PD and DOD	4	8
6	6	M	GB	H3K27M-m	Pons	IV	B/RT/VIN+NIM	PD	6	(7*)
7	7	M	AA	H3K27M-m	Pons	IV	B/RT/VIN+NIM/IRI/SIR	PD and DOD	7	10
8	8	F	AA	H3K27M-m	Pons	IV	B/RT/VIN+NIM	PD and DOD	6	12
9	16	F	AA	H3K27M-m	R-DMJ	IV	B/RT/TEM+BEV	PD and DOD	5	6
10	10	F	AA	H3K27M-m	R-Th	IV	B/RT/TEM+BEV	PD and DOD	10	18
11	3	F	AA	H3K27M-m	R-Th	IV	B/RT/TEM	PD and DOD	4	8
12	6	M	GB	H3K27M-wt	L-Th	IV	B/RT/TEM+BEV	PD and DOD	6	10
13	9	M	GB	H3K27M-wt	L-Th	IV	PS/RT/CAR+VC/TEM/BEV+ETO	PD and DOD	4	7
14	9	M	GB	H3K27M-wt	R-Th	IV	B/RT/TEM+BEV/POM/ETO	PD and DOD	5	11
15	11	M	AA	H3K27M-wt	R-Th/L-Th	III	B/RT/TEM	PD and DOD	6	12
16	17	F	AA	H3K27M-wt	R-Th/L-Th	III	PS/RT/TEM+BEV/VIN+RAP	PD and DOD	8	12
NF1 CEREBRAL HIGH-GRADE DIFFUSE MIDLINE GLIOMAS										
1	11	F	GB	H3K27M-m	L-Pons midbrain	IV	B/RT/VIN+NIM/ETO+TEM	PD and DOD	3	7
2	13	F	AA	H3K27M-wt	R-Th/L-Th	III	B/RT/TEM+VIN	PD and DOD	3	5

PFS: progression free survival, OS: overall survival, M: male, F: female, GB: glioblastoma, AA: anaplastic astrocytoma, m: mutant, wt: wild-type, R: right, L: left, Th: thalamus, DMJ: diencephalic-mesencephalic junction, B: biopsy, PS: partial surgery, RT: radiotherapy, VIN: Vinorelbine, NIM: Nimotuzumab, TEM: temozolomide, ETO: Etoposide, DAB: Dabrafenib, TRA: Trametinib, BEV: Bevacizumab, IRI: Irinotecan, SIR: Sirolimus, CAR: carboplatin, VC: vincristine, POM: Pomalidomide, RAP: Rapamycin, PD: progressive disease, DOD: death of disease
*: subject alive at the time of this publication

Figure Legend

Figure 1 Kaplan-Meier progression free survival and overall survival curves of sporadic H327M-mutant vs H3K27M-wildtype high-grade DMG, demonstrating no significant differences



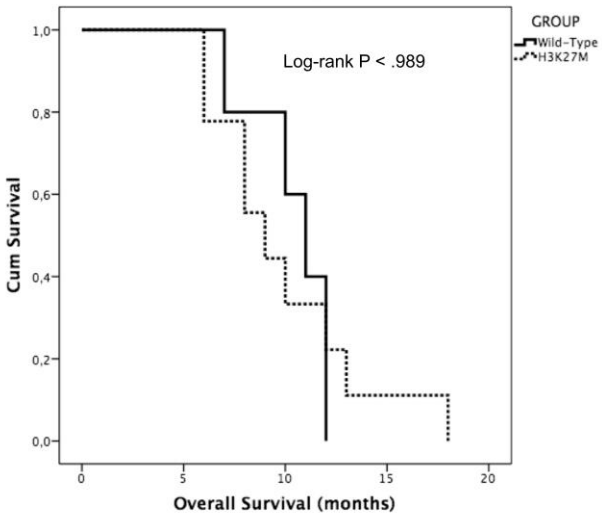


Figure 2 Neuroimaging findings in NF1 patient 1

At admission, brain axial T2-weighted and contrast-enhanced (CE) T1-weighted images show focal areas of signal abnormalities without contrast-enhancement in the globus pallidus bilaterally, in keeping with typical UBOs (thin arrows). Additional brain axial and sagittal T2-weighted and CE T1-weighted images show a mass lesions with a central necrotic area and irregular rim enhancement in the left ponto-mesencephalic region, along with (arrowheads) an adjacent expansile lesion involving the medulla without contrast-enhancement. Diffusion-weighted imaging (DWI) shows reduced diffusivity along the ventrolateral margin of the ponto-mesencephalic lesion (thick arrow). Post-contrast Fluid Attenuated Inversion Recovery (FLAIR-CE) image does not reveal cerebral leptomeningeal dissemination. Sagittal CE T1-weighted image of the spine does not show secondary lesions. Single voxel Magnetic Resonance Spectroscopy (MRS) with echo time of 144 ms of the ponto-mesencephalic lesion shows prominent increase of Cho/NAA and Cho/Cr ratios. Three months later, following radiotherapy and first line chemotherapy treatment with vinorelbine and nimotuzumab, sagittal brain CE T1-weighted image shows increased extension of the necrotic component within the ponto-mesencephalic lesion. Axial CE T1-weighted and post-contrast FLAIR images (FLAIR-CE), demonstrate the appearance of leptomeningeal contrast-enhancement, in keeping with secondary dissemination, along the sylvian fissures (thin arrow and thick arrows). Leptomeningeal dissemination is much more evident on sagittal CE T1-weighted image of the spine.

Seven months after diagnosis, following second line treatment with etoposide and temozolomide, axial CE T1-weighted and FLAIR-CE images show marked increase of nodular leptomeningeal dissemination with extensive brain edema and subependymal dissemination. Sagittal CE T1-weighted image of the spine show massive secondary involvement around and within the spinal cord.

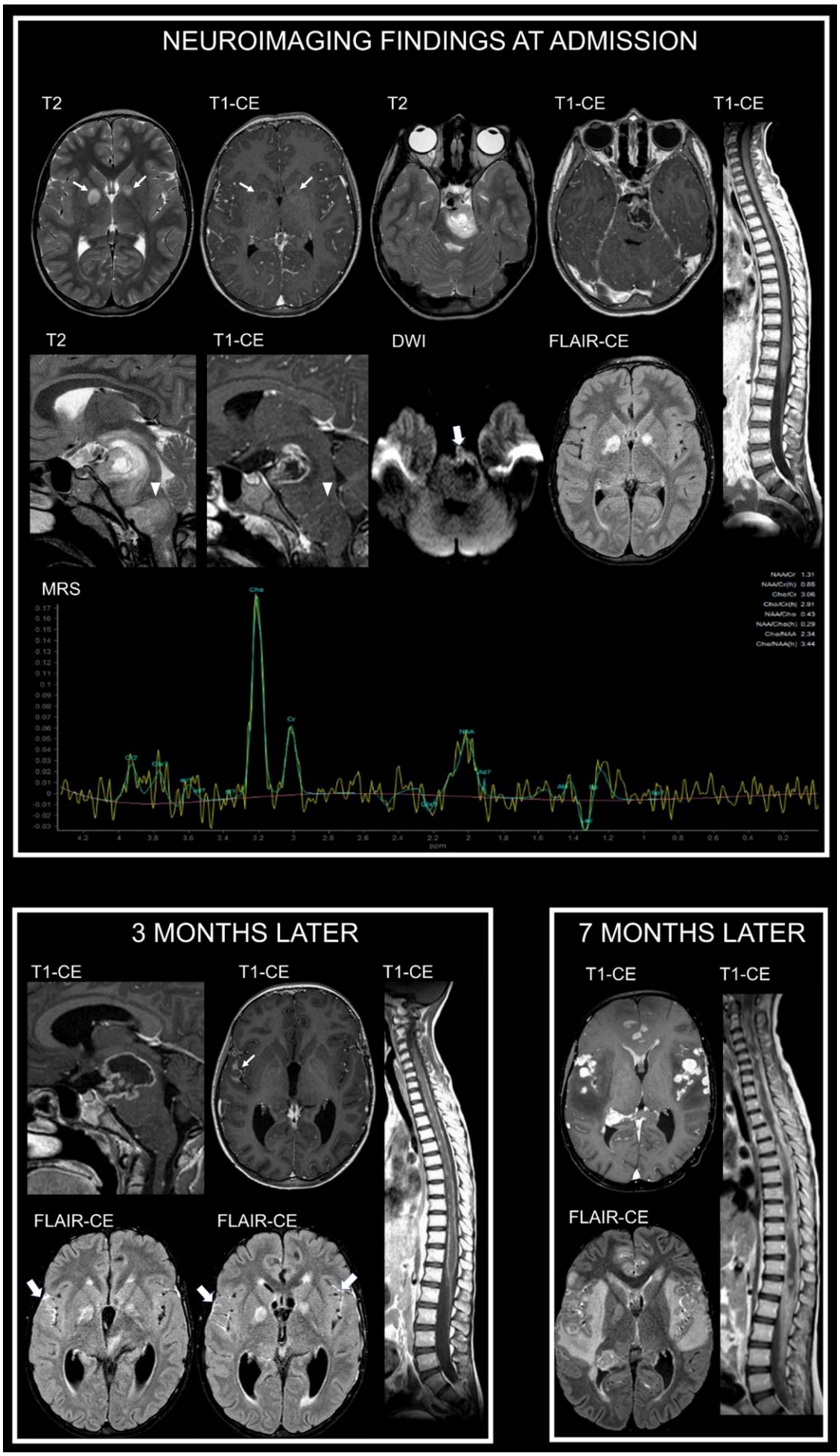

NEUROIMAGING FINDINGS AT ADMISSION
T2
T1-CE
T2
T1-CE
T1-CE
T2
T1-CE
DWI
FLAIR-CE
MRS
3 MONTHS LATER
T1-CE
T1-CE
T1-CE
FLAIR-CE
FLAIR-CE
7 MONTHS LATER
T1-CE
T1-CE
FLAIR-CE

Figure 3 Neuroimaging findings in NF1 patient 2

At admission, brain axial FLAIR and contrast-enhanced (CE) T1-weighted images, show an infiltrating and expansile lesion with epicentre in the right thalamus, partially involving the contralateral thalamus, with irregular contrast-enhancement. DWI and corresponding Apparent Diffusion Coefficient (ADC) map show restricted diffusivity of the right thalamic portion of the lesion. Arterial Spin Labelling (ASL) perfusion weighted imaging clearly demonstrates increased perfusion of the lesion (thick arrow). Axial T2-weighted image shows small focal hyperintense areas located in the dorsal pons and deep cerebellar white matter (thin arrows) in keeping with typical UBOs. Single voxel MRS with echo time of 144 ms of the right thalamic region shows marked increase of Cho/NAA and of Cho/Cr ratios. 18F-DOPA PET clearly demonstrates markedly increased uptake of the lesion.

Three months later, following radiotherapy and first line chemotherapy treatment with temozolomide and vinorelbine, axial FLAIR and coronal T2-weighted images show increased extension of the infiltrating components in the deep cerebral regions with prominent involvement of the brain stem (midbrain and dorsal pons). Axial and sagittal CE T1-weighted images show concomitant marked increase of contrast-enhancement. There was no evidence of leptomeningeal dissemination.

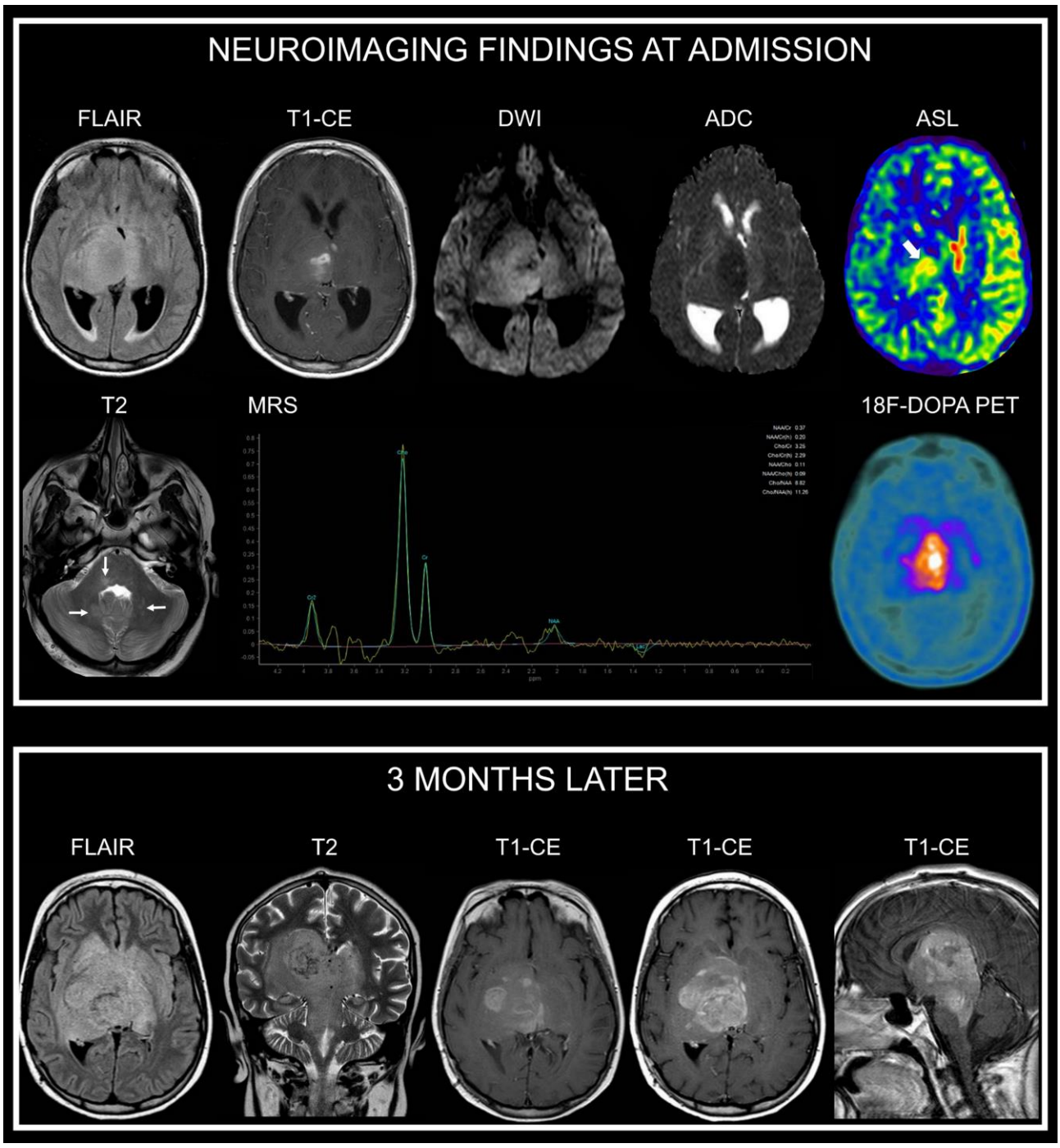
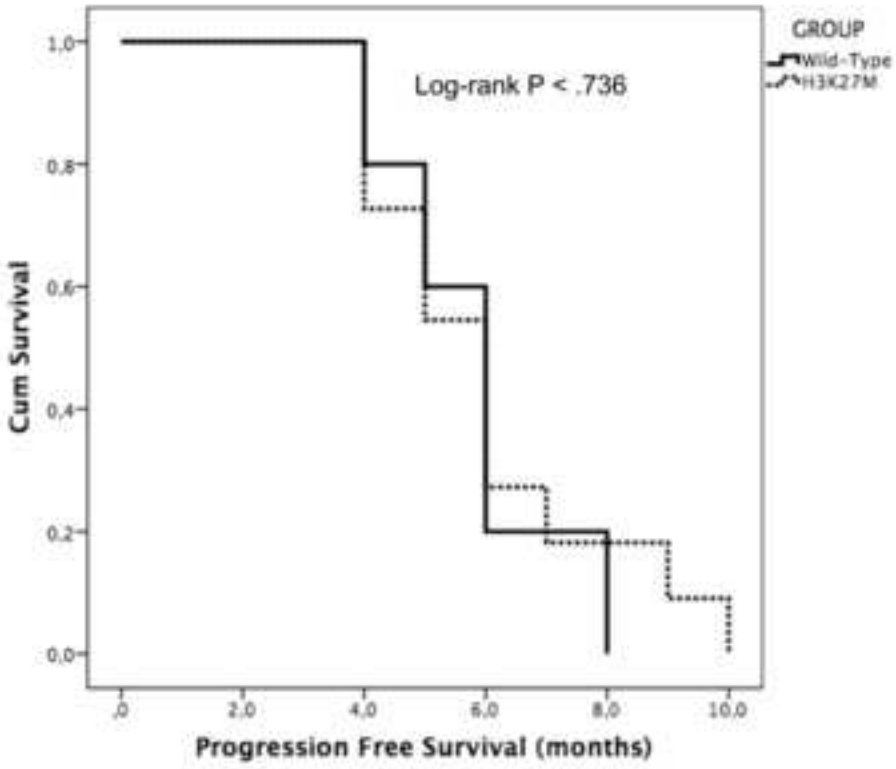

NEUROIMAGING FINDINGS AT ADMISSION
FLAIR
T1-CE
DWI
ADC
ASL
T2
MRS
18F-DOPA PET
3 MONTHS LATER
FLAIR
T2
T1-CE
T1-CE
T1-CE

Table 1 Summary of patient characteristics, treatments and outcome

Case	Age at diagnosis	Sex	Histological diagnosis	H3K27M status	Location	WHO Grade	Treatments	Outcome	PFS (months)	OS (months)
SPORADIC CEREBRAL HIGH-GRADE DIFFUSE MIDLINE GLIOMAS										
1	12	M	GB	H3K27M-m	L-Th	IV	PS/RT/VIN+NIM/TEM	PD	6	(10*)
2	3	F	GB	H3K27M-m	R-Th/L-Th	IV	B/RT/VIN+NIM	PD and DOD	9	13
3	10	M	GB	H3K27M-m	Pons	IV	B/RT/VIN+NIM/TEM+ETO	PD and DOD	4	6
4	12	F	GB	H3K27M-m	Medulla	IV	B/ RT/VIN+NIM/DAB+TRA	PD and DOD	5	9
5	6	F	GB	H3K27M-m	R-DMJ	IV	B/RT/TEM+BEV	PD and DOD	4	8
6	6	M	GB	H3K27M-m	Pons	IV	B/RT/VIN+NIM	PD	6	(7*)
7	7	M	AA	H3K27M-m	Pons	IV	B/RT/VIN+NIM/IRI/SIR	PD and DOD	7	10
8	8	F	AA	H3K27M-m	Pons	IV	B/RT/VIN+NIM	PD and DOD	6	12
9	16	F	AA	H3K27M-m	R-DMJ	IV	B/RT/TEM+BEV	PD and DOD	5	6
10	10	F	AA	H3K27M-m	R-Th	IV	B/RT/TEM+BEV	PD and DOD	10	18
11	3	F	AA	H3K27M-m	R-Th	IV	B/RT/TEM	PD and DOD	4	8
12	6	M	GB	H3K27M-wt	L-Th	IV	B/RT/TEM+BEV	PD and DOD	6	10
13	9	M	GB	H3K27M-wt	L-Th	IV	PS/RT/CAR+VC/TEM/BEV+ETO	PD and DOD	4	7
14	9	M	GB	H3K27M-wt	R-Th	IV	B/RT/TEM+BEV/POM/ETO	PD and DOD	5	11
15	11	M	AA	H3K27M-wt	R-Th/L-Th	III	B/RT/TEM	PD and DOD	6	12
16	17	F	AA	H3K27M-wt	R-Th/L-Th	III	PS/RT/TEM+BEV/VIN+RAP	PD and DOD	8	12
NF1 CEREBRAL HIGH-GRADE DIFFUSE MIDLINE GLIOMAS										
1	11	F	GB	H3K27M-m	L-Pons midbrain	IV	B/RT/VIN+NIM/ETO+TEM	PD and DOD	3	7
2	13	F	AA	H3K27M-wt	R-Th/L-Th	III	B/RT/TEM+VIN	PD and DOD	3	5

PFS: progression free survival, OS: overall survival, M: male, F: female, GB: glioblastoma, AA: anaplastic astrocytoma, m: mutant, wt: wild-type, R: right, L: left, Th: thalamus, DMJ: diencephalic-mesencephalic junction, B: biopsy, PS: partial surgery, RT: radiotherapy, VIN: Vinorelbine, NIM: Nimotuzumab, TEM: temozolomide, ETO: Etoposide, DAB: Dabrafenib, TRA: Trametinib, BEV: Bevacizumab, IRI: Irinotecan, SIR: Sirolimus, CAR: carboplatin, VC: vincristine, POM: Pomalidomide, RAP: Rapamycin, PD: progressive disease, DOD: death of disease
*: subject alive at the time of this publication

Figure 1



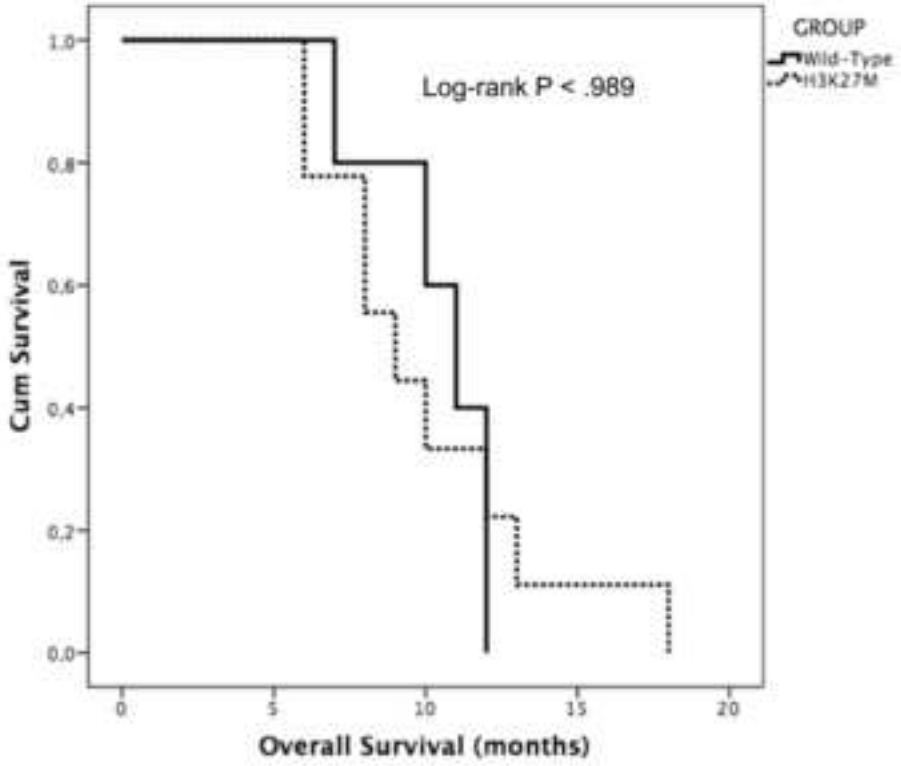


Figure 2

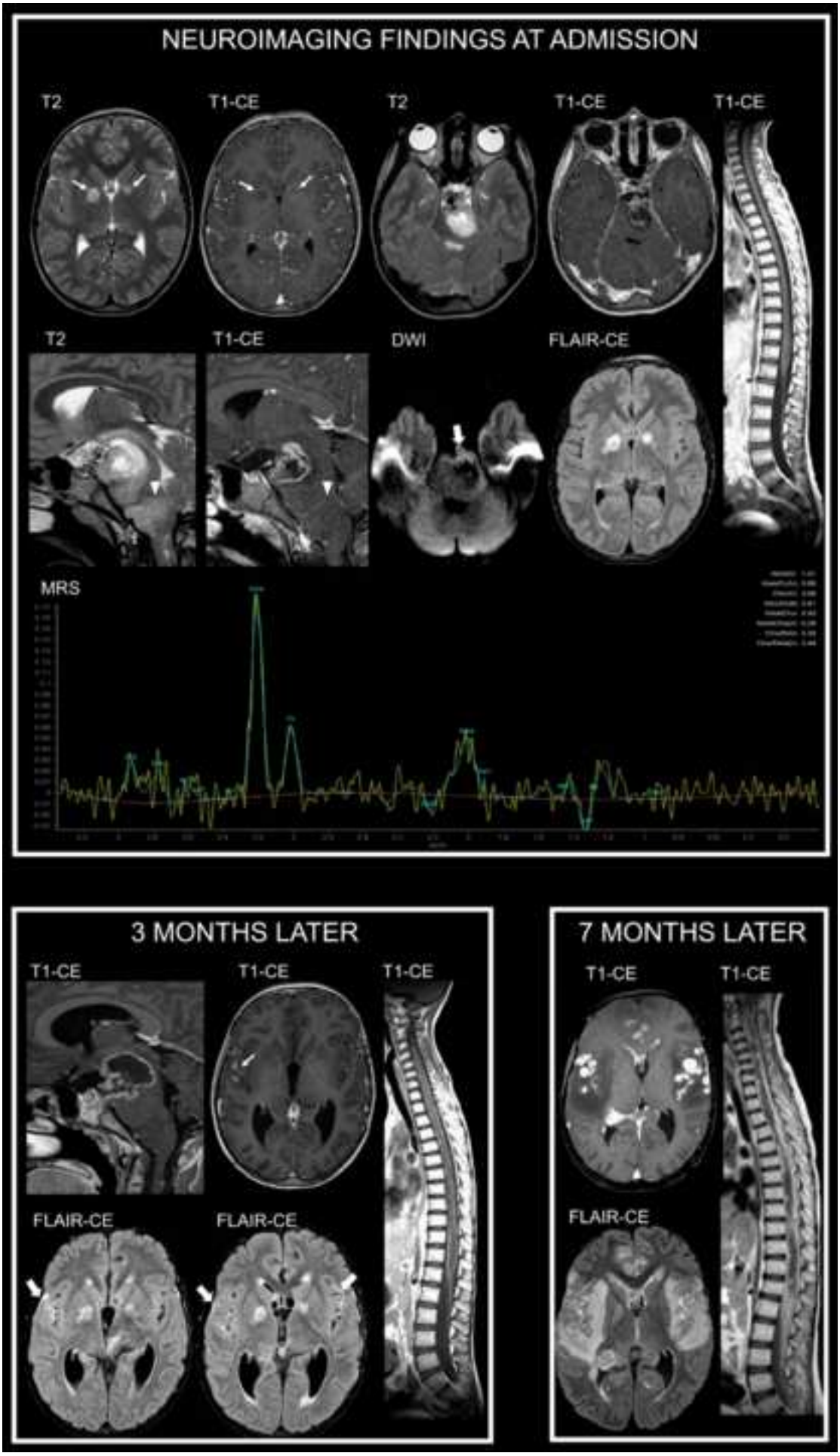


Figure 3