

# A homogeneous treatment for non-DIPG diffuse midline glioma

Tumori Journal

1-7

© Fondazione IRCCS Istituto Nazionale dei Tumori 2022




Article reuse guidelines:

[sagepub.com/journals-permissions](https://sagepub.com/journals-permissions)

DOI: 10.1177/03008916221099067

[journals.sagepub.com/home/tmj](https://journals.sagepub.com/home/tmj)

Elisabetta Schiavello<sup>1</sup> , Veronica Biassoni<sup>1</sup>, Giovanna Gattuso<sup>1</sup> ,  
Marta Podda<sup>1</sup>, Stefano Chiaravalli<sup>1</sup>, Francesco Barretta<sup>2</sup> ,  
Manila Antonelli<sup>3</sup>, Loris De Cecco<sup>4</sup>, Emilia Pecori<sup>5</sup>,  
Lorenza Gandola<sup>5</sup> and Maura Massimino<sup>1</sup>

## Abstract

**Introduction:** The H3K27M-mutant diffuse midline glioma (DMG) was first included in the World Health Organization (WHO) Classification of central nervous system (CNS) tumors in 2016, and confirmed in its fifth edition. The biological behavior and dismal prognosis of this tumor resemble diffuse intrinsic pontine gliomas (DIPG). Homogeneously-treated series are rarely reported.

**Methods:** From 2016 onwards, we treated patients with DMG with radiotherapy and concomitant/adjuvant nimotuzumab/vinorelbine, plus re-irradiation at relapse, as already done for DIPG.

**Results:** We treated nine patients, seven females, with a median age at diagnosis of 13 years. Tumor sites were: thalamic in five cases, pontocerebellar in two, pineal in one, and paratrighonal with nodular/leptomeningeal dissemination in one. Three patients were biopsied, and six had partial tumor resections. Central pathological review was always performed. The median time to local progression was 12.7 months, and the median overall survival was 17.8 months. Six patients died of tumor progression, one of cerebral bleeding at progression. Two were alive, one in continuous remission, the other after relapsing, at 38.6 and 46.3 months after diagnosis. Progression-free survival was 33.3% at one year. Overall survival was 88.9%, 33.3% and 22.2% at 1, 2 and 3 years, respectively.

**Conclusions:** This is a small series of homogeneously-treated DMG patients. The results obtained are comparable with those of DIPG patients. Given the phenotypically- and molecularly-defined setting of DMG and severe outcome in this orphan population, they should be treated and included in registries and protocols of DIPG.

## Keywords

Diffuse midline glioma, radiotherapy, nimotuzumab, vinorelbine, p53

Date received: 7 April 2022; revised: 8 April 2022; accepted: 12 April 2022

## Introduction

Pediatric high grade glioma (HGG; WHO grade III-IV), and diffuse intrinsic pontine glioma (DIPG) in particular, pose one of the great challenges in pediatric oncology, carrying the highest mortality in this patient population.<sup>1</sup> The H3K27M-mutant diffuse midline glioma was first included in the 2016 WHO Classification of CNS tumors as a distinct grade IV glioma<sup>2</sup> and it was confirmed in the latest 2021 edition.<sup>3</sup> Recent studies<sup>4-6</sup> found that, regardless of its morphological features, this tumor subtype has an aggressive clinical behavior and a dismal prognosis, sharing the

<sup>1</sup>Pediatric Oncology Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy

<sup>2</sup>Department of Clinical Epidemiology and Trial Organisation, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy

<sup>3</sup>Department of Radiological, Oncological and Anatomic-Pathological Sciences, Sapienza University Viale Regina Elena, Rome, Italy

<sup>4</sup>Integrated Biology Platform, Department of Applied Research and Technology Development, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy

<sup>5</sup>Pediatric Radiotherapy, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy

### Corresponding author:

Elisabetta Schiavello, IRCCS Fondazione Istituto nazionale dei Tumori, via Venezian 1, Milan, 20133, Italy.

Email: [elisabetta.schiavello@istitutotumori.mi.it](mailto:elisabetta.schiavello@istitutotumori.mi.it)

same outcomes as DIPG. The thalamus, pineal region, brainstem and spinal cord are the typical sites of origin of DMG, but the presence of the H3K27M mutation seems to be a negative prognostic indicator in pediatric HGG, whatever the site involved.<sup>5</sup>

The tumor cells contain a missense mutation caused by a lysine-to-methionine substitution at amino acid position 27, in either the HIST1H3B (H3.1) or the H3F3A (H3.3) histone variant. The mutation results in significant epigenetic reprogramming, which contributes to tumorigenesis and gives rise to a tumor phenotype with genetic, epigenetic and clinical features that differ from its wild-type counterpart and from other non-midline HGG. Both histone variants (H3.1 and H3.3) can be tested with CLIA-certified DNA sequencing or immunohistochemical staining.<sup>6</sup>

Genome analysis of K27M-mutant DMG has revealed a number of cooperating genetic alterations including: TP53 overexpression, ATRX loss, and none of the genetic alterations generally associated with a more favorable prognosis, such as IDH1/2 mutations or 1p/19q co-deletion.<sup>7,8</sup> DMGs have rarely been found to harbor both the histone H3-K27M and the BRAF-V600E mutation.<sup>6,9</sup> DMGs account for 10-20% of all pediatric brain tumors, with a median age at presentation ranging between six and nine years.

Due to the rarity of reports on their ad-hoc treatment to date, we describe our homogeneously-treated series of nine patients.

## Materials and methods

All patients were referred at our Institution (Fondazione IRCCS Istituto Nazionale dei Tumori, Milan) for adjuvant treatment after surgery performed elsewhere. Resections were classified as complete, subtotal (removal of >90% of the tumor) or partial (removal of >50% but <90%), while resections of <50% of the tumor were classified as non-debulking, including biopsies.<sup>5</sup> Central neuropathological review was performed according to standard Italian procedure, which is referring cases to the Neuropathology Unit at La Sapienza University in Rome. A central histopathological diagnosis of DMG according to the WHO 2016 Classification was necessary to include the patients.

All patients with DMG diagnosed between April 2016 and November 2018 were staged and treated in the same way. Tests performed within 14 days prior to any therapy included: cranial and spinal magnetic resonance imaging (MRI) with gadolinium (to identify the index lesion); full clinical and neurological examination, recording body weight and height, Karnofsky score or Lansky performance status; full blood counts, renal, kidney and coagulation parameters. Response to treatment was assessed using RECIST criteria,<sup>10</sup> considering the diameters measured on T2-weighted images.

Systemic treatment consisted of vinorelbine 20 mg/m<sup>2</sup> with nimotuzumab 150 mg/m<sup>2</sup> administered weekly during the first 12 weeks of treatment. Focal radiotherapy was generally delivered from weeks 3 to 9, for a total dose of 54 Gy (induction phase). Vinorelbine 25 mg/m<sup>2</sup> and nimotuzumab (same dosage) were given every other week thereafter (maintenance phase) until the tumor progressed, or for up to two years. In cases of local progression, re-irradiation consisted of 19.8 Gy, fractionated over 11 days (Figure 1). In cases of progression with tumor dissemination, craniospinal irradiation (CSI) doses and schedules varied, depending on the sites of disease already irradiated, but the total dose was generally 36 Gy, delivered with daily fractions of 1.8 Gy.

MRI was repeated every 12 weeks throughout the treatment, or sooner if symptoms worsened or pseudoprogression was suspected.

## Immunohistochemistry for detecting the H3.3 mutation

Formalin-fixed and paraffin-embedded material was available for all cases. Immunohistochemical studies were performed using a Leica BondMax immunostainer with the following antibodies: rabbit polyclonal anti-histone-H3K27M antibody (#ABE419 Millipore, 0.5 ug/ml, 1:1000); and rabbit monoclonal anti-trimethyl-histone-H3 (Lys27) antibody (C36B11, Cell Signaling Tech, 1:200). Slides were independently scored for H3K27M and H3K27me3 staining by two neuropathologists. Samples were considered positive when tumor cells showed strong nuclear staining for H3K27M, and non-neoplastic elements (such as endothelial cells or lymphocytes) were negative. H3K27me3 immunoreactivity and H3K27M positivity were mutually exclusive. Positive K27M samples did not stain for H3K27me3.

## Statistical analyses

The main endpoints of the study were overall survival (OS) and progression-free survival (PFS). Secondary endpoints were post-relapse survival (PRS) and local relapse-free survival (LRFS). Survival was calculated from the date of the first surgical procedure leading to the diagnosis for OS, PFS and LRFS, and from the date of first recurrence for PRS. OS and PRS were defined as time to death due to any cause, and censored at the latest follow-up for patients still alive. PFS was defined as the time to any radiological or clinical progression or death due to any cause (whichever came first), and censored at the latest follow-up for patients still alive and progression-free. LRFS was defined as the time to the first local recurrence, and censored at the time of dissemination or death due to any cause (whichever came first). All survival probabilities were estimated with the Kaplan-Meier method. The

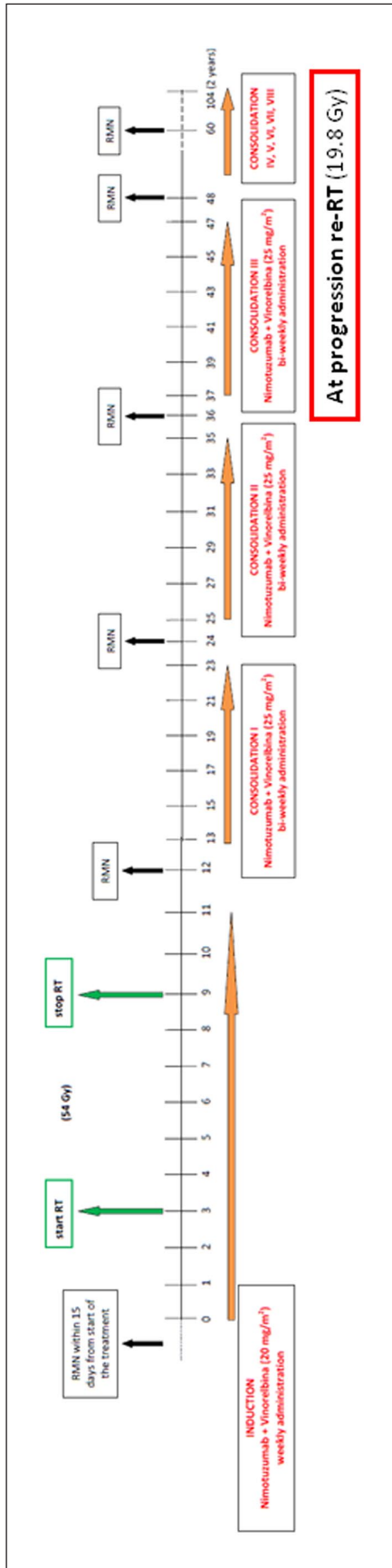


Figure 1. Treatment flow-chart for patients with DMG.

median follow-up was estimated on OS data with the reverse Kaplan-Meier method.

The protocol adopted was approved by the Institutional Review Board (IRB) as 149/18 and patients’ parents, or patients over 18 years old signed a consent form before receiving treatment.

## Results

### Neuropathological assessment

The first pathological diagnoses (performed elsewhere, before referral for adjuvant treatment) were: glioblastoma in four cases, anaplastic astrocytoma, diffuse astrocytoma and glial tumor with oligodendrogial features in one case each and DMG (H3K27M mutant) in two cases. Central pathology review revealed H3.3 K27M mutation on immunohistochemical staining in all nine cases. The Ki67 value varied, ranging between 2% and 70%. No ATRX loss was seen in five cases tested. Four samples were found positive for the Tp53 mutation, while five tested negative. The BRAFV600E mutation was identified in one of seven patients tested with PCR sequencing. FISH for TRK1/3 fusions was negative in four patients tested. IDH1-2 mutations were negative in three patients tested. An EGFR mutation was found in the patient with bithalamic tumor.<sup>11</sup>

### Patients’ characteristics

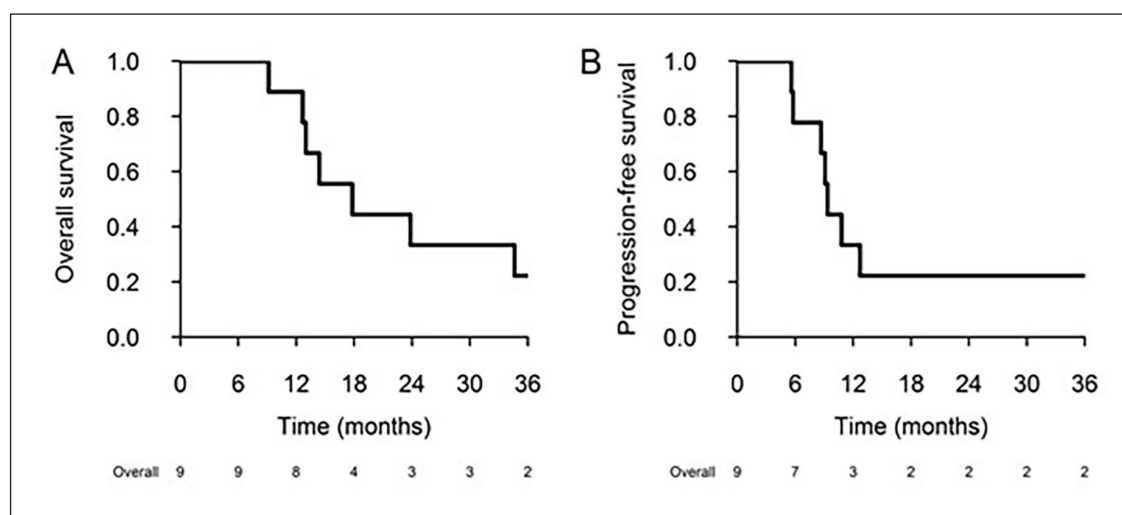
Nine consecutive patients with DMG were diagnosed and treated from May 2016 to December 2018. Seven were females. The median age at diagnosis was 13 years (range 1-26). The median duration of symptoms at the time of diagnosis was 40 days (range 10 days to 4 months). Eight patients had localized disease, while one had nodular and leptomeningeal metastases at diagnosis. Five patients with localized disease had thalamic tumors (bithalamic in one), two had tumors in the pontocerebellar structures (one with two distinct lesions), one had tumor in the pineal gland, and the paratrigenal area was involved in one (with nodular and leptomeningeal metastases).

Six patients had their tumors partially resected, while three only had biopsies (Table 1). The eight patients with localized tumor all completed the prescribed radiotherapy (total dose 54 Gy). The patient with disseminated disease received 39.6 Gy of CSI fractionated over 22 days. Response after the induction phase was stable disease or a minor volume reduction in five patients and possible pseudoprogression in the other four, who were reassessed with MRI after 45 days, with tumor dissemination found in one case and stable disease in three. One of the patients with pseudoprogression (and disseminated tumor at diagnosis) concomitantly developed hydrocephalus and needed a ventriculo-peritoneal shunt.

With a median observation period of 42.5 months (1<sup>st</sup> and 3<sup>rd</sup> quartiles: 38.6-46.3 months), the PFS and OS

**Table 1.** Patient Characteristics.

patient	age	sex	tumor location	type of surgery	outcome (days)		I <sup>^</sup> radiotherapy	reirradiation
					PFS	OS		
#1	3	F	pineal	partial	193	300	focal	yes craniospinal
#2	15	F	thalamic	partial	180	460	focal	yes focal
#3	3	F	bithalamic	biopsy	260	390	focal	yes focal
#4	13	F	thalamic	partial	270	545	focal	yes focal
#5	14	F	thalamic	biopsy	300	305	focal	no
#6	26	M	paratrighonal+ leptomeningeal	partial	366	402	craniospinal 39.6 Gy	no
#7	6	F	ponto-cerebellar	partial	385	434	focal	no
#8	14	M	thalamic	biopsy	1095	1095	focal	no
#9	1	F	pontine and cerebellar (2 lesions)	partial	1151	1215	focal (posterior fossa and medulla up to C2)	yes focal

**Figure 2.** Overall survival (OS) and Progression free survival (PFS) across the patient cohort. In the x-axis the detailed number of alive patients.

(95%CI) at 1 year were 33.3% (13.2-84.0%) and 88.9% (70.6-100), respectively. At 2 and 3 years, the OS was 33.3% (13.2-84.0%) and 22.2% (6.6-75.4%), respectively (Figure 1). No patients died of toxicity (Figure 2).

Six patients had local progressions after a median of 12.7 months (range 5.6-38.6 months) from diagnosis. Three were re-irradiated with a total dose of 19.8 Gy; one relapsing with disseminated tumor had CSI, receiving a total dose of 32.4 Gy delivered in 18 days. Among the other patients with progressive disease, two underwent re-surgery at other centers, and were then treated with second-line chemotherapy, one developed a cerebral hemorrhage after radiological evidence of progressive disease, and one (treated with CSI at diagnosis) died during second-line chemotherapy two months after disease progression. Hydrocephalus requiring a shunt was present at diagnosis in five patients, while it developed during

treatment in one, and after tumor progression in one. The patient with BRAF-V600E mutation progressed six months after starting the treatment and further progressed after re-irradiation, she was then hospitalized due to deteriorating neurological conditions. She showed a rapid clinical and radiological response to dabrafenib and trametinib association, administered for compassionate purposes, which persisted for four months. One patient refused further treatment after six months, when her tumor was stable. Median survival after relapse was 6.4 months (range 1.7-25.6 months).

Two patients are long-term survivors. One was diagnosed at 20 months of age with two tumor localizations, one bulbo-pontine and the other (which was completely removed) cerebellar hemispheric. After radiotherapy, a reduction in the residual tumor persisted all along the two years of treatment, then a local relapse was observed after

38.6 months. Tumor control was achieved again with partial surgery, re-irradiation and the same systemic treatment. The other patient, with hereditary neurofibromatosis type 1, was diagnosed with thalamic disease at 14 years old. After completing the two years of treatment with stable disease, he is in good clinical conditions 38.6 months after primary surgery.

### Toxicity

Hematological profile of safety of this regimen has been already reported elsewhere in DIPG protocol.<sup>12</sup> However no patient delayed or modified treatment because of hematological or other toxicities. The cases of pseudoprogression, hydrocephalus and cerebral hemorrhage have already been described above; all these events were surely not drug-correlated.

### Discussion

H3K27M-mutant DMG carries a very grim prognosis. Overall survival is typically 7-11 months in children, and 8-19 months in adults.<sup>6</sup> Maximal safe resection is recommended, if feasible, but the role of surgery is often only diagnostic, radical removal being impossible due to the tumor's critical location. No standard therapy has been clearly identified as yet, but patients are generally given the same focal radiotherapy as for all aggressive gliomas.<sup>13,14</sup> While the effects of radiotherapy seem to be very short-lived, the role of chemotherapy still appears to be marginal. Temozolomide is indicated for adult glioblastoma, and still used as standard in pediatric patients as well. It seems to have no effect, however, partly because H3K27M-mutant gliomas are all MGMT(O<sup>6</sup>-methylguanine-DNA methyltransferase)-unmethylated, and therefore probably all resistant.<sup>15</sup> Over an accrual period of two years, we adopted the same strategy as for DIPG<sup>12</sup> for the few DMG patients referred to us. Having obtained results that compare favorably with those reported in previous trials<sup>5,16</sup> we have now included non-DIPG DMG patients in our current ongoing protocol as well (Eudract Number: 2015-002185-23). One of these was a multicentric randomized trial<sup>16,17</sup> designed for newly diagnosed pediatric high grade glioma that demonstrated a clear negative impact on survival for subgroup of 24 patients with midline K27M tumors [median event free survival (EFS)/OS: 7.9 and 14.2 months respectively]. The other was a large retrospective series<sup>5</sup> on diffuse midline glioma K27M-mutant (including DIPG) taken from German HIT-HGG registry that extrapolated data on the thalamic DMG cohort (24 patients) showing one year EFS and OS of 21 and 63% respectively. While there were no toxic deaths in our series, the treatment was associated with pseudoprogression in nearly half the patients after the first course of radiotherapy. When using target therapies like the anti-EGFR nimotuzumab, response to radiation may be

associated with tumor cell death, immune activation, and edema, leading to pseudoprogression.<sup>18</sup>

In line with some previous reports,<sup>19,20</sup> more than 40% of our patients (four of our nine patients) expressed Tp53-mutant tumors. This would seem to suggest a link with the severe prognosis for cases of DMG, but this remains to be seen in larger studies.

Some papers describe a favorable prognostic effect of pseudoprogression that we were unable to examine in our small series.<sup>21</sup> We also noted a relatively high ventricular shunting rate in our series, due to the large, deep-seated lesions involved, and the frequent tumor dissemination at both diagnosis and relapse, making our patients particularly fragile both at diagnosis and during the course of their disease. Re-irradiation nonetheless proved just as feasible in this subgroup of DMG patients as in cases of DIPG,<sup>12</sup> and is a second-line therapy always worth considering.

The identification of the H3K27M mutation in DMG and DIPG has prompted the testing of new potential target drugs in ongoing clinical trials. The hypomethylation associated with the H3K27M mutation suggests a correlation between chromatin dysregulation and malignancy in pediatric HGG. Novel agents that target the processes involved in epigenetic regulation are now under investigation, including histone deacetylase (HDAC) inhibitors like panobinostat and BET bromodomain inhibitors.<sup>22,23,24</sup> A phase 1 trial is currently examining the safety of administering panobinostat in children and young adults with DIPG, HGG and medulloblastoma (PBTC-047). Another ongoing phase 1 trial focuses on combinations with vorinostat and temsirolimus in children and young adults with DIPG (Eudract NCT02420613). ONC201 is a small molecule selective antagonist of dopamine receptor D2/3 (DRD2/3) that has shown anticancer activity in preclinical models of K27 mutant HGG.<sup>25</sup> DRD2 expression is highest in midline structures of the brain. Few reports<sup>26,27</sup> have described ONC201 as a potential target for DMG in recurrent adult and pediatric setting (including DIPG). A phase I study of oral ONC201 in pediatric patients with newly diagnosed DIPG and recurrent/refractory H3K27M gliomas is actually ongoing (Eudract NCT03416530).

Convection-enhanced delivery (CED) is also being studied as an attractive means of delivering therapeutics directly to DMG, as it bypasses the blood-brain barrier and enables the treatment of a relatively large volume of tissue with small amounts of infusate.<sup>28</sup> A recent study found that cell cultures from patients' H3K27M-mutant gliomas strongly expressed disialoganglioside (GD2) and, in cultured cells, anti-GD2 CAR T cells revealed a significant antigen-dependent cytokine production and killing of H3K27M-mutant DIPG cells that was not seen in a H3K27M-wild type cell culture. Anti-GD2 CAR T cells proved effective in orthotopically engrafted H3K27M tumors, although a significant toxicity was observed in treated mice.<sup>29</sup> Whether this strategy is

applicable to patients and sustainable remains to be seen. The co-occurrence of histone H3K27M and BRAF V600E mutations had already been described,<sup>9</sup> but is extremely rare in DMG. BRAF V600E mutation inhibitors should nonetheless be used when indicated. While the prospects of new findings emerging and being rapidly applied in clinical practice is exciting, we feel that our strategy – which has led to two of our nine patients achieving a remarkably long survival - remains an acceptable option until such time as the results of other trials become available.

## Table of abbreviations

CART cells	chimeric antigen receptor T cells
CED	convection-enhanced delivery
CNS	central nervous system
CSI	craniospinal irradiation
DIPG	diffuse intrinsic pontine gliomas
DMG	H3K27M-mutant diffuse midline glioma
EFS	event free survival
HGG	high grade glioma
IRB	institutional review board
LRFS	local relapse-free survival
MGMT	O <sup>6</sup> -methylguanine-DNA methyltransferase
MRI	magnetic resonance imaging
OS	overall survival
PFS	progression-free survival
PRS	post-relapse survival

## Acknowledgements

We thank Lega Italiana per la Lotta contro i Tumori (LILT Milano Monza Brianza) for continuous assistance of patients and family during treatment.

## Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

## Funding

The author disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was partially supported by the Italian Ministry of Health and by Regione Lombardia (project ID NET-2019-12371188 to Maura Massimino).

## ORCID iDs

Elisabetta Schiavello  <https://orcid.org/0000-0002-8049-7445>

Giovanna Gattuso  <https://orcid.org/0000-0002-8337-2752>

Francesco Barretta  <https://orcid.org/0000-0002-7797-161X>

## References

1. Roux A, Pallud J, Saffroy R, et al. High-grade gliomas in adolescents and young adults highlight histomolecular

- differences from their adult and pediatric counterparts. *Neuro Oncol* 2020; 22: 1190-1202.
2. Louis DN, Perry A, Reifenberger G, et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. *Acta Neuropathol* 2016; 131: 803-820.
3. Louis DN, Perry A, Wesseling P, et al. The 2021 WHO Classification of Tumors of the Central Nervous System: a summary. *Neuro Oncol* 2021; 23: 1231-1251.
4. Lu VM, Alvi MA, McDonald KL, et al. Impact of the H3K27M mutation on survival in pediatric high-grade glioma: a systematic review and meta-analysis. *J Neurosurg Pediatr* 2018; 23: 308-316.
5. Karremann M, Gielen GH, Hoffmann M, et al. Diffuse high-grade gliomas with H3 K27M mutations carry a dismal prognosis independent of tumor location. *Neuro Oncol* 2018; 20: 123-131.
6. Khuong-Quang DA, Buczkowicz P, Rakopoulos P, et al. K27M mutation in histone H3.3 defines clinically and biologically distinct subgroups of pediatric diffuse intrinsic pontine gliomas. *Acta Neuropathol* 2012; 124: 439-447.
7. Buczkowicz P, Hoeman C, Rakopoulos P, et al. Genomic analysis of diffuse intrinsic pontine gliomas identifies three molecular subgroups and recurrent activating ACVR1 mutations. *Nat Genet* 2014; 46: 451-456.
8. Wu G, Diaz AK, Paugh BS, et al. The genomic landscape of diffuse intrinsic pontine glioma and pediatric non-brainstem high-grade glioma. *Nat Genet* 2014; 46: 444-450.
9. Pagès M, Beccaria K, Boddaert N, et al. Co-occurrence of histone H3 K27M and BRAF V600E mutations in paediatric midline grade I ganglioglioma. *Brain Pathol* 2018; 28: 103-111.
10. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 2020; 92: 205-216.
11. Mondal G, Lee JC, Ravindranathan A, et al. Pediatric bithalamic gliomas have a distinct epigenetic signature and frequent EGFR exon 20 insertions resulting in potential sensitivity to targeted kinase inhibition. *Acta Neuropathol* 2020; 139: 1071-1088.
12. Massimino M, Biassoni V, Miceli R, et al. Results of nimotuzumab and vinorelbine, radiation and re-irradiation for diffuse pontine glioma in childhood. *J Neurooncol* 2014; 118: 305-312.
13. Cohen KJ, Jabado N and Grill J. Diffuse intrinsic pontine gliomas - current management and new biologic insights. Is there a glimmer of hope? *Neuro Oncol* 2017; 19: 1025-1034.
14. Hargrave D, Bartels U and Bouffet E (2006) Diffuse brainstem glioma in children: critical review of clinical trials. *Lancet Oncol* 2006; 7: 241-248
15. Cohen KJ, Pollack IF, Zhou T, et al. Temozolomide in the treatment of high-grade gliomas in children: a report from the Children's Oncology Group. *Neuro Oncol* 2011; 13: 317-323.
16. Grill J, Massimino M, Bouffet E, et al. Phase II, open-label, randomized, multicenter trial (HERBY) of bevacizumab in pediatric patients with newly diagnosed high-grade glioma. *J Clin Oncol* 2018; 36: 951-958.
17. Mackay A, Burford A, Molinari V, et al. Molecular, pathological, radiological, and immune profiling of non-brain-

- stem pediatric high-grade glioma from the HERBY Phase II randomized trial. *Cancer Cell* 2018; 33: 829-842.
18. Qiu B, Kline C and Mueller S. Radiation in combination with targeted agents and immunotherapies for pediatric central nervous system tumors - progress, opportunities, and challenges. *Front Oncol* 2021; 11: 674596.
  19. Dufour C, Perbet R, Leblond P, et al. Identification of prognostic markers in diffuse midline gliomas H3K27M-mutant. *Brain Pathol* 2020; 30: 179-190.
  20. Manjunath N, Jha P, Singh J, et al. Clinico-pathological and molecular characterization of diffuse midline gliomas: is there a prognostic significance? *Neurol Sci* 2021; 42: 925-934.
  21. Kruser TJ, Mehta MP and Robins HI. Pseudoprogression after glioma therapy: a comprehensive review. *Expert Rev Neurother* 2013; 13: 389-403.
  22. Grasso CS, Tang Y, Truffaux N, et al. Functionally defined therapeutic targets in diffuse intrinsic pontine glioma. *Nat Med* 2015; 21: 827.
  23. Piunti A, Hashizume R, Morgan MA, et al. Therapeutic targeting of polycomb and BET bromodomain proteins in diffuse intrinsic pontine gliomas. *Nat Med* 2017; 23: 493-500.
  24. Vitanza NA, Biery MC, Myers C, et al. Optimal therapeutic targeting by HDAC inhibition in biopsy-derived treatment-naïve diffuse midline glioma models. *Neuro Oncol* 2021; 23: 376-386.
  25. Cantor E, Wierzbicki K, Tarapore RS, et al. Serial H3K27M cell-free tumor DNA (cf-tDNA) tracking predicts ONC201 treatment response and progression in diffuse midline glioma. *Neuro Oncol*. Epub ahead of print 6 February 2022. DOI: 10.1093/neuonc/noac030.
  26. Chi AS, Tarapore RS, Hall MD, et al. Pediatric and adult H3 K27M-mutant diffuse midline glioma treated with the selective DRD2 antagonist ONC201. *J Neurooncol* 2019; 145: 97-105.
  27. Duchatel RJ, Mannan A, Woldu AS, et al. Preclinical and clinical evaluation of German-sourced ONC201 for the treatment of H3K27M-mutant diffuse intrinsic pontine glioma. *Neurooncol Adv* 2021; 3: vdab169.
  28. Himes BT, Zhang L and Daniels DJ. Treatment strategies in diffuse midline gliomas with the H3K27M mutation: the role of convection-enhanced delivery in overcoming anatomic challenges. *Front Oncol* 2019; 9: 31.
  29. Mount CW, Majzner RG, Sundaresh S, et al. Potent antitumor efficacy of anti-GD2 CAR T cells in H3-K27M<sup>+</sup> diffuse midline gliomas. *Nat Med* 2018; 24: 572-579.