Received: 19 May 2020 | Revised: 28 October 2020 | Accepted: 5 November 2020

DOI: 10.1002/ijc.33438

CANCER THERAPY AND PREVENTION

IJC International Journal of Cancer

Upfront treatment with mTOR inhibitor everolimus in pediatric low-grade gliomas: A single-center experience

Antonella Cacchione[1] | Mariachiara Lodi[1] | Andrea Carai[2] | Evelina Miele[1] | Marco Tartaglia[3] | Giacomina Megaro[1] | Giada Del Baldo[1] | Iside Alessi[1] | Giovanna Stefania Colafati[4] | Alessia Carboni[4] | Luigi Boccuto[5,6] | Francesca Diomedi Camassei[7] | Giuseppina Catanzaro[8] | Agnese Po[9] | Elisabetta Ferretti[8] | Lucia Pedace[1] | Simone Pizzi[3] | Valentina Folgiero[1] | Marco Pezzullo[10] | Tiziana Corsetti[11] | Domitilla Elena Secco[12] | Maria Giuseppina Cefalo[1] | Franco Locatelli[1,13] | Angela Mastronuzzi[1]

[1]Department of Paediatric Haematology/Oncology, Cell and Gene Therapy, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy

[2]Neurosurgery Unit, Department of Neuroscience, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy

[3]Genetics and Rare Diseases Research Division, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy

[4]Neuroradiology Unit, Department of Imaging, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy

[5]School of Nursing, College of Behavioral, Social and Health Sciences, Clemson University, Clemson, South Carolina

[6]JC Self Research Institute of the Greenwood Genetic Center, Greenwood, South Carolina

[7]Department of Laboratories, Pathology Unit, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy

[8]Department of Experimental Medicine, Sapienza University, Rome, Italy

[9]Department of Molecular Medicine, Sapienza University, Rome, Italy

[10]Research Laboratories, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy

[11]Hospital Pharmacy Unit, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy

[12]PsD of Department of Paediatric Haematology/Oncology, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy

[13]Sapienza University of Rome, Italy

Correspondence
Angela Mastronuzzi, Neuro-Oncologist, Department of Onco-hematology, Cell and Gene Therapy, Ospedale Pediatrico Bambino Gesù, Piazza Sant'Onofrio, 4, 00165 Rome, Italy.
Email: angela.mastronuzzi@opbg.net

Abstract

Pediatric low-grade gliomas (pLGGs) are the most frequent brain tumor in children. Adjuvant treatment, consisting in chemotherapy and radiotherapy, is often necessary if a complete surgical resection cannot be obtained. Traditional treatment approaches result in a significant long-term morbidity, with a detrimental impact on quality of life. Dysregulation of the mitogen-activated protein kinase (MAPK) pathway is the molecular hallmark of pLGGs and hyperactivation of the downstream mammalian target of rapamycin (mTOR) pathway is frequently observed. We report clinical and radiological results of front-line treatment with everolimus in 10 consecutive patients diagnosed with m-TOR positive pLGGs at the Bambino Gesù Children's Hospital in Rome, Italy. Median duration of treatment was 19 months (range from 13-60). Brain

Abbreviations: AG, angiocentric glioma; BR, best radiological response; BSA, bovine serum albumin; CNS, central nervous system; CR, complete response; CTCAE, Common Terminology Criteria for Adverse Events; DNET, dysembryoplastic neuroepithelial tumor; EPIC, HumanMethylation EPIC BeadChip; FFPE, formalin-fixed paraffin-embedded; GG, ganglioglioma; IHC, immunohistochemistry; IMP, improvement; MAPK, mitogen-activated protein kinase pathway; MRI, magnetic resonance imaging; mTOR, mammalian target of rapamycin; NF1, neurofibromatosis type 1; NS, Noonan syndrome; PAs, pilocytic astrocytomas; PD, progressive disease; pLGGs, pediatric low-grade gliomas; p-mTOR, phosphorylated mTOR; PR, partial response; RT, room temperature; SD, stable disease; SEGA, sub-ependymal giant astrocytoma; SIOP, International Society of Paediatric Oncology; TS, tuberous sclerosis.

magnetic resonance imaging showed stable disease in 7 patients, partial response in 1 and disease progression in 2. Therapy-related adverse events were always reversible after dose reduction or temporary treatment interruption. To the best of our knowledge, this is the first report of everolimus treatment for chemo- and radiotherapy-naïve children with pLGG. Our results provide preliminary support, despite low sample size, for the use of everolimus as target therapy in pLGG showing lack of progression with a manageable toxicity profile.

KEYWORDS

everolimus, MAPK pathway, mTOR, pediatric low-grade gliomas

1 | INTRODUCTION

Pediatric low-grade gliomas (pLGGs) represent the most common tumors of the central nervous system (CNS). Median age at diagnosis is around 6 to 8 years, and about 7% of patients are younger than 1 year of age.[1,2] Histologically, they are a heterogeneous group of Grade I and II tumors of glial origin, according to the latest version of 2016 WHO classification.[3]

Surgery is the mainstay of pLGG treatment. Complete resection of the tumor alone is feasible in 60% of cases,[4] and a diagnostic biopsy is possible in over 95%.[5] However, unresectable residual tumors might have a tendency to progress clinically and radiologically, requiring further treatment[6] in a percentage ranging from 25% to 35% of patients.[4] Adjuvant treatment is based on chemotherapy and the standard of care is the scheme combining carboplatin and vincristine.[7] Well-known morbidities related to chemotherapy consist of neuropathy, myelosuppression and consequent high risk of infections requiring hospitalization and a worsening of quality of life, ototoxicity, nausea and vomiting, carboplatin hypersensitivity reaction.

Radiation therapy is reserved to 30% of patients, particularly in certain scenarios like older children or after failure of multiple treatment lines.[8] Despite their overall good prognosis, pLGGs can cause significant long-term morbidity due to the disease and its treatments. Particularly, radiotherapy may cause a decline in neurocognitive function, vasculopathy and malignant transformation of primary tumor. All these sequelae have a loud for patients, their families and society. Therefore, side effect reduction and quality of life preservation are increasingly been valorized as the main goal of any proposed therapy.

The main molecular alterations shown by LGG relate to the activation of the mitogen-activated protein kinase pathway (MAPK) pathway. In the majority of these cases, either duplication or point mutations of the BRAF gene[9] are identified, allowing to consider signal transducers belonging to the MAPK pathway as potential target for therapy. Patients negative for *BRAF* mutations/rearrangements, instead, are not eligible for these treatments. Interestingly, evidence of mechanistic target of rapamycin (mTOR) iper-activation was observed in pLGGs associated with syndromic conditions, as tuberous sclerosis (TS) and neurofibromatosis type 1 (NF1) which has paved the way toward the clinical use of mTOR inhibitors, like everolimus.[10-16]

What's new?

Traditional therapeutic approaches for pediatric low-grade glioma (pLGG) center on surgery and radiotherapy. Radiotherapy in particular, however, can have long-term, detrimental impacts on patient health. Here, the authors investigated the use of everolimus as a post-surgical, front-line targeted therapy in a group of 10 chemo- and radiotherapy-naïve children with pLGGs harboring alterations in mTOR signaling pathways. Front-line everolimus therapy was associated with a high rate of clinical response, with disease stabilization in the majority of patients. Adverse effects were resolved following temporary everolimus interruption. These preliminary findings warrant further investigation of everolimus as a targeted therapeutic strategy for mTOR-positive pLGG.

However, activation of the mTOR pathway is not restricted to pLGGs associated with the aforementioned conditions. In fact, mTOR activation has been reported in sporadic Pilocytic Astrocytomas (PAs), the most frequent pLGG histotype, as well as in in vitro primary models of pLGG belonging to less frequent histological variants, such as angiocentric glioma (AG), dysembryoplastic neuroepithelial tumor (DNET) and ganglioglioma (GG).[11,12]

In the current study, we present preliminary results on the first ever reported use of everolimus as upfront postsurgery treatment in mTOR/phospho-mTOR-positive pLGGs.

2 | MATERIALS AND METHODS

2.1 | Study design

This is a retrospective, single-center study to evaluate the efficacy of everolimus as front-line therapy for pLGGs after surgery.

2.2 | Patients' characteristics

Patients referred to Bambino Gesù Children's Hospital in Rome from September 2014 to September 2019 with a pLGG requiring adjuvant treatment, according to International Society of Paediatric Oncology (SIOP)[4] criteria (Table 1), and positive (any score) for mTOR/phosphorylated mTOR (p-mTOR^{Ser2448}) on immunohistochemistry (IHC) were treated with everolimus as upfront therapy. We excluded subependymal giant cell astrocytoma (SEGA) associated with TS histology and BRAFV600-mutated tumors. All patients had undergone a surgical procedure at the time of presentation, maximal safe resection was performed when it was possible and otherwise patients received a surgical biopsy.

The eligibility to treatment for each patient was approved by the institutional ethical committee. Written informed consent was obtained from parents or legal guardians according to our ethical committee guidelines before enrolment.

2.3 | Histopathological and molecular analysis

All tumor samples were evaluated for activation of the mTOR pathway. IHC was performed for mTOR/p-mTOR^{Ser2448}. Briefly, 3-μm paraffin-embedded (FFPE) sections were dehydrated, pretreated with avidin and biotin block for 15 minutes at room temperature (RT), then incubated with bovine serum albumin (BSA) for 30 minutes at RT and overnight at 4°C with monoclonal rabbit antibodies against mTOR (Cell Signaling Technology, 1:50 dilution, PT-link pretreatment at high pH) and p-mTOR^{Ser2448} (Cell Signaling Technology, 1:100 dilution, PT-link antigen retrieval at high pH). Incubation with biotinylated secondary antibodies for 15 minutes at RT and with alkaline phosphatase-conjugated streptavidin (DAB) for 15 minutes at RT was performed. IHC was also performed in six tumor samples for one epitope of phosphorylated S6 ribosomal protein (p-S6Ser$^{235/236}$), based on the availability of the samples. Staining for each stain was scored on a scale between 0 and 3, assessing both the percentage of positive tumor cells (extension) and the staining intensity (Table 2). The expression of mTOR, phospho-mTOR and pS6 was examined by a specialized neuropathologist (FDC) without any information regarding clinicopathologic features or prognosis. Immunostaining was semi-quantitatively evaluated by using the percentage score of positive cells (extension): negative 0% to 5% (0); weakly positive 6% to 25% (1); moderately positive 26% to 50% (2); and strongly positive 51% to 100% (3). Since cytoplasmic intensity on neoplastic cells was not homogeneous, we arbitrarily grouped cases by their overall median intensity, as weak (1), moderate (2) and strong staining intensity (3).

Both peripheral venous blood and FFPE material were available from subject #2, #3,#4, #8 and #9. Genomic DNA was extracted using the automatic MagPurix method (Zinexts, Life Science Corporation) according to the manufacturer protocols. In particular, 250 ng and 135 ng of DNA were used as input material, respectively, from FFPE tissues and peripheral blood.

TABLE 1 Time point evaluation to start everolimus (modified from SIOP-E-BTG and GPOH Guidelines for diagnosis and treatment of children and adolescents with low-grade glioma[4]

Significant neurological symptoms (at diagnosis and/or following observation)
• Diencephalic syndrome
• Focal neurologic deficits
• Drug resistant seizures subsequent to tumor growth
• Focal increased intracranial pressure subsequent to tumor growth
• Symptomatic metastases
Ophthalmologic symptoms (at diagnosis and/or following observation)
• Definitive anamnestic loss of vision
• Borderline vision
• Reduction of residual low level vision/visual field
• Nystagmus subsequent to visual impairment in infants
• Any visual loss in the second eye when the first eye is blind
Radiological criteria
• Increase of tumor volume of >25% (the increase of the diameter of the optic nerve should be indicated separately)
• Involvement of previously uninvolved areas
• Appearance of new lesions
• Increase of the number and/or size of metastases
Prognostic risk factor
• Neurofibromatosis type 1
• Age
• Diencephalic syndrome
• Diffuse glioma WHO grade II
• Dissemination
• Tumor location (brain stem, chiasm/hypothalamus and thalamus)
• Extent of resection
• Biomarkers

For FFPE samples, tumor areas with the highest tumor cell content (≥70%) were selected for DNA extraction. Tumor samples were analyzed for DNA methylation profiling, according to protocols approved by our institutional review board with written consent obtained from patient's parents. The samples were analyzed using Illumina Infinium HumanMethylationEPIC BeadChip (EPIC) arrays according to the manufacturer's instructions as previously described.[13,14] Generated methylation data were compared to the Heidelberg brain tumor classifier 11b4[3] to assign a subgroup score for the tumor compared to 91 different brain tumor entities. Table 2 summarizes methylation classes and their relative score.

DNA from both peripheral blood and brain tumor was amplified and sequenced by 150-bp paired-end sequencing on Illumina NextSeq550 (Illumina, San Diego, CA) to characterize single-nucleotide variants and loss of heterozygosity. A total of 554 genes were included in the sequencing panel (available on request) using custom-designed NimbleGen SeqCap probe hybridization (Roche

TABLE 2 Pathological and clinical features

N. Pts	Sex	Age	Tumor location	Symptoms at diagnosis	Histology	WHO grade	Inherited conditions	mTOR staining (extension)	mTOR staining (intensity)	Phospho mTOR staining (extension)	Phospho mTOR staining (intensity)	p-S6%	p-S6 intensity	BRAF status	BRAF fusion	Methylation profile	Simptomps before everolimus start	Dose mg/day
1	F	4 y 5 mo	Mesencephalic tectum	Macrocrania, triventricular hydrocephalus	Grade I glioneuronal tumor, NOS	I	NE	3	1	1	1	2	2	WT	NE	NE	Radiological progression	2,5 mg
2	F	18 y 6 mo	Right ventriculus	Right hemianopsia	Grade I glioneuronal tumor, NOS	I	NE	3	3	3	3	NE	NE	WT	KIAA1549: BRAF 16:11	Pilocytic astrocytoma (score 0.33) LGG, subclass hemispheric pilocytic astrocytoma and ganglioglioma (score 0.28)	Visual impairmen	5 mg
3	F	7 y 5 mo	Right thalamus	Hyposthenia to the left hemisome	Pilocytic astrocytoma	I	NE	2	2	1	3	1	2	WT	NO fusion	Pilocytic astrocytoma (score 0.79) LGG, subclass posterior fossa pilocytic astrocytoma (score 0.73)	Focal motor deficit	2,5 mg to >5 mg
4	F	10 years 2 mo	Right tempomedial cortex	Seizures	Grade I glioneuronal tumor, NOS	I	Noonan Syndrome PTPN11 *c.922A> G*	3	2	2	2	NE	NE	WT	NE	Pilocytic astrocytoma (score 0.71) LGG, subclass hemispheric pilocytic astrocytoma and ganglioglioma (0.69)	Visual impairmen	5 mg to >2, 5 mg to >5 mg and 2,5 mg every other day
5	F	7 y	3rd ventriculus	Macrocrania	Pilocytic astrocytoma	I	*NF1*	3	2\3	3	2\3	NE	NE	WT	NE	NE	Radiological progression	2.5 mg to ≥5 mg
6	F	6 y 11 mo	Left frontoparietal cortex	Seizures	DNET	I	NE	3	1	3	2	2	2	WT	NO fusion	Low grade glioma, dysembryoplastic neuroepithelial tumor (score 0.99)	Worsening seizures	5 mg
7	M	12 y 11 mo	Optic pathway—diencephalic	Visual impairment	Pilocytic astrocytoma	I	NE	2	2	1	1	NE	NE	WT	NE	NE	Visual impairmen	5 mg
8	M	4 y 10 mo	Right talamus	Headache	Pilocytic astrocytoma	I	NE	3	2	2	2	2	3	WT	NE	NE	Focal neurological deficits	2,5 mg
9	M	4 y 10 mo	Left frontal lobe, corpus callosum	Development delay	Diffuse glioma	II	Absence of NF1 mutations	3	1	1	3	2	2	NE	NE	Low-grade glioma, MYB/MYBL1 (score 0.38)	Worsening of neurological deficit	2,5 mg
10	F	8 y 1 mo	Suprasellar/ chiasmatic	Visual impairment	Pilocytic astrocytoma	I	NE	2	2	3	3	1	3	WT	NE	NE	Visual impairmen	5 mg

Note: All patients' tumor tissues were evaluated for activation of the mTOR pathway. Immunohistochemistry (IHC) was performed for mTOR/phosphorylated mTOR (p-mTOR). Staining for extension was scored on a scale between 0 and 3: negative 0% to 5% (0), weakly positive 6% to 25% (1), moderately positive 26% to 50% (2) and strongly positive 51% to 100% (3). Staining for median intensity was scored on a scale between 0 and 3: weak (1), moderate (2) and strong (3). RAS/RAF/MEK pathway genes including $BRAF^{V600E}$ mutation were also investigated for the indicated patients.

Abbreviations: DA, diffuse astrocytoma, DNET, neuroepithelial dysembryoplastic tumor; F, female; GN, glioneuronal; M, male; NE: not evaluated, PA, pilocytic astrocytoma.

NimbleGen, Inc., Madison, WI). Comparison between blood and brain tumor from the same patient allowed us to discriminate somatic from germline variants. To this aim, both the HaplotypeCaller and muTect2 algorithms were used in the bioinformatic analysis workflow (GATK v3.7, http://www.broadinstitute.org/gatk/). Variants of possible clinical interest were extracted by setting filtering criteria on population frequency as well as disease associations and in silico prediction of impact, as reported in Table 5. When available, genomic DNA from parents' blood specimens was used to perform segregation analysis using Sanger sequencing.

2.4 | Treatment

Everolimus was administered orally once per day at the dose of 2.5 mg for children <30 kg or 5 mg for children ≥30 kg (ie, 5 mg/m^2).[10] Dosing was subsequently adjusted to attain blood concentrations ranging between 3 and 8 ng/mL.[15]

Treatment duration was not predetermined. Reasons to discontinue everolimus administration included evidence of disease progression and/or unacceptable Grade 4 toxicity, according to Common Terminology Criteria for Adverse Events, CTCAE v5.0.[16] Temporary suspension of the treatment with everolimus was recommended in case of thrombocytopenia (Grade 2), neutropenia (Grade 3) or any nonhematologic Grade 3 toxicity. Discontinuation of treatment was mandatory in case of any Grade 4 toxicity.

2.5 | Treatment response evaluation

Neurological evaluations were scheduled every 3 months during the first year of treatment and every 6 months thereafter; in children with optic pathway gliomas, ophthalmologic assessments were included.

Each evaluation was performed according to the European SIOP Brain Tumor Imaging Group consensus for LGG.[4] Complete response (CR) was defined as no evidence of all measurable disease (residual or new lesion); partial response (PR) as a reduction of tumor volume ≥ 50% compared to the pretreatment MRI; improvement (IMP) as reduction of the tumor volume between 50% and ≥ 25%; stable disease (SD) as tumor volume between +25% and −25%; progressive disease (PD) as an increase of tumor volume of ≥25% or appearance of a new lesion. Growth of cystic parts was not considered as PD.[15]

3 | RESULTS

3.1 | Patients population

Ten patients (7 females, 3 males) received everolimus as front-line treatment postsurgery. Five patients received a surgical biopsy, and five patients underwent to maximal safe resection. Median age was 7.3 years (range 4-18). Time interval between diagnosis and treatment initiation was 21 months (range 5-40).

Clinical suspicion of NF1 and NS in two patients was confirmed by genetic analysis. Detailed features of each case are summarized in Table 2.

3.2 | Histology and molecular features

Tumor location was supratentorial in all cases, with three patients having optic pathway involvement.

Histologically, nine (90%) tumors were WHO grade I and one (10%) was grade II (Table 2).

Details of tumor immunostaining for mTOR, p-mTOR ^{Ser2448} and p-S6$^{Ser235/236}$ are shown in Table 2 and Figure 1.

In five patients, there was tumor tissue sufficient to perform methylation profiling and molecular analysis (Table 2). A methylation class was attributed in four cases, with an optimal score in two cases. Molecular analysis revealed clinically/functionally relevant somatic and germline variants in Patients #2, #3 and #4 (Table 3). Patient #2 was previously analyzed for constitutional molecular alteration, without any finding about MAPK molecular alteration. The tumor sample was negative for TSC1/2 mutations (although radiologically no SEGA, according to the inclusion criteria).

3.3 | Treatment

Two patients were treated after a ≥25% increase in tumor volume on MRI. Six patients, in the absence of radiological progression, started everolimus for clinical worsening. Among them, three had visual impairment due to optic pathway lesions and one for a large temporal tumor compressing visual tracts. One child had drug-resistant epilepsy and one showed worsening of focal neurological deficit. In two cases, everolimus was started at the time of diagnosis for focal motor deficit due to thalamic location of the lesions (Table 2). Oral treatment with everolimus continued for at least 1 year, with a median duration of 19 months (range 13-60 months).

At the time of best radiological response (BR), follow-up MRI evaluations showed SD in seven patients (median time of 9 months), PR in one patient (after 18 months of treatment) and PD in two cases (respectively at 18 and 36 months of therapy) (Figure 2 and Table 4). No CR was observed.

Among the two patients enrolled due to radiological progression, Patient #1 showed an MRI stability of 6 months after everolimus start treatment and experienced this result for 18 months; Patient #5 obtained a radiological SD without any worsening of symptoms for 60 months (Table 2 and Table 4).

The two patients with documented radiological PD did not show concomitant clinical worsening of preexisting ocular motility impairment in one case and of hemiparesis in the second one. The only patient showing PR after 18 months of treatment had a glioneuronal tumor of the right lateral ventricle. Average time to BR was 9 months (range 6-48 months). The longest lasting response was obtained in an NF1 patient with PA of the III ventricle, who

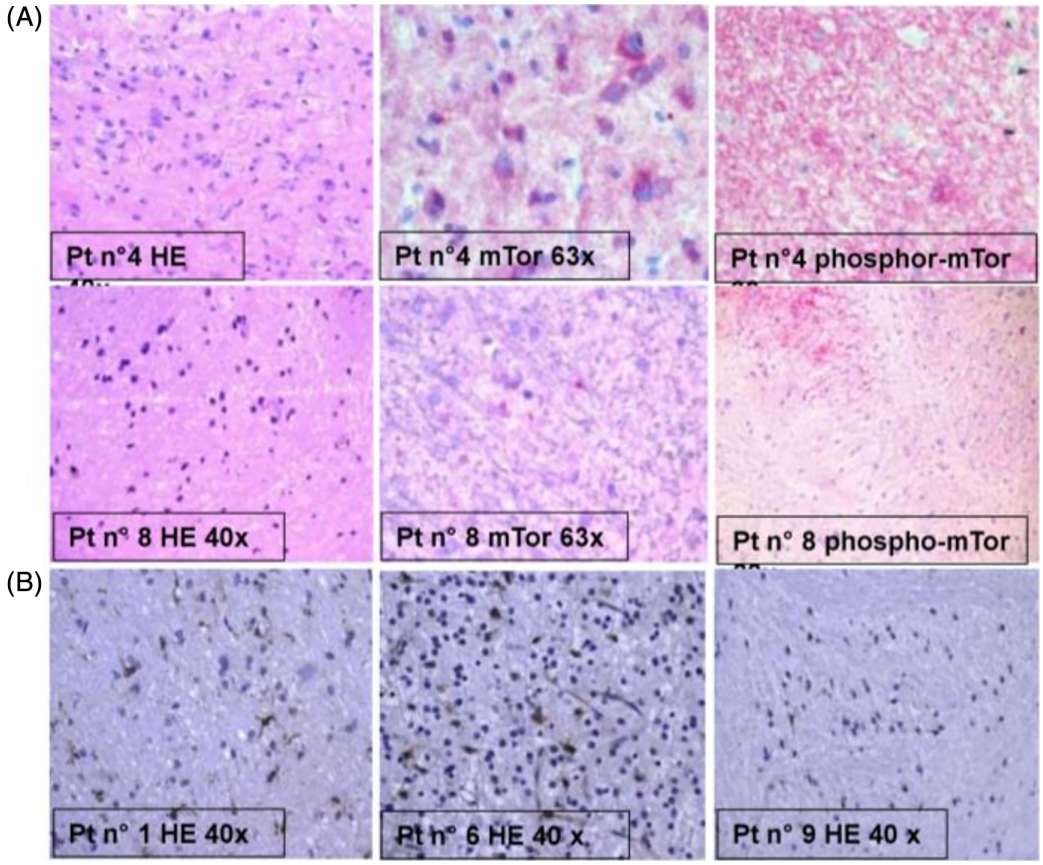

FIGURE 1 A, Low-grade gliomas and activation of the mTOR pathway (left column: hematoxylin eosin; middle column: mTor stain; right column: phospho-mTor stain. Alkaline phosphatase chromogen, hematoxylin counterstain). Pt n°4: from left to right: low-grade glioneuronal tumor showing intense/multifocal mTor positivity (+++/++) and strong/diffuse phospho-mTor positivity (+++/+++). Pt n°8: from left to right: pilocytic astrocytoma showing mild/focal mTor positivity (+/+) and moderate/focal phospho-mTor positivity (++/+). B, Low-grade gliomas and p -S6Ser$^{235/236}$ expression. Pt n°1, n°6, n°9 express a moderate p-S6 positivity

TABLE 3 Clinical and radiological evaluation after treatment with everolimus

Pt n°	Clinical features during treatment	Time of best radiological response (mo)	B.R.	F.U. (mo)	D.T. (mo)	Stop therapy
1	Clinical stability	6	PD	18	18	18 mo for radiological progression
2	Clinical stability	18	PR	48	49	on going
3	Clinical stability	12	PD	36	36	36 mo for radiological progression
4	Clinical stability	18	SD	36	38	on going
5	Clinical stability	48	SD	60	60	60 mo for medical decision
6	Clinical stability	9	SD	12	12	12 mo for parental decision
7	Clinical stability	9	SD	18	21	on going
8	Clinical stability	6	SD	12	14	on going
9	Clinical stability	9	SD	12	14	on going
10	Improvement of visual field	9	SD	12	13	on going

Abbreviations: BR, best response; DT, duration of treatment; mo, months; PD, disease progression; PR, partial response; SD, stable disease; y, years.

presented SD at 60 months and interrupted treatment thereafter, with evidence of stable disease at the latest follow-up visit after further 8 months.

Treatment was also interrupted in two patients after evidence of radiological PD, after 18 months in one case and after 36 months in the second one, and in a third child with persistent pharmacoresistant epilepsy whose parents finally reconsidered the option of a second surgical procedure (Table 4). Six patients are still on everolimus monotherapy and no rebound effect has been observed after therapy discontinuation in others.

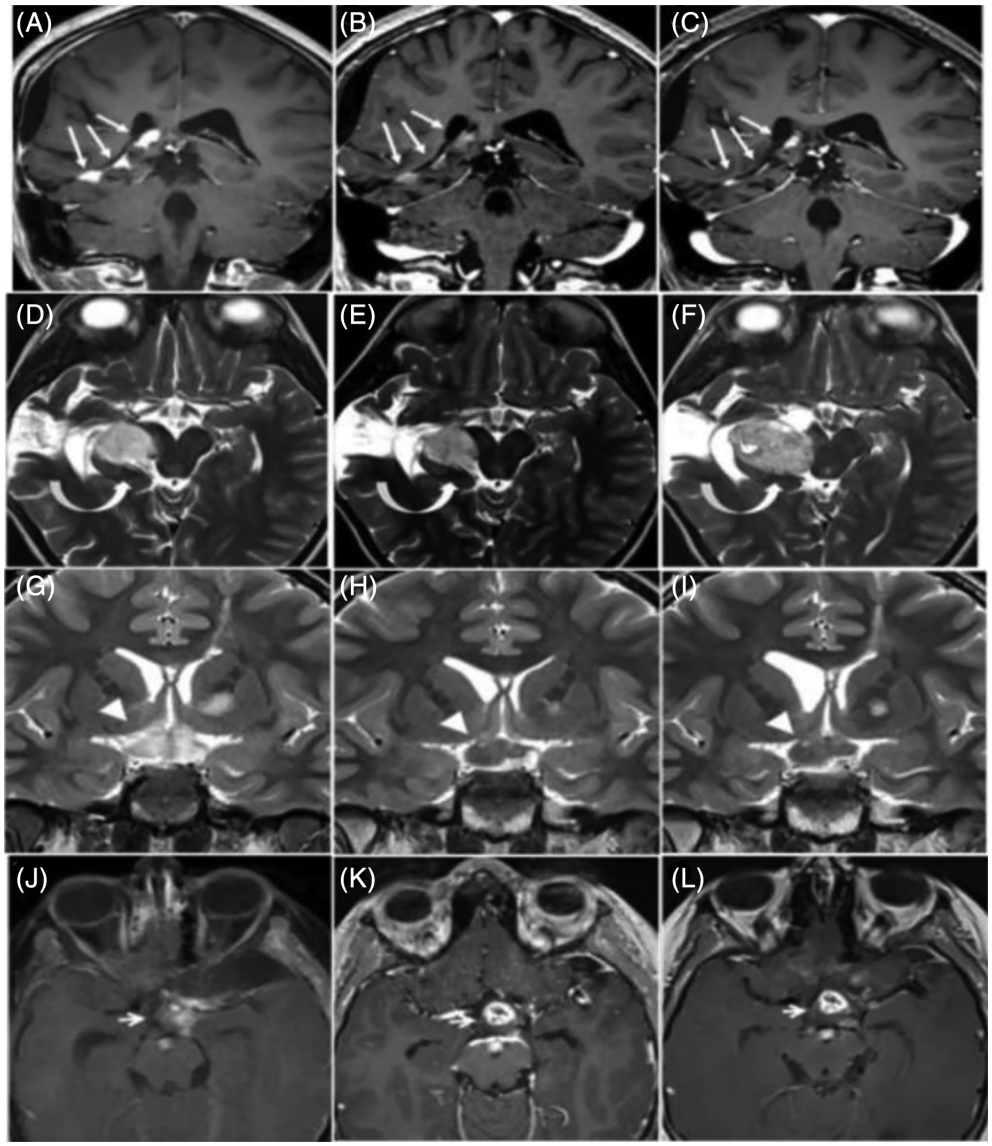

FIGURE 2 MRI. A, Pt #2 (a,b, c): Coronal T1w images show the progressive reduction, PR (more than 50%) of the residual tumor areas (arrows) from the baseline (a) and during treatment at 18 mo (BR, b) and at 4 years (c). B, Pt #3 (d,e,f): Axial T2w images demonstrate the progressive tumor growth, PD, (curved arrows) during the treatment, from the baseline (d), at 12 mo (BR, e) and at 3 years (f). C, Pt #5 (g,h,i): Coronal T2w images show the tumor volume (arrow head) at baseline (g), and during treatment at 48 months (BR, h) and at 5 years (i), demonstrating stable disease, SD. D) Pt #10 (l,m,n): Axial Gd T1 images demonstrate the presence of the residual lesion (arrows) after surgery (l), at the baseline (m) before the treatment and at 1 year after treatment (SD, n)

3.4 | Tolerability

All patients had at least one adverse event (Table 5) and some patients experienced multiple side effects. Grade 1/2 stomatitis and dry skin were the most frequent adverse events. Grade 3/4 stomatitis was observed in two patients. Five patients had G2 alterations in lipid metabolism (hypertriglyceridemia/hypercholesterolemia).

One patient each had Grade 3/4 joint pain and bacterial pneumonia (20%) without neutropenia, which did not require hospital admission. Resolution of symptoms was observed after temporary suspension of the drug with no recrudescence after resumption of everolimus. No life-threatening events were observed. At the time of the submission of our article, only two of the seven cases in SD have suspended the treatment with everolimus. In one case, the patients' parents requested to withdraw after 12 months of treatment with no medical reasons: after 13 months from the suspension of everolimus, we have only detected a minor degree of tumor progression via imaging, but no clinical effects. The other patient interrupted the treatment after 60 months and after another 12 months is still in SD. We believe that both the number of cases and the time occurred since the interruption of the treatment are not sufficient to draw significant

TABLE 4 Adverse events during treatment with everolimus

Adverse events	Grades 1–2 (n of pts)	Grades 3–4 (n of pts)
Stomatitis	5	2
Dry skin	7	0
Lipid metabolism alteration	5	0
Acneiform dermatitis	4	0
Joint pain	2	1
Respiratory tract infections	0	1
Diarrhea	0	1
Headache	1	0
Decreased white-cell count	1	0

Note: Common Terminology Criteria for Adverse Events (version 5.0).

TABLE 5 High-quality, functionally relevant variants with unknown, private or low population frequency (gnomAD MAF < 0.1% and frequency < 1% within our ~2000 exomes in-house database)

N. Pts	Gene	Nt change	Aa change	Somatic/germline	Allelic fraction (%)	gnomAD MAF	dbSNP	OMIM	InterVar	CADD
2	***RAF1***	**c.97G>A**	**p.Val33Ile**	**g**	**46**	—	—	**611 553, 611 554, 615 916**	**VoUS**	**22.8**
	NCOA2	c.2795T>C	p.Leu932Ser	g	46	—	—	—	VoUS	23.6
	SYVN1	c.1811G>A	p.Arg604His	g	43	0.000026	rs762623189	—	VoUS	24.9
	PALB2	c.3428T>A	p.Leu1143His	g	47	0.0005	rs62625284	114 480,610 832,613 348	VoUS	27.1
	RPL5	c.545G>A	p.Gly182Asp	s	5	0.000008	rs749153960	612 561	VoUS	32
	NOS3	c.2651C>T	p.Pro884Leu	s	3	—	—	104 300, 189 800, 601 367	VoUS	23.3
	PARN	c.647C>T	p.Thr216Ile	s	3	—	—	616 353, 616 371	VoUS	24.9
3	*KDR*	c.3686G>A	p.Arg1229Gln	g	52	0.00004	rs776015468	602 089	VoUS	22.8
	FBXW7	**c.925C>T**	**p.Arg309Cys**	**g**	**45**	—	—	—	**Likely pathogenic**	**33**
	ATM	c.6248G>A	p.Gly2083Glu	g	48	0.00005	rs1060501559	208 900	VoUS	27.6
	CHEK2	**c.1035A>C**	**p.Glu345Asp**	**g**	**46**	**0.0001**	**rs587780190**	**114 480,176 807,259 500**	**VoUS**	**24.3**
4	*IGF2BP2*	c.1415C>T	p.Ala472Val	g	48	0.00005	rs751050074	—	VoUS	23.1
	PTPN11	**c.922A>G**	**p.Asn308Asp**	**g**	**44**	**0.0002**	**rs28933386**	**151 100,156 250,163 950**	**Likely pathogenic**	**23.7**
	SETD1A	c.2432A>G	p.Asn811Ser	g	50	0.0003	rs201168236	618 832	VoUS	22.9
	NF1	**c.1885G>A**	**p.Gly629Arg**	**s**	**33**	—	**rs199474738**	**114 500,162 200,162 210**	Pathogenic	**20.7**
8	*ZKSCAN7*	c.753_756delTCAG	p.Ser251fs	g	43	0.0001	rs755376386	—	—	23.6
	SETD2	c.3244G>A	p.Glu1082Lys	g	53	0.00004	rs764499297	144 700, 601 626	VoUS	26.9
	WEE1	c.218_219delCC	p.Pro73fs	s	12	—	—	—	—	27
	MCM5	c.204C>G	p.Tyr68*	g	52	—	—	617 564	Pathogenic	36
	BCL11A	c.1112A>C	p.Gln371Pro	s	18	0.0008	—	617 101	VoUS	23.7
9	*NTRK1*	c.482G>A	p.Arg161His	g	53	0.0007	rs150271893	256 800	VoUS	20.5
	CTNNB1	c.238G>C	p.Ala80Pro	g	40	—	—	114 500,132 600,155 255	VoUS	22.8
	EGFR	c.2996G>A	p.Arg999His	g	42	0.0005	rs149248025	211 980,616 069	VoUS	28.3

Note: gnomAD MAF: Genome Aggregation Database v2.1.1: https://gnomad.broadinstitute.org/. dbSNP: NCBI database of genetic variation (https://www.ncbi.nlm.nih.gov/snp/). Functional impact of variants was assessed by Combined Annotation Dependent Depletion v.1.4 (CADD score > 20) (http://cadd.gs.washington.edu/) and Intervar v2.0.1 (http://intervar.wglab.org), which classifies variants according to the American College of Medical Genetics and Genomics guideline. VoUS: Variant of Uncertain significance. Variants in bold are discussed in the main text.

conclusions on the effects of the suspension of everolimus, although an abrupt worsening of the clinical presentation does not seem likely.

4 | DISCUSSION

Low-grade gliomas are the most common variant of pediatric brain tumors, accounting for 40% to 50% of CNS neoplasms, with an excellent long-term survival rate.[17] The treatment paradigm consists of maximal safe surgical resection and subsequent adjuvant chemoradiotherapy for symptomatic or progressive residual disease.[18]

The most widely used chemotherapy regimen consists of a combination of carboplatin and vincristine[7] that has been included in the latest LGG protocol elaborated by the SIOP,[19] with a 3- and 5-year progression-free survival rate of 68 ± 7% and 35% to 45%, respectively.

More recently, a combinational regimen of thioguanine, procarbazine, lomustine, and vincristine, monotherapy with etoposide, bevacizumab, temozolomide or weekly vinblastine[19-23] have been proposed, none of these proving to be superior to the standard of care.[1,20]

Radiation therapy is currently limited to non-NF1 older children, because long-term radio-induced side effects on the developing nervous system, including cognitive impairment, have been extensively documented.[24] However, radiotherapy remains an option for small volume lesions not involving functionally relevant brain structures to radiation damage or in cases of progressive disease after multiple treatment lines.[4]

In fact, experiences accumulated in the last two decades suggest a significant window of improvement both on efficacy and on toxicity profile. As pLGG proves to be a chronic condition, functional outcomes are progressively gaining importance as a treatment endpoint.[25]

Recently, the extensive molecular profiling of pLGG has unveiled the possibility of different targeted therapy options with promising results. Targeted therapy regimens offer the theoretical benefit of a chemo-free approach, with potential advantages on treatment burden and quality of life. On the other hand, the need of tumor tissue to investigate potential targets requires a surgical procedure, exposing some children to additional risks.

Enhanced activation of the MAPK pathway is the hallmark of pLGGs. In most cases, increased signal flow through this cascade results from activated mutations or rearrangements of the *BRAF* gene. Interestingly, mTOR activation has also been documented in most of these tumors.[21,26] Consistently, in these patients, therapies targeting the BRAF kinase in currently used with relatively positive response. Alteration of the BRAF/MEK/MAPK pathway with activation of the phosphatidylinositol 3-kinase (PI3K) pathway is the hallmark of pLGGs and mTOR activation has been documented in most of these tumors.[18,27] mTOR is a downstream target of the PI3K/AKT pathway and its unregulated activation leads to abnormal cell growth, proliferation and angiogenesis.[28,29]

Immunohistochemistry for downstream targets of PI3K, including mTOR/pmTOR, phosphorylated ribosomal S6 (p-S6) is seen in over half of patients with LGG.[26] Furthermore, PS6 pathway activation is associated with worse overall survival. Together, these data suggest that PI3K pathway activation is an important driver of glioma progression, and thus may be a promising avenue for targeted therapy pS6.[26]

Use of the mTOR inhibitor everolimus in the treatment of children with tuberous sclerosis and subependymal giant cell astrocytoma has shown a tumor response in about 75% of cases.[10] These data suggest that mTOR-positive pLGGs may also benefit from everolimus treatment. In fact, multiple clinical trials are ongoing to test its efficacy in recurrent or progressive LGG in both adults and children.[30,31]

To the best of our knowledge, our retrospective series is the first to report everolimus in chemotherapy and radiotherapy-*naïve* children. Five patients, based on the localization of the brain tumor, were not eligible for surgery, and therefore a diagnostic biopsy was performed. Of the five patients who underwent surgery, none achieved complete remission. Hence, everolimus was proposed as an upfront postsurgery treatment.

Treatment with everolimus was evaluated on the basis of three criteria: (a) radiological progression as in the case of Patients #1 and #5; (b) thalamic localization with consequently associated motor deficit; (c) worsening of tumor-related symptoms.

We observed SD in 7 of 10 patients (70%) and PR in one case (10%), with an overall favorable outcome in 80% of the children. The BR was documented after a median time of 9 months of treatment. No patient developed signs of increased intracranial pressure, hydrocephalus or visual impairment during treatment. In the two children (20%) presenting PD, it occurred after 18 and 36 months of treatment, respectively. Nine out of 10 patients did not show during treatment a worsening of the clinical symptoms already present at onset; this finding was also observed in the two patients with documented progression of radiological disease. It should be noted that Patient #10, enrolled for visual impairment, showed improvement in the visual field.

Only two of the seven cases in SD have suspended the treatment with everolimus: one patient after 12 months of treatment, due to parental decision, and after 13 months we have only detected some tumor progression via imaging, but no clinical effects; the other patient interrupted the treatment after 60 months and after another 12 months is still in SD. We believe that both the number of cases and the time occurred since the interruption of the treatment are not sufficient to draw significant conclusions on the effects of the suspension of everolimus, although an abrupt worsening of the clinical presentation does not seem likely.

These efficacy data are promising compared to the current standard of care[22] despite the limitations related to the small population investigated and the limited follow-up. Moreover, they are similar to what has recently been reported for everolimus use in NF1 patients.[12]

The molecular hallmark of pLGGs is activating somatic mutations in genes of the RAS/MAPK pathway, leading to downstream activation of the PI3K/AKT pathway and ultimately of the mTOR pathway.

The assessment of the mTOR activation was a critical step of our study because it allowed us to confirm the molecular target for our hypothesis based on treatment with everolimus. However, since we did not screen the cancer cells for mutations in the PI3K/AKT/mTOR genes, we can only hypothesize that the major genetic alterations involve the RAS/MAPK pathway and that the mTOR pathway activation is a secondary event. Therefore, inhibition of such pathway by everolimus may only block one of the downstream pathways activated by RAS/MAPK. For this reason, we preferred to not limit our hypothesis to a specific outcome of the everolimus treatment on the pLGG clinical course. Instead, we elected to maintain an unbiased position, reasoning that everolimus might have induced a positive response in cancer cells, affecting to various extents the cancer growth and proliferation: this could have been reflected in either disease arrest, stabilization or regression. After conducting the study and collecting the data, we reported that indeed the disease stabilization was the most common outcome.

Considering that the inhibition of the PI3K/AKT pathway by everolimus blocks only one of the downstream pathways regulated by the activated RAS/MAPK in pLGGs, it was challenging to predict a specific outcome of the everolimus treatment on the pLGG clinical course. Indeed, the everolimus inhibitory effect might have affected to various extents the cancer growth and proliferation, causing either disease arrest, stabilization, or regression of the neoplasia. For this reason, we elected to evaluate the effect of the treatment after collecting the data without establishing preventively the expected outcome. The results of the study indicated the disease stabilization as the most common outcome of the treatment with everolimus in children with pLGGs.

To note that Patient #5 enrolled due to a radiological progression showed the longest SD duration (60 months) (Table 4 and Figure 2). Interestingly, we did not document any rebound effect after treatment discontinuation. This is different from what has been previously reported for other target drugs[32,33] and might be a specific feature of everolimus, a hypothesis that remains to be confirmed in a larger series and with a longer follow-up.

Semi-quantitative IHC assessment of mTOR, p-mTOR^{Ser2448} and p-S6$^{Ser235/236}$ is a cheap and readily available technique. Evidence of mTOR and p-mTOR^{Ser2448} positivity as an inclusion criterion resulted with a good outcome in 70% of our cases.

The expression in terms of both extension and intensity of mTOR and pmTOR is particularly high in the patient who presented a partial response (#2). This finding would seem to correlate with the good radiological response to treatment. A correlation between the intensity of mTOR and the outcome has not been demonstrated in the literature. Since Patient #2 underwent a biopsy, the scarce material available did not allow the analysis of p-S6 to be performed. All six tumors analyzed for p-S6 by IHC (#1, #3, #6, #8, #9, #10) were positive. The two patients who showed radiological progression (#1 and #3) had histological expression weak and moderate positive for mTOR and moderate positive for pS6, respectively. A correlation between increased p-SE expression and a lower PSF has already been found in studies conducted on a population of adult and LGG patients. However, it is not possible from the data at our disposal to demonstrate a certain correlation between the greater expression of p-S6 and a worse prognosis as previously demonstrated in the literature.[34]

On the other hand, in line with the literature, clinical/radiological response did not correlate to IHC intensity score (Table 2).[15] This observation suggests that combination of IHC technique with systematic mutation analysis focused on the PI3K/mTOR pathway might allow a more refined stratification of patients for targeted therapies in the future. The screening of the MAPK and PI3K/mTOR pathways could provide useful information for therapies targeting these pathways or for identifying patients that could be eligible for combined therapies.[35]

Molecular analysis identified one germline variant of uncertain significance (VoUS) c.97G>A (p.[Val33Ile]) in the gene *RAF1* in Patient #2. The gene encodes a paralogous of BRAF kinase (MAP3K), which functions downstream of the Ras family of membrane-associated GTPases. Furthermore, although the variant does not fall in a functional domain, it is extremely rare in the population and affects a conserved amino acid. Interestingly, gain of function mutations in *RAF1* is associated with Noonan syndrome 5 (OMIM# # 611553).[36]

Although Patient #2 does not present evident clinical signs of Noonan syndrome, we cannot exclude an altered cellular phenotype and a role of this variant in response to everolimus therapy.

In Patient #3, genetic analysis highlighted one germline VoUS (c.1035A>C; p.Glu345Asp, rs587780190) in the tumor-suppressor gene *CHEK2*. Interestingly, mutations in *CHEK2* are linked with Li-Fraumeni syndrome and are considered to confer a predisposition to sarcomas, breast cancer and brain tumors.[37] Moreover, in Patient #3 was detected one likely pathogenetic variant in the gene *FBXW7* (c.925C>T, p.Arg309Cys). *FBXW7* is a tumor-suppressor gene encoding a member of the F-box protein, one of the four subunits of ubiquitin protein ligase complex called SCFs (SKP1-cullin-F-box), which function in phosphorylation-dependent ubiquitination and subsequent proteasomal degradation of target proteins. Identified substrates include oncoproteins such as cyclin E, c-Myc, Mcl-1, mTOR, Jun and Notch1.[38] This evidence supports the hypothesis of a critical role of FBW7 in cancer development and progression including glioma.[35,37] The variant affects a highly conserved and structurally relevant amino acid within the F-box domain, suggesting a putative deleterious role in overall structure rearrangement of the ubiquitin complex. Finally, molecular analysis confirmed in Patient #4 the presence of the pathogenic germline variant in *PTPN11* (c.922A>G, p.Asn308Asp, rs28933386). In this patient, clinical diagnosis of Noonan syndrome was previously formulated, but molecular analysis of genomic DNA failed to identify point mutations and large deletions/duplications in the *NF1* gene.[39]

Several limitations of our study should be noted. We have described a limited cohort of patients; molecular analyses were not available for all tumor and peripheral blood sample, thus we were not able to perform a critical correlation between clinical, radiological response and a possible activated molecular pathway. Unfortunately, DNA specimens from patients' parents were not available for genotyping.

Our study confirms the previously reported high treatment compliance and manageable toxicity profile of everolimus.[9,10,30] The few adverse effects recorded (of which stomatitis was the most frequent) promptly resolved after temporary drug suspension and did not recur thereafter without any adjusted dose. No life-threatening events were observed.

At the time of this report, six patients are still on everolimus monotherapy. Among the three children where treatment was discontinued for evidence of progression, two were finally reconsidered for a further surgical debulking and one switched to chemotherapy according to LGG2004 protocol.

Our study underlines the need of a surgical biopsy to molecularly characterize brain tumors and identify possible target treatments. However, this has been shown to be feasible with an acceptable risk profile by dedicated pediatric neurosurgical teams.[40]

Several MEK inhibitors are currently in development for pLGG such as trametinib, selumetinib and cobimetinib. Selumetinib was the first MEK inhibitor showing promising activity in a pediatric.

LGG phase I/II trial was efficacious and well tolerated in a subsequent phase II trial comprising different clinical and molecular strata with good safety profile.[41,42] Results of trametinib confirm these observations[43,] indicating in-class drug activity of MEK inhibitors in sporadic progressive LGGs. In BRAFV600-mutated pLGG, the use of vemurafenib has been well explored.[42,43]

Compared to the literature, our study evaluated everolimus as front-line therapy. Among different MAP-K pathways exists a crosstalk leading to compensatory activation of one of the pathways if the other pathway is inhibited.[44] Blocking different pathways simultaneously may represent an important treatment strategy in pLGG.[45]

At the time of the publication, no other studies about everolimus as front-line adjuvant therapy have been published; a phase II trial about everolimus as single drug in recurrent refractory pLGG is still ongoing,[46] and no results are currently available.

At time of writing, all patients are alive; the extension phase of our study is still ongoing, and efficacy and safety data will continue to be monitored.

5 | CONCLUSIONS

Our data, albeit preliminary, support the use of everolimus in children with mTOR-positive LGG, making it a viable alternative when needed to first-line conventional adjuvant treatment.

The greatest positive impact observed in association with the treatment with everolimus was the arrested progression of the disease. We believe this still represents a favorable outcome for an adjuvant pharmacological therapy, or, even more so, for the management of unresectable LGGs: as many other forms of brain tumors, preventing the mass expansion may prevent a series of dramatic side effects and may have a relevant impact on the patients' quality of life. Only two of the seven cases in SD have suspended the treatment with everolimus: one patient after 12 months of treatment, due to parental decision, and after 13 months we have only detected some tumor progression via imaging, but no clinical effects; the other patient interrupted the treatment after 60 months and after another 12 months is still in SD. We believe that both the number of cases and the time occurred since the interruption of the treatment are not sufficient to draw significant conclusions on the effects of the suspension of everolimus, although an abrupt worsening of the clinical presentation does not seem likely.

This "chemo- and radio-free" first-line therapeutic approach results in a clinical and radiological stabilization of the disease in the majority of the children in our cohort, reducing side effects, hospitalization and potentially delaying the need for conventional treatments.

ACKNOWLEDGEMENTS

We would like to thank all patients, their families, Megan Eckley and the HEAL Foundation for their support.

CONFLICT OF INTEREST

The authors have no competing interests to declare.

DATA AVAILABILITY STATEMENT

Data will be made available upon reasonable request.

ETHICS STATEMENT

Authors obtained written informed consent from the patients (or their legal representatives) and from Ethical Committee for the publication. Such consent has been designed in accordance with the internal policy approved by the ethical committee of the Bambino Gesù Hospital (approval code 349/RA, n°1301/2014).

REFERENCES

1. Gnekow AK, Falkenstein F, von Hornstein S, et al. Long-term follow-up of the multicenter, multidisciplinary treatment study HIT-LGG-1996 for low-grade glioma in children and adolescents of the German speaking Society of Pediatric Oncology and Hematology. *Neuro Oncol*. 2012;14(10):1265-1284. https://doi.org/10.1093/neuonc/nos202.
2. Stokland T, Liu J-F, Ironside JW, et al. A multivariate analysis of factors determining tumor progression in childhood low-grade glioma: a population-based cohort study (CCLG CNS9702). *Neuro Oncol*. 2010; 12(12):1257-1268. https://doi.org/10.1093/neuonc/noq092.
3. Wesseling P, Capper D. WHO 2016 classification of gliomas. *Neuropathol Appl Neurobiol*. 2018;44(2):139-150. https://doi.org/10.1111/nan.12432.
4. Gnekow AK, Kandels D, Tilburg VC, et al. SIOP-E-BTG and GPOH guidelines for diagnosis and treatment of children and adolescents with low grade glioma. *Klin Padiatr*. 2019;231(3):107-135. https://doi.org/10.1055/a-0889-8256.
5. Ater JL, Zhou T, Holmes E, et al. Randomized study of two chemotherapy regimens for treatment of low-grade glioma in young children: a report from the Children's Oncology Group. *J Clin Oncol*. 2012;30(21):2641-2647. https://doi.org/10.1200/JCO.2011.36.6054.
6. Terashima K, Chow K, Jones J, et al. Long-term outcome of centrally located low-grade glioma in children. *Cancer*. 2013;119(14):2630-2638. https://doi.org/10.1002/cncr.28110.
7. Packer RJ, Lange B, Ater J, et al. Carboplatin and vincristine for recurrent and newly diagnosed low-grade gliomas of childhood. *J Clin Oncol*. 1993;11(5):850-856. https://doi.org/10.1200/JCO.1993.11.5.850.

8. Bitterman DS, MacDonald SM, Yock TI, et al. Revisiting the role of radiation therapy for pediatric low-grade glioma. *J Clin Oncol off J Am Soc Clin Oncol*. 2019;37(35):3335-3339. https://doi.org/10.1200/JCO.19.01270.
9. Franz DN, Belousova E, Sparagana S, et al. Efficacy and safety of everolimus for subependymal giant cell astrocytomas associated with tuberous sclerosis complex (EXIST-1): a multicentre, randomised, placebo-controlled phase 3 trial. *Lancet Lond Engl*. 2013;381(9861):125-132. https://doi.org/10.1016/S0140-6736(12)61134-9.
10. Krueger DA, Care MM, Holland K, et al. Everolimus for subependymal giant-cell astrocytomas in tuberous sclerosis. *N Engl J Med*. 2010;363(19):1801-1811. https://doi.org/10.1056/NEJMoa1001671.
11. Hütt-Cabezas M, Karajannis MA, Zagzag D, et al. Activation of mTORC1/mTORC2 signaling in pediatric low-grade glioma and pilocytic astrocytoma reveals mTOR as a therapeutic target. *Neuro Oncol*. 2013;15(12):1604-1614. https://doi.org/10.1093/neuonc/not132.
12. Catanzaro G, Besharat ZM, Miele E, et al. The miR-139-5p regulates proliferation of supratentorial paediatric low-grade gliomas by targeting the PI3K/AKT/mTORC1 signalling. *Neuropathol Appl Neurobiol*. 2018;44(7):687-706. https://doi.org/10.1111/nan.12479.
13. Capper D, Jones DTW, Sill M, et al. DNA methylation-based classification of central nervous system tumours. *Nature*. 2018;555(7697):469-474. https://doi.org/10.1038/nature26000.
14. Petruzzellis G, Alessi I, Colafati GS, et al. Role of DNA methylation profile in diagnosing astroblastoma: a case report and literature review. *Front Genet*. 2019;10:391. https://doi.org/10.3389/fgene.2019.00391.
15. Fouladi M, Laningham F, Wu J, et al. Phase I study of everolimus in pediatric patients with refractory solid tumors. *J Clin Oncol off J Am Soc Clin Oncol*. 2007;25(30):4806-4812. https://doi.org/10.1200/JCO.2007.11.4017.
16. Common Terminology Criteria for Adverse Events (CTCAE) Protocol Development CTEP. https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm. Accessed May 1, 2020.
17. Pachow D, Wick W, Gutmann DH, Mawrin C. The mTOR signaling pathway as a treatment target for intracranial neoplasms. *Neuro Oncol*. 2015;17(2):189-199. https://doi.org/10.1093/neuonc/nou164.
18. Sievert AJ, Fisher MJ. Pediatric low-grade gliomas. *J Child Neurol*. 2009;24(11):1397-1408. https://doi.org/10.1177/0883073809342005.
19. Gnekow AK, Walker DA, Kandels D, et al. Corrigendum to "A European randomised controlled trial of the addition of etoposide to standard vincristine and carboplatin induction as part of an 18-month treatment programme for childhood (≤16 years) low grade glioma—A final report" [Eur J of Canc (2017) 206–225]. *Eur J Cancer Oxf Engl 1990*. 2018;90:156-157. https://doi.org/10.1016/j.ejca.2017.11.017.
20. Chintagumpala M, Eckel SP, Krailo M, et al. A pilot study using carboplatin, vincristine, and temozolomide in children with progressive/symptomatic low-grade glioma: a Children's Oncology Group study. *Neuro Oncol*. 2015;17(8):1132-1138. https://doi.org/10.1093/neuonc/nov057.
21. Packer RJ, Ater J, Allen J, et al. Carboplatin and vincristine chemotherapy for children with newly diagnosed progressive low-grade gliomas. *J Neurosurg*. 1997;86(5):747-754. https://doi.org/10.3171/jns.1997.86.5.0747.
22. Lassaletta A, Scheinemann K, Zelcer SM, et al. Phase II weekly vinblastine for chemotherapy-naïve children with progressive low-grade glioma: a Canadian Pediatric Brain Tumor Consortium Study. *J Clin Oncol*. 2016;34(29):3537-3543. https://doi.org/10.1200/JCO.2016.68.1585.
23. Hwang EI, Jakacki RI, Fisher MJ, et al. Long-term efficacy and toxicity of bevacizumab-based therapy in children with recurrent low-grade gliomas. *Pediatr Blood Cancer*. 2013;60(5):776-782. https://doi.org/10.1002/pbc.24297.
24. Williams NL, Rotondo RL, Bradley JA, et al. Late effects after radiotherapy for childhood low-grade glioma. *Am J Clin Oncol*. 2018;41(3):307-312. https://doi.org/10.1097/COC.0000000000000267.
25. Ater JL, Xia C, Mazewski CM, et al. Nonrandomized comparison of neurofibromatosis type 1 and non-neurofibromatosis type 1 children who received carboplatin and vincristine for progressive low-grade glioma: a report from the Children's Oncology Group. *Cancer*. 2016;122(12):1928-1936. https://doi.org/10.1002/cncr.29987.
26. McBride SM, Perez DA, Polley M-Y, et al. Activation of PI3K/mTOR pathway occurs in most adult low-grade gliomas and predicts patient survival. *J Neurooncol*. 2010;97(1):33-40. https://doi.org/10.1007/s11060-009-0004-4.
27. Pfister S, Janzarik WG, Remke M, et al. BRAF gene duplication constitutes a mechanism of MAPK pathway activation in low-grade astrocytomas. *J Clin Invest*. 2008;118(5):1739-1749. https://doi.org/10.1172/JCI33656.
28. Boulay A, Lane HA. The mammalian target of rapamycin kinase and tumor growth inhibition. *Recent Results Cancer Res*. 2007;172:99-124. https://doi.org/10.1007/978-3-540-31209-3_7.
29. Sandsmark DK, Pelletier C, Weber JD, Gutmann DH. Mammalian target of rapamycin: master regulator of cell growth in the nervous system. *Histol Histopathol*. 2007;22(8):895-903. https://doi.org/10.14670/HH-22.895.
30. Ullrich NJ, Prabhu SP, Reddy AT, et al. A phase II study of continuous oral mTOR inhibitor everolimus for recurrent, radiographic-progressive neurofibromatosis type 1-associated pediatric low-grade glioma: a Neurofibromatosis Clinical Trials Consortium Study. *Neuro Oncol*. 2020;22:1527-1535. https://doi.org/10.1093/neuonc/noaa071.
31. Kieran MW, Yao X, Macy M, et al. Final results of a prospective multi-institutional phase ii study of EVEROLIMUS (RAD001), an MTOR inhibitor, in pediatric patients with recurrent or progressive low-grade glioma. A poetic CONSORTIUM trial. *Neuro Oncol*. 2014;16(suppl 3):iii27-iii27. https://doi.org/10.1093/neuonc/nou208.15.
32. Karajannis MA, Legault G, Fisher MJ, et al. Phase II study of sorafenib in children with recurrent or progressive low-grade astrocytomas. *Neuro Oncol*. 2014;16(10):1408-1416. https://doi.org/10.1093/neuonc/nou059.
33. Sievert AJ, Lang S-S, Boucher KL, et al. Paradoxical activation and RAF inhibitor resistance of BRAF protein kinase fusions characterizing pediatric astrocytomas. *Proc Natl Acad Sci U S A*. 2013;110(15):5957-5962. https://doi.org/10.1073/pnas.1219232110.
34. Wahl M, Chang SM, Phillips JJ, et al. Probing the phosphatidylinositol 3-kinase/mammalian target of rapamycin pathway in gliomas: a phase 2 study of everolimus for recurrent adult low-grade gliomas. *Cancer*. 2017;123(23):4631-4639. https://doi.org/10.1002/cncr.30909.
35. Yang Y, Gao X, Zhang M, et al. Novel role of FBXW7 circular RNA in repressing glioma tumorigenesis. *JNCI J Natl Cancer Inst*. 2018;110(3):304-315. https://doi.org/10.1093/jnci/djx166.
36. Pandit B, Sarkozy A, Pennacchio LA, et al. Gain-of-function RAF1 mutations cause Noonan and LEOPARD syndromes with hypertrophic cardiomyopathy. *Nat Genet*. 2007;39(8):1007-1012. https://doi.org/10.1038/ng2073.
37. Wang Z, Inuzuka H, Zhong J, et al. Tumor suppressor functions of FBW7 in cancer development and progression. *FEBS Lett*. 2012;586(10):1409-1418. https://doi.org/10.1016/j.febslet.2012.03.017.
38. Yeh C-H, Bellon M, Nicot C. FBXW7: a critical tumor suppressor of human cancers. *Mol Cancer*. 2018;17:115. https://doi.org/10.1186/s12943-018-0857-2.
39. Lodi M, Boccuto L, Carai A, et al. Low-grade gliomas in patients with Noonan syndrome: case-based review of the literature. *Diagn Basel Switz*. 2020;10(8):582. https://doi.org/10.3390/diagnostics10080582.
40. Goodden J, Pizer B, Pettorini B, et al. The role of surgery in optic pathway/hypothalamic gliomas in children. *J Neurosurg Pediatr*. 2014;13(1):1-12. https://doi.org/10.3171/2013.8.PEDS12546.

41. Banerjee A, Jakacki RI, Onar-Thomas A, et al. A phase I trial of the MEK inhibitor selumetinib (AZD6244) in pediatric patients with recurrent or refractory low-grade glioma: a pediatric brain tumor Consortium (PBTC) study. *Neuro Oncol*. 2017;19(8):1135-1144. https://doi.org/10.1093/neuonc/now282.
42. Fangusaro J, Onar-Thomas A, Young Poussaint T, et al. Selumetinib in paediatric patients with BRAF-aberrant or neurofibromatosis type 1-associated recurrent, refractory, or progressive low-grade glioma: a multicentre, phase 2 trial. *Lancet Oncol*. 2019;20(7):1011-1022. https://doi.org/10.1016/S1470-2045(19)30277-3.
43. Kondyli M, Larouche V, Saint-Martin C, et al. Trametinib for progressive pediatric low-grade gliomas. *J Neurooncol*. 2018;140(2):435-444. https://doi.org/10.1007/s11060-018-2971-9.
44. Conciatori F, Bazzichetto C, Falcone I, et al. Role of mTOR signaling in tumor microenvironment: an overview. *Int J Mol Sci*. 2018;19(8):2453. https://doi.org/10.3390/ijms19082453.
45. Arnold A, Yuan M, Price A, Harris L, Eberhart CG, Raabe EH. Synergistic activity of mTORC1/2 kinase and MEK inhibitors suppresses pediatric low-grade glioma tumorigenicity and vascularity. *Neuro Oncol*. 2020;22(4):563-574. https://doi.org/10.1093/neuonc/noz230.
46. PNOC 001: Phase II Study of Everolimus for Recurrent or Progressive Low-grade Gliomas in Children—Full Text View—ClinicalTrials.gov. 2020. https://clinicaltrials.gov/ct2/show/NCT01734512. Accessed Apr 5, 2020

How to cite this article: Cacchione A, Lodi M, Carai A, et al. Upfront treatment with mTOR inhibitor everolimus in pediatric low-grade gliomas: A single-center experience. *Int. J. Cancer*. 2021;148:2522–2534. https://doi.org/10.1002/ijc.33438