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Journal

Cancer, 123(23)

ISSN

0008-543X

Authors

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Publication Date

2017-12-01

DOI

10.1002/cncr.30909

Peer reviewed

HHS Public Access

Author manuscript

Cancer. Author manuscript; available in PMC 2017 December 01.

Published in final edited form as:

Cancer. 2017 December 01; 123(23): 4631–4639. doi:10.1002/cncr.30909.

Probing the PI3K/mTOR Pathway in Gliomas: A Phase II Study of Everolimus for Recurrent Adult Low Grade Gliomas

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Abstract

Background—Activation of the PI3K/mTOR pathway is common in patients with low-grade gliomas (LGG), but agents that inhibit this pathway, including mTOR inhibitors, have not been studied in this population.

Methods—58 patients with pathologic evidence of recurrence after initially diagnosed WHO grade II gliomas were enrolled on a prospective phase II clinical trial and received daily everolimus (RAD001) for 1 year or until progression. Tissue at time of enrollment was analyzed for markers of PI3K/mTOR pathway activation. 38 patients underwent serial multiparametric MRI, with tumor volume and perfusion metrics (capillary density, fBV; vascular permeability, K_{ps})

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Conflicts of Interest

Michael Wahl: Employment, Illumina, Inc (Family Member); Stock ownership (Family Member)
Daphne Haas-Kogan: Leadership, Cellworks
Joseph Costello: Stock Ownership, Telo Therapeutics
Nicholas Butowski: Honoraria, Genentech, VBL Therapeutics, Omniox; Consulting or advisory role, Genentech; Research funding, VBL Therapeutics, Bristol-Myers Squibb, Stemline Therapeutics, Merrimack, Celldex
Michael Prados: Honoraria, Actelion; Consulting, Actelion
Susan Chang: Consulting, Neonc Technologies

measured during treatment. The primary endpoint was progression-free survival after 6 months (PFS-6) in patients with WHO II disease at enrollment.

Results—For WHO II gliomas at enrollment, PFS-6 was 84%, meeting the primary endpoint ($p<0.001$ for improvement from historical rate of 17%). Evidence of PI3K/mTOR activation by immunohistochemistry for phosphorylated S6 (p-$S6^{Ser240/244}$) was associated with worse PFS (HR 3.03, p=0.004) and overall survival (HR 12.7; p=0.01). Tumor perfusion decreased after 6 months (median decrease: fBV 15% p=0.03; K_{ps} 12%, p=0.09), with greater decreases associated with improved PFS (HR for each 10% fBV decrease: 0.71, p=0.01; HR for each 10% K_{ps} decrease: 0.82, p=0.04).

Conclusion—Patients with recurrent LGG demonstrate a high degree of disease stability during treatment with everolimus. PI3K/mTOR activation as measured by immunohistochemistry for p-S6 was associated with worse prognosis. Tumor vascular changes were observed consistent with antiangiogenic effects of mTOR inhibitors. Our results support further study of everolimus in LGG.

Keywords

Low-Grade Gliomas; PI3K Pathway; Everolimus; Perfusion Imaging; Clinical Trial

Introduction

The treatment paradigm for low-grade gliomas (LGG) has evolved rapidly in recent years to include systemic therapy. RTOG 9802 demonstrated a survival benefit with the addition of procarbazine, CCNU and vincristine (PCV) chemotherapy to adjuvant radiotherapy[1] and several phase II studies demonstrated efficacy with either PCV or temozolomide (TMZ) chemotherapy for patients with newly diagnosed or recurrent LGG[2–6]. However, there is no standard of care for patients with recurrent LGG, particularly for those who have undergone prior radiation, rendering a compelling need for effective therapy in this population.

While prior studies examined the use of cytotoxic chemotherapy, targeted molecular therapy is largely unstudied in LGG, with limited prior studies showing negative results[7]. Activation of the phosphatidylinositol 3-kinase (PI3K) pathway is commonly seen LGGs and secondary malignant gliomas, often through inactivation of PTEN, an inhibitory regulator of the pathway[8,9]. Immunohistochemistry for downstream targets of PI3K, including phosphorylated ribosomal S6 (p-S6) is seen in over half of patients with LGG, and is associated with PTEN promoter methylation[10]. Furthermore, pathway activation, as measured by these markers, 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.

Mammalian target of rapamycin (mTOR) is a downstream target of PI3K that, when activated, leads to abnormal cell growth, proliferation and angiogenesis[11,12]. Targeted therapy with the mTOR inhibitor everolimus (RAD001) is highly effective in the treatment of subependymal giant-cell astrocytomas (SEGAs) in patients with mutations in the tuberous sclerosis complex (TSC), a key inhibitor of mTOR[13], as well as in other solid malignancies

with PI3K pathway activation[14–16]. Here we present the results of a phase II clinical trial investigating the efficacy of everolimus in patients with recurrent LGG.

Patients and Methods

Patient Characteristics

Eligibility criteria included patients over age 18 with histologically proven supratentorial WHO grade II oligodendroglioma, astrocytoma or oligoastrocytoma at initial diagnosis, with any number of recurrences prior to enrollment and any WHO grade at the time of enrollment. Patients were required to have undergone either subtotal resection or biopsy for recurrence or progression within 4 months prior to enrollment, with unequivocal pathologic evidence for tumor recurrence as determined by a neuropathologist at our institution. If the most recent histology demonstrated progression to WHO grade III or IV glioma, patients were required to have undergone prior radiotherapy. Additional inclusion criteria were KPS (Karnofsky Performance Status) ≥ 60, adequate hematologic, liver and renal function on laboratory testing, a fasting cholesterol ≤ 300 mg/dL or ≤ 7.75 mmol/L and fasting triglycerides ≤ 2.5 times the upper limit of normal. Exclusion criteria included uncontrolled significant medical illness, history of malignancy in remission for less than 3 years, known HIV positive status or AIDS-related illness, gastrointestinal disease altering the absorption of everolimus, impaired lung function with O_2 saturation ≤ 88% on room air, patients who had received prior treatment with an mTOR inhibitor, or women who were pregnant, breastfeeding or of childbearing potential not using contraception. Our institutional review board approved the protocol, and all patients provided informed consent.

Treatment

Everolimus was provided by Novartis (Basel, Switzerland) and administered orally, 10 mg daily in a fasting state, continuously until disease progression, unacceptable toxicity or 12 months of treatment. Further treatment was allowed by investigator discretion. Dose delay and modifications were required for persistent thrombocytopenia ($<75 \times 19^9$/L), neutropenia (ANC $<1000/mm^3$), or any persistent non-hematologic grade 2 or 3 toxicity. Discontinuation of everolimus was required for any grade 4 toxicity, or any toxicity requiring treatment interruption for ≥ 28 days.

Patient Evaluations

Within 14 days prior to initiating therapy, all patients underwent a baseline evaluation, including medical history, neurologic examination, KPS, laboratory testing, pulse oximetry, chest radiograph, and brain MRI. Patients were assessed with neurologic examination, laboratory testing, and MRIs every 2 months during the first 12 months of treatment, and every 3 months thereafter.

Treatment Response Evaluation

Assessment of treatment response was based on RANO criteria[17,18], determined by MRI in conjunction with steroid requirement. Complete response was defined as disappearance of all measurable disease while on minimal or no steroids, partial response as a 50% reduction in the sum of products of perpendicular diameters of all measurable lesions, with no new

lesions on stable or decreasing doses of steroids, and progressive disease as a 25% increase in the sum of products of all measurable lesions, clear worsening of any evaluable disease, or any new lesions. All other situations were considered stable disease.

Molecular Analysis

All patients with available tissue were evaluated for activation of the PI3K/mTOR pathway. Immunohistochemistry (IHC) was performed for phosphorylated PRAS40 (p-PRAS40^{Thr246}), phosphorylated mTOR (p-mTOR^{Ser2448}), two epitopes of phosphorylated S6 ribosomal protein (p-S6$^{Ser240/244}$ and p-S6$^{Ser235/236}$), and phosphorylated 4EBP1 (p-4EBP1$^{Thr37/46}$); see Supplemental Data. Staining for each marker was scored on a continuous scale between 0 and 100%, denoting the percent of tumor cells staining positive. The evaluating neuropathologists were blinded to patient outcome. Patients also underwent testing for 1p/19q codeletion by fluoresence *in situ* hybridization (FISH) and for IDH1-R132H mutation by IHC, and were placed into three molecular subgroups according to 2016 WHO classification[19]: 1p/19q codeleted/IDH1 mutated (1p/19q codel), 1p/19q intact/IDH1 mutated (IDH1mut), and 1p/19q intact/IDH1 wild type (IDH1wt).

Multiparametic Imaging

A subset of 38 patients underwent research imaging at our institution with multiparametric MRI at the time of study enrollment, then every 2 months during treatment, including T2 fluid-attenuated inversion-recovery (FLAIR) imaging, T1 with/without gadolinium contrast, dynamic contrast-enhanced perfusion-weighted imaging (DCE-PWI) and diffusion-weighted imaging (DWI). Lesion volume was defined as the hyperintense region on FLAIR along with any contrast-enhancing region, and was manually defined. Quantitative metrics were then assessed within the tumor region at each timepoint, including K_{ps} (transfer coefficient) and fBV (fractional blood volume) from DCE-PWI, and ADC (apparent diffusion coefficient) from DWI.

Statistical Design

The primary endpoint was progression-free survival at 6 months (PFS-6), measured in patients with WHO grade II glioma at enrollment. Secondary endpoints included radiographic response rate, overall survival from the time of study enrollment (OS), and toxicity. Due to a dearth of clinical data in recurrent LGG at the time of study design, a null hypothesis was extrapolated from studies of malignant glioma showing a PFS-6 of 17%[20]; the study was powered to detect a difference in PFS-6 from 17% if the true PFS-6 was 40% with 90% power, using a one-tailed binomial exact test with α of 0.05. Based on this sample size calculation, enrollment of 40 patients with low-grade histology at enrollment was planned, with accrual of additional patients with high-grade histology allowed for correlative studies but not included in the primary and secondary endpoint analyses. The influence of clinical, molecular and radiographic parameters on survival was assessed by Cox proportional hazards analysis and changes in imaging parameters were assessed with Wilcoxon rank-sum test. Adjustment for testing of multiple imaging and molecular parameters was not performed, as results were considered to be hypothesis generating. Classification and regression tree (CART) analysis was performed to generate binary thresholds for continuous molecular markers. To minimize cohort heterogeneity, the

relationships between survival endpoints and clinical and molecular factors were assessed only in patients with WHO II glioma at enrollment.

Results

Patient Characteristics

Fifty-eight patients were enrolled on the trial from 2009 to 2015, with patient characteristics summarized in Table 1. 47 patients (81%) had WHO grade II disease at study enrollment, while 11 (19%) had WHO grade III/IV disease. Only one patient with WHO II disease was in the IDHwt molecular subgroup, so the 1p/19q intact/IDHmut and IDHwt groups were combined into a single group (1p/19q intact) for subsequent analysis.

Survival Endpoints

Median follow-up for all patients was 3.5 years; there were 45 progression events and 16 deaths during follow-up. All patients were evaluable for the primary endpoint of PFS-6. In patients with WHO Grade II disease at enrollment, we observe a PFS-6 of 84% (95% CI 71% – 94%), meeting the primary endpoint ($p<0.001$ for improvement from historical rate of 17%). The PFS-6 for patients with WHO grade III/IV disease at enrollment was 55% (95% CI 24% – 83%). Median PFS was 1.4 years (95% CI 1.2 – 1.9 years) and 0.6 years (95% CI 0.1 years – NA), while median OS was not reached (95% lower CI, 4.0 years) and 2.9 years (95% CI 1.6 years – NA), for WHO II and III/IV, respectively (Figure 1 A, B). Table 2 shows survival outcomes for patients with WHO II disease at enrollment according to clinical, molecular and radiographic factors.

Treatment Response and Disease Progression

There were no complete or partial radiographic responses. Seventeen patients (29%) progressed during the first year of therapy, including 10 with WHO II (21%) and 7 with WHO III/IV (63%) disease at enrollment. Twenty-seven patients (46%) completed at least one year of treatment without progression, of which 23 had WHO II disease at enrollment (48%). 12 patients discontinued treatment during the first year without progression, and 2 patients remain on treatment with less than 1 year of follow-up.

Treatment Compliance and Toxicity

The median treatment duration was 11 months (range, 1 – 28 months); eight patients (14%) discontinued treatment due to toxicity. There were no grade 4 or 5 toxicities; lesser grade toxicities are shown in Table 3.

PI3K/mTOR Pathway Activation

Activation of PI3K pathway at the time of recurrence was assessed for 50 patients (86%), including 41 of 47 (87%) with WHO II disease at enrollment, using IHC for multiple markers (Table 1). In general, patients with 1p/19q codeletion had lower median staining for PI3K activation compared with 1p/19q intact patients (Supplemental Table 1).

While staining for p-mTOR, p-PRAS40 and p-4EBP1 showed no relationship with survival outcomes (Supplemental Table 2), we observe a significant relationship between staining for

the p-S6$^{Ser240/244}$ epitope and shorter PFS (HR for each 10% increase in staining 1.23; 95% CI, 1.05 to 1.43) and OS (HR for each 10% increase 1.50; 95% CI, 1.17 to 1.91). p-S6$^{Ser240/244}$ staining was also associated with shorter OS as measured from the time of initial diagnosis (HR for each 10% increase 1.37; 95% CI, 1.12 to 1.67). Using a binary threshold determined by CART analysis with OS as endpoint, p-S6$^{Ser240/244}$ positivity (≥19%) was prognostic of shorter PFS (HR 3.03; 95% CI 1.42 to 6.47) and OS (HR 12.7; 95% CI 1.57 to 102) from time of study enrollment (Figure 1 E,F). Staining for a second p-S6 epitope (p-S6$^{Ser235/236}$) was performed independently, yielding similar results (Table 2). Positivity for p-S6 was the only clinical or molecular factor found to be prognostic of PFS.

Multiparametric MRI

In an exploratory analysis, multiparametric MRI was obtained at baseline and every two months on therapy for 38 patients (32 WHO II, 6 WHO III/IV), and quantitative metrics of tumor size (volume of FLAIR hyperintensity), cellular density (ADC from DWI) and vascular characteristics including capillary density and vascular permeability (fBV and K_{ps}, respectively, from DCE-PWI) were assessed. Baseline radiographic characteristics were not significantly associated with PFS (Supplementary Table 2). However, there was a significant decrease in median fBV after 6 months of therapy (median decrease 15%, IQR 4 – 39%, p=0.026) and a trend towards a significant decrease in median K_{ps} (median decrease 12%, IQR 2 – 23%, p=0.087). Decrease in both fBV and K_{ps} after 6 months was associated with improved PFS (HR for each 10% decrease in fBV 0.71, 95% CI 0.53 to 0.92; HR for each 10% decrease in K_{ps} 0.82, 95% CI 0.67 to 0.99). There were no significant changes in tumor volume or ADC during therapy (Table 4).

Discussion

While adjuvant radiotherapy and chemotherapy are now considered standard of care for most patients with newly diagnosed LGG[1], there is no standard treatment for patients at disease recurrence, particularly for patients who have already received radiotherapy. Thus there is a significant need for effective systemic therapy in patients with recurrent LGG, and an increasing interest in the incorporation of systemic agents into adjuvant therapy. Activation of the PI3K/mTOR pathway is common in adults with LGG[10], but the efficacy of agents inhibiting this pathway has not been prospectively studied.

We report results from a phase II study investigating the use of everolimus (RAD001), an mTOR inhibitor, in patients with recurrent LGG. Despite our study population consisting of pretreated patients, our study met the primary endpoint with a 6-month progression-free survival of 84% in patients with WHO II disease at enrollment. We also observe a high rate of disease stability, with 48% of patients completing one year of therapy without progression. Treatment compliance was high, with a manageable toxicity profile.

While the prespecified primary endpoint was met, conclusions regarding clinical efficacy are limited by the combination of our single arm study design and the lack of an appropriate historical control population at the time the study was designed, requiring extrapolation from data for patients with malignant glioma[20]. As a result, the population used to generate our

null hypothesis likely comprised a molecularly distinct group, predominantly IDH wild-type, in contrast to our study population of predominantly IDH mutated patients.

However, our median PFS of 17 months compares favorably to a median of 10 months from a study of recurrent LGG, reported after our study was designed[7]. Only 38% and 19% of patients in that study had received prior chemotherapy and radiotherapy, respectively, compared with 72% and 28%, respectively, in our study. While authors do not report PFS-6, precluding a direct comparison with our primary endpoint, power analysis for median PFS indicates that our study had 89% power to detect a significant difference in median PFS, with an alpha of 0.05. Thus, had our study been designed with this more recent data as a null hypothesis, it would have been powered to detect a significant improvement in median PFS. However, given that this was a post hoc analysis using an endpoint other than our primary endpoint, these results should be interpreted with caution. Thus, compared to the limited prior literature in recurrent LGG, our study shows encouraging results in a more heavily pretreated cohort. Given the dearth of data in this population, our results may serve as a historical comparator for future investigations.

To our knowledge, this is the first study evaluating mTOR inhibition in LGG. Prior studies evaluating mTOR inhibition in glioblastoma have yielded mixed results, with modest rates of response and scant evidence of improvement in clinical outcomes[21–24]. However, in contrast to LGG, glioblastomas are characterized by a higher mutational burden, with alteration of multiple redundant cell signaling pathways[25], suggesting that mTOR inhibition as monotherapy may provide limited clinical efficacy. Agents inhibiting mTOR may provide a benefit in higher grade tumors only in combination with other agents, an approach that has been successful in other solid tumors[14].

A unique strength of our study was that tissue confirming disease recurrence was obtained for all patients within 4 months prior to enrollment, allowing for comprehensive prospective assessment of molecular diagnosis[19] and of PI3K/mTOR pathway activation status at the time of treatment, which is of critical importance due to the known evolution of tumor genomic properties during the course of therapy[26]. Several markers of the PI3K/mTOR pathway were examined, but only p-S6 was found to be of significant prognostic value, with p-S6$^{Ser240/244}$ epitope positivity associated with worse PFS and OS from the time of study enrollment, and with worse OS from the time of initial diagnosis, indicating that activation of the PI3K/mTOR pathway may negatively affect overall disease prognosis. These results were also validated through independent staining for a separate epitope (S6$^{Ser235/236}$). Our results are consistent with prior retrospective studies demonstrating the prognostic value of p-S6[10], and thus further support the utility of p-S6 as a clinically meaningful marker of PI3K/mTOR pathway activation.

The fact that p-S6 remains a negative prognostic factor despite the use of mTOR inhibition does not imply a lack of drug efficacy, since our study lacked a control arm of p-S6 positive patients not receiving mTOR to serve as a comparator. Further work is needed to determine whether p-S6 serves as a predictive marker for efficacy of PI3K/mTOR pathway inhibition. Furthermore, there were too few patients in the IDHwt molecular subgroup to assess whether p-S6 remains prognostic after accounting for molecular status based on the 2016

WHO classification[19]; further work is also needed to validate p-S6 as an independent prognostic marker in addition to standard molecular markers.

In an exploratory analysis, we prospectively performed multiparametric MRI for 38 patients on the study, assessing tumor perfusion and diffusion properties at baseline and during treatment to allow for assessment of *in vivo* modulation of tumor physiologic properties with therapy. We found a substantial decrease in tumor vascular permeability and capillary density after 6 months of treatment, as measured by perfusion MRI. These alterations are consistent with the known antiangiogenic properties of mTOR inhibitors via modulation of downstream HIF-1α signaling[27,28]. We also found that larger decreases in both capillary density and vascular permeability during therapy were associated with improved PFS, suggesting these changes correspond to clinically meaningful outcomes. Given the lack of volumetric response commonly seen in LGG, assessment of validated radiographic markers probing tumor microstructure and vascular properties may be important components of future studies.

Several limitations to our study should be mentioned. First, while molecular and radiographic analyses were performed prospectively, data were not available for analyses for all patients on the study. Second, the binary threshold for p-S6 positivity derived from CART analysis has not been validated on an independent dataset. In addition, since patients underwent resection prior to study enrollment, our finding of decreased tumor perfusion during treatment is potentially confounded by post-operative changes unrelated to the effect of treatment. However, decreased perfusion was shown to be associated with improved PFS, which would not be expected from standard post-operative changes. These molecular and radiographic results should be considered exploratory in nature, and thus require further validation. Finally, there were few OS events in our cohort, so results relating to this endpoint should be interpreted with caution.

In conclusion, in a pretreated population of patients with recurrent LGG, we demonstrate a high rate of disease stability during therapy with everolimus, but conclusions from the study regarding clinical efficacy are limited by the lack of a control arm. However, we provide radiographic evidence of alteration in tumor vasculature during therapy, consistent with antiangiogenic properties of mTOR inhibitors. Prospective molecular analysis supports the use of p-S6 as a clinically meaningful prognostic marker in recurrent LGG. Our results support further investigation of the utility of everolimus in this population; a study for patients with newly diagnosed LGG is underway at our institution.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding: SPORE (P50 CA097257), Novartis and Dabbiere Family

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Summary

Inhibition of mTOR with everolimus yielded a high rate of clinical stability in a phase II clinical trial of patients with recurrent low-grade gliomas. Activation of the PI3K/mTOR pathway appears to be an important molecular prognostic marker of clinical outcomes.

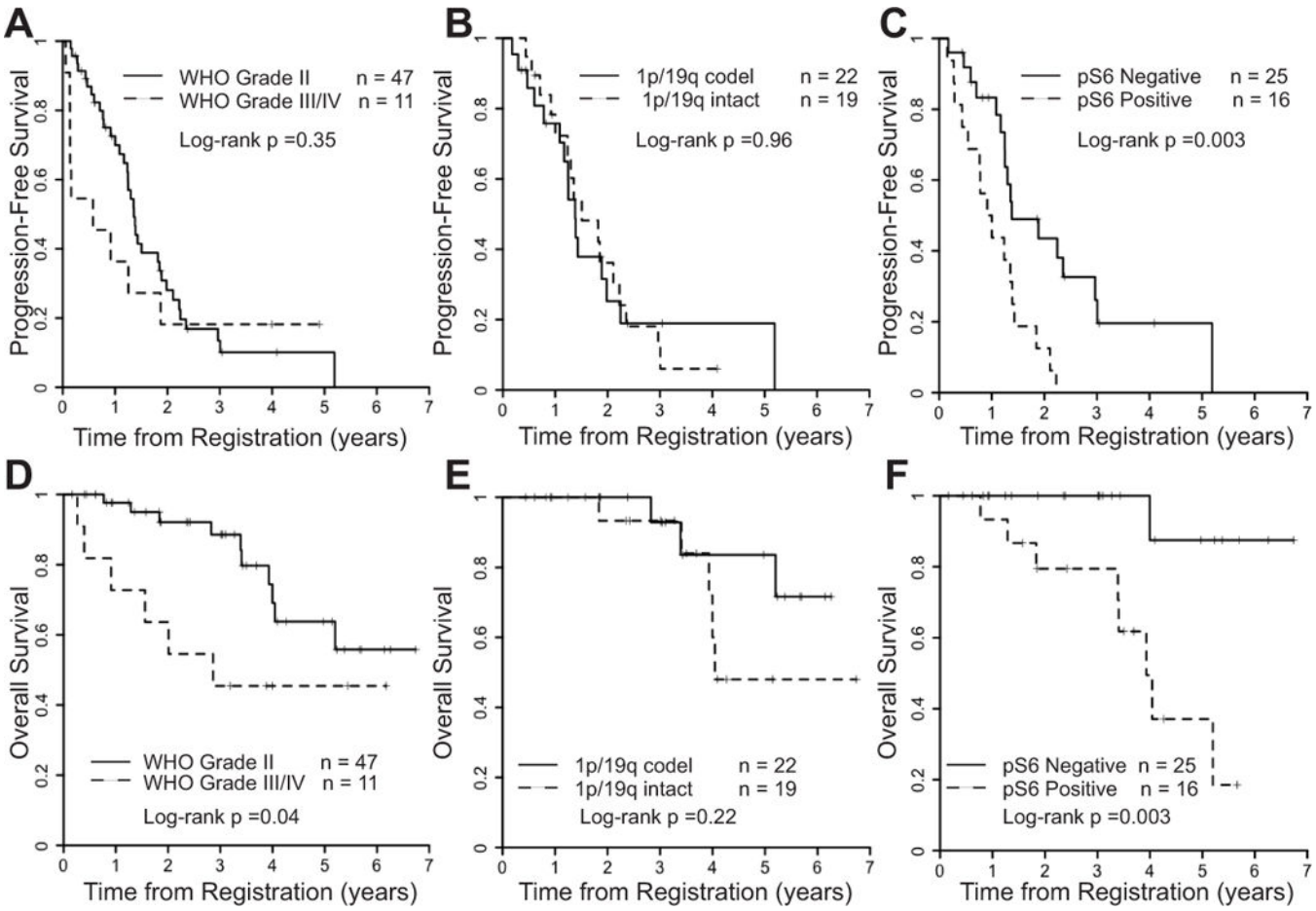

Figure 1.

Kaplan-Meier curves demonstrating PFS and OS by WHO Grade (A,D), 1p/19q codeletion (B,E) and PI3K/mTOR pathway activation based on the p-S6Ser240/244 epitope (C,F). Patients with WHO grade III or IV at recurrence excluded from B-F. Threshold for pS6 positivity of 19% derived from CART analysis.

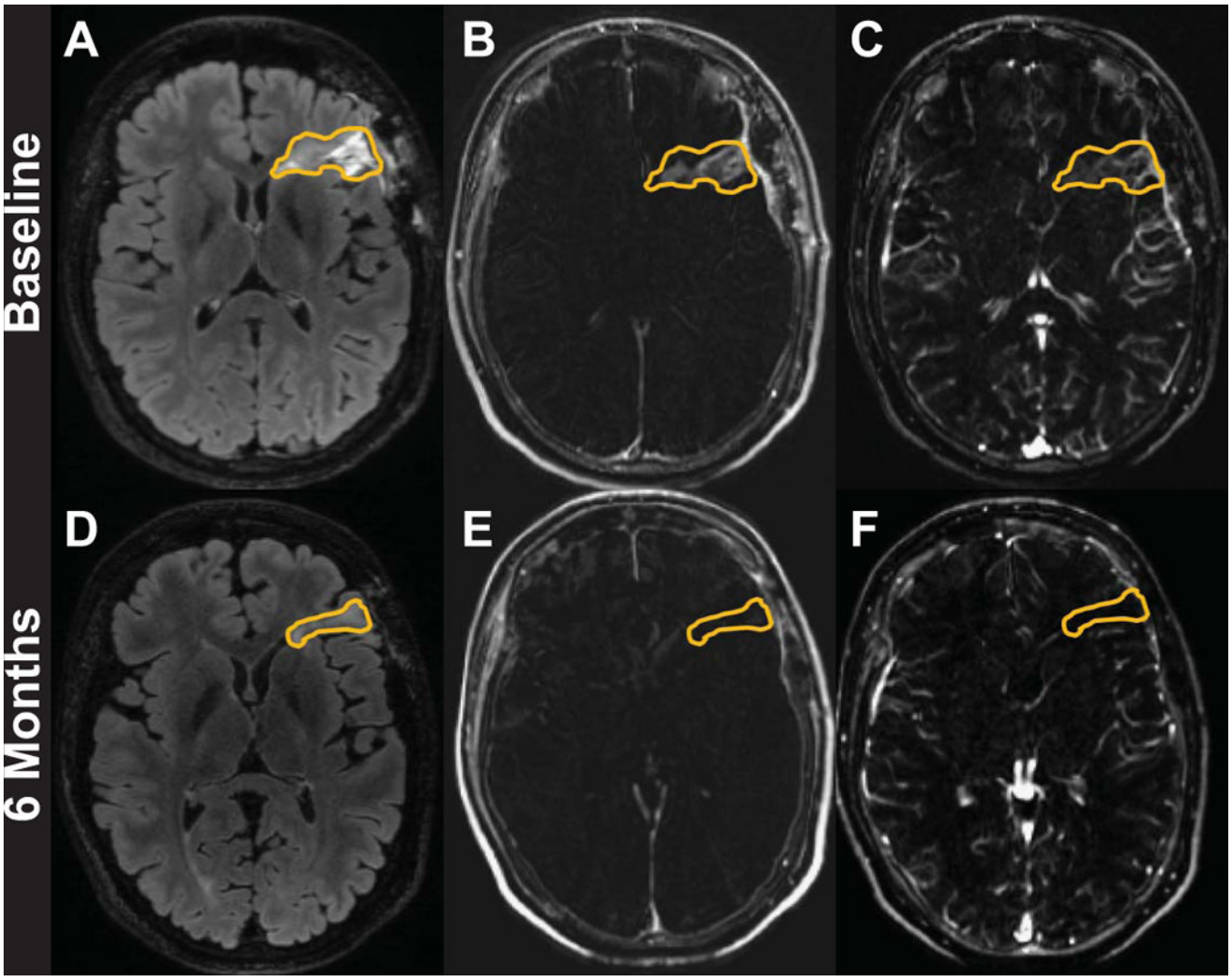

Figure 2.

Example patient MRI at study enrollment (top) and after 6 months of therapy (bottom). T2 FLAIR (A,D) and DCE-PWI perfusion maps of Kps (B,E) and fBV (C,F) are shown, with tumor volume as delineated on T2 FLAIR sequence shown in yellow.

Table 1

Baseline characteristics for patients with WHO II and WHO III/IV disease at recurrence. Number of recurrences includes the recurrence prompting study enrollment. Median and interquartile range for percentage staining with IHC for each marker of PI3K/mTOR pathway activation reported.

Characteristics	WHO II, n=47 (%)	WHO III/IV, n=11 (%)
Age (years)		
Median	46	42
Range	27–66	31–66
Sex		
Male	24 (51)	5 (45)
Female	23 (49)	6 (55)
KPS		
90–100	38 (81)	10 (91)
<90	9 (19)	1 (9)
Time Since Initial Diagnosis (years)		
Median	7	9
Range	1–20	1–26
# Recurrences		
1	23 (49)	0
2	15 (32)	5 (45)
>2	9 (19)	6 (55)
Time Since Prior Recurrence (years)		
Median	3.5	1.6
Range	0.4–14.3	0.5–4.5
# Prior Surgeries		
Median	2	3
Range	1–5	2–3
Prior Therapy		
Chemotherapy and RT	9 (19)	11 (100)
RT Alone	4 (9)	0
Chemotherapy Alone	25 (53)	0
Surgery Alone	9 (19)	0
Extent of Resection		
STR	35	8
Biopsy	12	3
Molecular Subtype		
1p19q Codeleted	22 (47)	1 (9)

Characteristics	**WHO II, n=47 (%)**	**WHO III/IV, n=11 (%)**
1p19q Intact/IDH mut	18 (38)	5 (45)
1p19q Intact/IDH wild type	1 (2)	4 (36)
Unknown	6 (13)	1 (9)
PI3K/mTOR Immunohistochemistry		
p-PRAS40 (n=50)	20 (4–70)	10 (8–54)
p-mTOR (n=48)	10 (4–23)	7.5 (1–45)
p-S6$^{Ser240/244}$ (n=50)	12.5 (5–20)	27.5 (15–30)
p-S6$^{Ser235/236}$ (n=43)	13.5 (7–25)	30 (15–35)
p-4EBP1 (n=44)	30 (8–88)	17.5 (18–70)
Pretreatment Tumor Volume (cm^3)	19.6 (9.0 – 30.4)	29.5 (11.6 – 49.8)

Table 2

Median PFS and OS by clinical, molecular and radiographic characteristics for patients with recurrent WHO II disease. Hazard ratios and p-values calculated using univariate Cox analysis. Thresholds for continuous variables derived from CART analysis. NR: Not Reached.

Characteristic (n)	Median PFS (y) (95% CI)	Hazard Ratio (95% CI)	p	Median OS (y) (95% CI)	Hazard Ratio (95% CI)	p
Age						
>=40 years (35)	1.3 (1.2 – 1.9)	1		5.2 (4.0 – NA)	1	
< 40 years (12)	1.9 (0.7 – NA)	0.85 (0.40–1.78)	0.66	NR (NA – NA)	0.67 (0.14–3.19)	0.62
Prior Radiotherapy						
No (34)	1.4 (1.2 – 1.9)	1		NR (4.0 – NA)	1	
Yes (13)	1.9 (0.7 – NA)	1.20 (0.58–2.45)	0.62	5.2 (3.4 – NA)	1.57 (0.40–6.15)	0.52
# Recurrences						
1 (23)	1.8 (1.4 – 3.0)	1		NR (NA – NA)	1	
2 or more (24)	1.3 (1.1 – 1.9)	1.89 (0.95–3.78)	0.07	5.2 (3.9 – NA)	2.36 (0.60–9.21)	0.22
Extent of Resection						
STR (35)	1.4 (1.2 – 2.1)	1		1.4 (1.2 – 2.1)	1	
Biopsy (12)	1.3 (0.8 – NA)	1.63 (0.76–3.51)	0.21	1.3 (0.8 – NA)	7.13 (1.72–29.6)	0.007
Molecular Subgroup						
1p19q Codel (22)	1.4 (1.2 – NA)	1		NR (5.2 – NA)	1	
1p19q Intact (19)	1.5 (1.2 – 3.0)	1.02 (0.50–2.07)	0.96	4.0 (3.9 – NA)	2.47 (0.55 – 11.1)	0.24
pS6 Staining						
p-S6$^{Ser240/244}$ <19% (25)	1.4 (1.2 – NA)	1		NR(NA – NA)	1	
p-S6$^{Ser240/244}$ >19% (16)	0.96 (0.55 – 1.9)	3.03 (1.42–6.47)	0.004	3.9 (3.4 – NA)	12.7 (1.57–102)	0.01
Continuous (p-S6$^{Ser240/244}$)		1.23 (1.05–1.43)	0.01		1.50 (1.17–1.91)	0.001
Continuous (p-S6$^{Ser235/236}$)		1.16 (1.01–1.34)	0.04		1.44 (1.12–1.87)	0.005
Pretreatment Volume						
<53 cm^3 (25)	1.4(1.2–2.0)	1		NR (4.0–NA)	1	
≥ 53 cm^3 (3)	0.5 (NA–NA)	5.35 (0.55–52.4)	0.15	2.3 (1.8–NA)	23.9 (2.14 – 267)	0.01
Continuous		1.11 (0.86–1.45)	0.40		1.51 (1.08–2.12)	0.02

Table 3

Most common toxic effects by grade.

Toxicity Type	Grade 2, n (%)	Grade 3, n (%)
Hematologic		
Thrombocytopenia	1 (2)	1 (2)
Neutropenia	0	2 (3)
Leukopenia	0	3 (5)
Hyperlipidemia	4 (7)	0
Transaminitis	0	3 (5)
Hypophosphatemia	0	3 (5)
Pneumonitis	0	2 (3)
Mucositis	6 (13)	1 (2)
Other Non-hematologic Toxicity	12 (21)	7 (12)
Total	23 (40)	22 (38)

Table 4

Changes in radiographic parameters during therapy. Median parameter value within T2 FLAIR tumor volume calculated for diffusion and perfusion parameters. For each parameter, percentage change after 6 months of therapy is reported, along with p-value for significant change from baseline (Wilcoxon rank-sum test). Cox proportional hazards model was performed to evaluate the relationship between radiographic changes and PFS, with hazard ratios reported for each 10% decrease in parameter.

Parameter (n)	Median % Change (IQR)	p	PFS Hazard Ratio (95% CI)	p
Tumor Volume (22)	−18 (−36 – 21)	0.47	1.03 (0.93–1.14)	0.53
Median Kps (19)	−12 (−23 – −2)	0.087	0.82 (0.67–0.99)	0.044
Median fBV (19)	−15 (−39 – −4)	0.026	0.71 (0.53–0.92)	0.011
Median ADC (22)	0.3 (−5.8 – 6.6)	0.97	1.01 (0.77–1.33)	0.92