Everolimus for Children With Recurrent or Progressive Low-Grade Glioma: Results From the Phase II PNOC001 Trial

Daphne A. Haas-Kogan, MD, MBA[1]; Mariam S. Aboian, MD, PhD[2]; Jane E. Minturn, MD, PhD[3,4]; Sarah E.S. Leary, MD[5,6,7]; Mohamed S. Abdelbaki, MD[8]; Stewart Goldman, MD[9,10]; Jennifer D. Elster, MD[11]; Adam Kraya, PhD[12]; Matthew R. Lueder, MSc[12,13]; Divya Ramakrishnan, BS[2]; Marc von Reppert, MD[2,14]; Kevin X. Liu, MD, DPhil[1]; Jo Lynne Rokita, PhD[12]; Adam C. Resnick, PhD[12]; David A. Solomon, MD, PhD[15]; Joanna J. Phillips, MD, PhD[15,16]; Michael Prados, MD[16,17]; Annette M. Molinaro, PhD[16]; Sebastian M. Waszak, PhD[18,19,20]; and Sabine Mueller, MD, PhD, MAS[16,19,17,21]

DOI https://doi.org/10.1200/JCO.23.01838

ABSTRACT

PURPOSE The PNOC001 phase II single-arm trial sought to estimate progression-free survival (PFS) associated with everolimus therapy for progressive/recurrent pediatric low-grade glioma (pLGG) on the basis of phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway activation as measured by phosphorylated-ribosomal protein S6 and to identify prognostic and predictive biomarkers.

PATIENTS AND METHODS Patients, age 3-21 years, with progressive/recurrent pLGG received everolimus orally, 5 mg/m² once daily. Frequency of driver gene alterations was compared among independent pLGG cohorts of newly diagnosed and progressive/recurrent patients. PFS at 6 months (primary end point) and median PFS (secondary end point) were estimated for association with everolimus therapy.

RESULTS Between 2012 and 2019, 65 subjects with progressive/recurrent pLGG (median age, 9.6 years; range, 3.0-19.9; 46% female) were enrolled, with a median follow-up of 57.5 months. The 6-month PFS was 67.4% (95% CI, 60.0 to 80.0) and median PFS was 11.1 months (95% CI, 7.6 to 19.8). Hypertriglyceridemia was the most common grade ≥3 adverse event. PI3K/AKT/mTOR pathway activation did not correlate with clinical outcomes (6-month PFS, active 68.4% *v* nonactive 63.3%; median PFS, active 11.2 months *v* nonactive 11.1 months; $P = .80$). Rare/novel *KIAA1549::BRAF* fusion breakpoints were most frequent in supratentorial midline pilocytic astrocytomas, in patients with progressive/recurrent disease, and correlated with poor clinical outcomes (median PFS, rare/novel *KIAA1549::BRAF* fusion breakpoints 6.1 months *v* common *KIAA1549::BRAF* fusion breakpoints 16.7 months; $P < .05$). Multivariate analysis confirmed their independent risk factor status for disease progression in PNOC001 and other, independent cohorts. Additionally, rare pathogenic germline variants in homologous recombination genes were identified in 6.8% of PNOC001 patients.

CONCLUSION Everolimus is a well-tolerated therapy for progressive/recurrent pLGGs. Rare/novel *KIAA1549::BRAF* fusion breakpoints may define biomarkers for progressive disease and should be assessed in future clinical trials.

ACCOMPANYING CONTENT

Appendix

Protocol

Accepted October 17, 2023

Published November 17, 2023

J Clin Oncol 42:441-451

View Online Article

INTRODUCTION

Pediatric low-grade gliomas (pLGGs) are common CNS tumors in pediatric patients, often showing 10-year overall survival (OS) rates exceeding 85%.[1-4] However, patients with incomplete tumor resection remain at risk for progression,[1,5-11] necessitating additional therapy.[5,9,12-14] Optimal management for progressive/recurrent pLGGs lacks consensus, and given extended survival, it remains essential to consider quality of life and treatment-associated morbidities along with cure potential when weighing treatment options.[5]

Recent molecular studies have identified key pathways contributing to pLGG tumorigenesis, including phosphatidylinositol 3-kinase (PI3K), AKT, and mammalian target of rapamycin (mTOR).[15-17] Activation of this pathway was detected in 43.8% and 50% of pLGGs by phosphorylated-ribosomal protein S6 (p-RPS6) and phosphorylated-4EBP1

CONTEXT

Key Objective

To our knowledge, the PNOC001 phase II trial is the first multi-institutional trial to integrate molecular biomarkers into assessment of targeted agents for pediatric low-grade glioma (pLGG), to evaluate the efficacy of everolimus for progressive/recurrent pLGGs and identify biomarkers of disease progression, focusing on phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) activation.

Knowledge Generated

Everolimus is well tolerated, with 67.4% 6-month progression-free survival (PFS) and 11.1-month median PFS, both independent of PI3K/AKT/mTOR pathway activation. Rare/novel *KIAA1549::BRAF* fusion breakpoints were associated with supratentorial pilocytic astrocytomas, progressive/recurrent disease, and poor clinical outcomes. Rare/novel *KIAA1549::BRAF* breakpoints may define biomarkers for progressive disease (PD) and should be assessed in future clinical trials.

Relevance *(S. Bhatia)*

This multi-institutional, biology-driven trial demonstrates the feasibility of integrating molecular biomarkers in assessing the efficacy of a targeted agent for treating patients with pLGGs and identifies a novel biomarker associated with PD. These findings can inform future risk-based targeted approach in a multi-institutional setting.*

*Relevance section written by *JCO* Associate Editor Smita Bhatia, MD, MPH, FASCO.

(p-4EBP1), respectively, and expression of p-RPS6 and p-4EBP1 correlated with worse progression-free survival (PFS).[16] Activated mTOR, a downstream target of the PI3K/AKT pathway, promotes increased protein synthesis, cell proliferation, angiogenesis, and cell survival.[18-20]

Previous trials have found that the mTOR inhibitor everolimus is safe and efficacious for recurrent adult LGGs and pLGGs.[21-23] However, biomarkers predicting everolimus response in pLGG remain uncertain, and several factors, such as young age at presentation,[6] supratentorial midline tumor location,[24] and rare *KIAA1549::BRAF* fusion breakpoints,[17] contribute to worse outcomes. A subset of cases are associated with genetic tumor syndromes, yet with unclear frequency or prognostic relevance.[25]

Assessing treatment response in pLGGs remains challenging because of tumor heterogeneity, variable enhancement patterns, and irregular margins.[26] The Response Assessment in Neuro-Oncology (RANO) criteria were adapted for LGG[27] but were insufficient for pediatric cases, leading to the creation of the Response Assessment in Pediatric Neuro-Oncology (RAPNO) guidelines[28] that account for unique pLGG characteristics. Volumetric analysis, although used in brain tumor response assessment, is currently not standard clinical practice, with limited literature relevant to pLGGs.[28,29]

Here, we report a multicenter phase II trial by the Pacific Pediatric Neuro-Oncology Consortium (PNOC), investigating the efficacy of everolimus in pediatric and adolescent patients with recurrent or progressive pLGG and ask whether response to everolimus is dependent on PI3K/AKT/mTOR pathway activation. Post hoc analysis investigated the role of genomic biomarkers in clinical outcomes and volumetric tumor assessments.

PATIENTS AND METHODS

Eligibility

This study (ClinicalTrials.gov identifier: NCT01734512) was reviewed and approved by the institutional review boards of participating institutions. All participants or their parents provided verbal and written informed consent, with assent for appropriately aged patients. Patients age 3-21 years, with histologically confirmed WHO grade 1 or 2 primary CNS pLGG (2016 WHO Classification of Tumors) with documented progression, were eligible (Appendix 1, online only).

Treatment Regimen

Patients self-administered everolimus orally once daily at 5 mg/m^2. Dose modification guidelines are outlined in Appendix 1.

Imaging Review and Definition of Response

Magnetic resonance imaging assessments were performed at baseline and every 2 months. Tumor measurements were performed per protocol criteria. The pretreatment scan was used as baseline to define response. Retrospective central imaging review was compared with institutional review using protocol-defined criteria (see Appendix 1 for details and response criteria).

Molecular Tumor Characterization

PI3K/AKT/mTOR pathway activation was assessed by p-RPS6 expression.[30] A blinded neuropathologist (J.J.P.) scored the percentage of positive tumor cells on the basis of review of hematoxylin and eosin and p-RPS6 immunostaining. Tumors with ≥25% positive tumor cells were scored as p-RPS6–positive. Two tumors did not have adequate material for analysis. Pathology reports and genomic data from clinical care were reviewed to extract genetic alterations in driver genes (Appendix 1).

KIAA1549::BRAF Fusion Breakpoint Analysis

KIAA1549::BRAF fusion breakpoint analysis was based on RefSeq transcripts NM_004333 and NM_001164665. Analysis of *BRAF* fusion partners and breakpoints was feasible for 21 PNOC001 patients. *KIAA1549::BRAF* fusion breakpoints were defined as common if their frequency was >5% in pLGG cohorts.[17] Common *KIAA1549::BRAF* fusion breakpoints involved 16:09 (exon 16 in *KIAA1549* and exon 9 in *BRAF*), 15:09, 16:11, and 18:10. All other breakpoints were considered rare/novel.

Independent Cohorts

KIAA1549::BRAF fusion breakpoints were assessed in independent retrospective and molecular pLGG cohorts (Appendix 1). Population frequency of rare pathogenic germline variants in *BRCA1*, *BRCA2*, and *PALB2* was obtained from the prospective UK Biobank cohort.[31]

Statistical Design and Analysis

The trial's primary objective was to determine whether everolimus was efficacious, defined by PFS at 6 months (PFS6), only in patients with PI3K/AKT/mTOR pathway activation (measured by p-RPS6 positivity), or independent of pathway activation. An adaptive Simon two-stage design for phase II studies of targeted therapies was used to assess the primary objective. Treatment with everolimus would be deemed unworthy of further investigation if the true PFS6 was <50%. If, in the first stage, with a combined sample size of 25, there was preliminary evidence to suggest that the efficacy of everolimus was restricted to patients with PI3K/AKT/mTOR activation as measured by p-RPS6 positivity, a total of 45 patients were enrolled, and the design had 81% statistical power to detect an actual disease stabilization rate ≥70%. If, in the first stage, there was preliminary evidence to suggest that the efficacy of everolimus was independent of PI3K/AKT/mTOR activation, a total of 65 patients were enrolled, and the design had >95% statistical power to detect an actual disease stabilization rate ≥70%. PFS was calculated from the date of first treatment to the date of progression or death (if progression was not noted) or date of last follow-up. Patients who died or were lost to follow-up before reaching the 6-month point were considered failures for the PFS6 end point. OS was calculated from the date of study entry to death or the date of last follow-up. The Kaplan-Meier method analyzed PFS and OS; two-sided *P* values were reported using log-rank test. A false discovery rate was calculated with the Benjamini-Hochberg procedure for all genomic biomarker tests. Statistical analyses were performed in R (R Foundation for Statistical Computing, Vienna, Austria; version 4.2.1).

RESULTS

Patient Characteristics

In PNOC001, 65 patients received everolimus (Fig 1A; Appendix Table A1). Median age at enrollment was 9.6 years (range, 3.0-19.9), 46.2% were female, and median follow-up was 57.5 months (range, 3.7-69.0). Supratentorial midline was the most frequent tumor location on the basis of central imaging review. Pilocytic astrocytoma (PA) was the most frequent tumor histology (Appendix Table A1). Twelve patients completed the 24-month protocol, with progression (n = 32) as the primary reason for discontinuation. Median number of previous chemotherapy regimens was two (range, 0-9); four patients had previous radiation therapy (end of radiation to start of PNOC001; range, 71-376 weeks). Hypertriglyceridemia was the most common grade ≥3 adverse event (Appendix Table A2).

PI3K/AKT/mTOR activation, determined by p-RPS6 expression, was present in 60.3% (38/63) of tumors. No significant differences were observed in pathway activation with respect to histology ($\chi^2 = 7.6$; $P = .18$), *BRAF* alteration status ($\chi^2 = 0.63$; $P = .73$), or age at enrollment (Mann-Whitney U test; $P = .87$). Supratentorial cortical tumors showed less frequent pathway activation (11%, n = 1) compared with brainstem (60%, n = 3), spinal cord (67%, n = 4), or supratentorial midline tumors (68%, n = 36; $\chi^2 = 10.0$; $P < .05$).

Clinical Outcomes and Predictive Biomarkers

PFS6 was 67.4% (95% CI, 60.0 to 80.0) by site review and 78.1% (95% CI, 68.6 to 88.9) by central imaging review for the entire cohort (Figs 2A and 2B). Median PFS per site review was 11.1 months (95% CI, 7.6 to 19.8) while not reached by central review. When stratified by PI3K/AKT/mTOR pathway activation (n = 63), there was no difference in PFS6 (active 68.4% *v* nonactive 63.3%) and median PFS (active 11.2 *v* nonactive 11.1 months; $P = .80$) according to site review (Fig 2C). PI3K/AKT/mTOR pathway activation was also not associated with OS and median PFS as defined by central imaging review (Appendix Figs A1A and A1B). OS for the entire cohort at 6 and 48 months was 100% and 91.9% (95% CI, 85.3 to 99.0), respectively, with nine subjects dying during follow-up time, due to tumor progression (n = 2), intratumoral hemorrhage possibly related to bevacizumab (n = 1), hydrocephalus and stroke (n = 1), cardiorespiratory arrest (n = 1), and unknown reasons (n = 4).

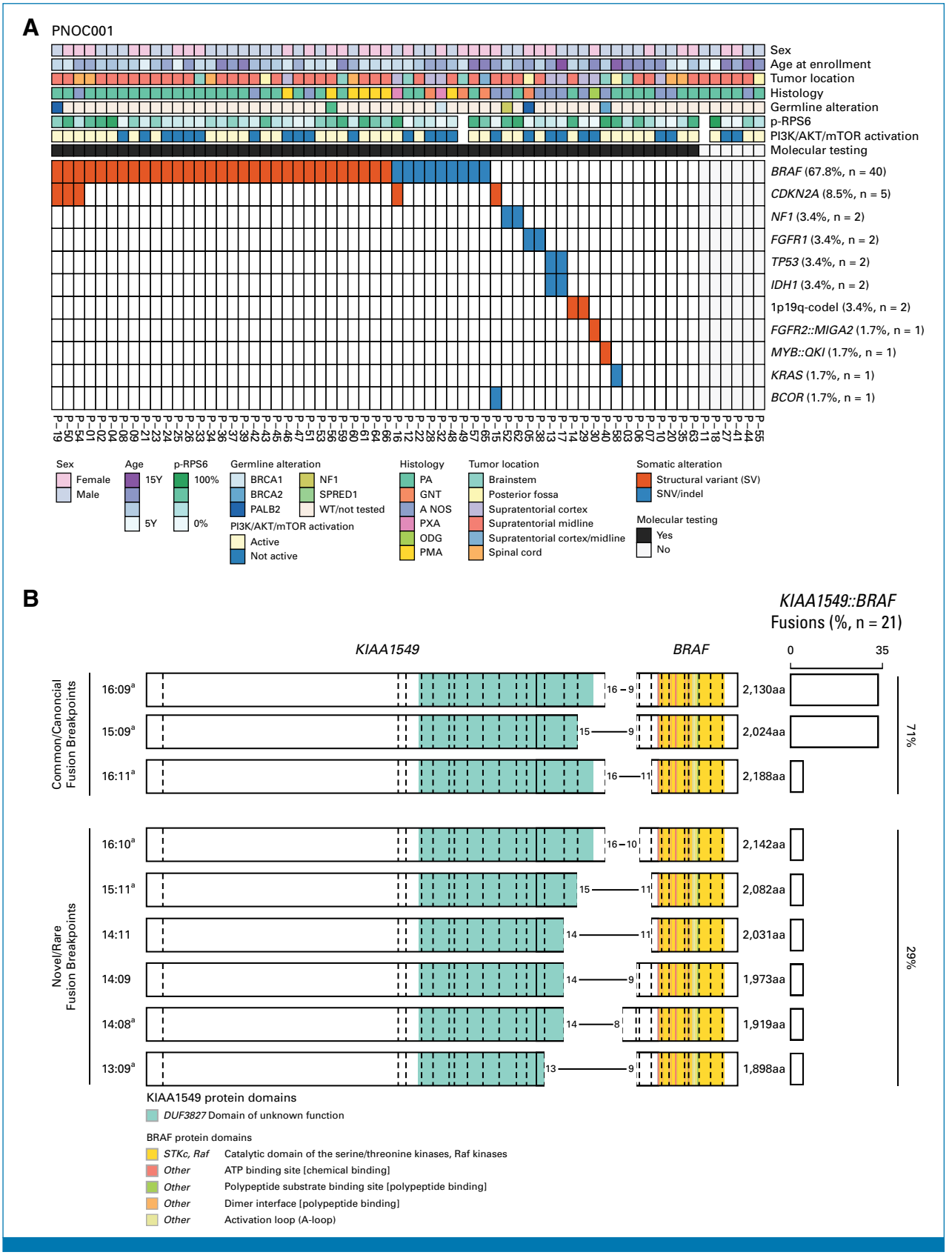

FIG 1. Overview of clinical and molecular characteristics. (A) Oncoplot with information about somatic driver gene mutations and PNOC001 patient characteristics. (B) Structure and frequency of common and rare/novel *KIAA1549::BRAF* fusion breakpoints in PNOC001. [a]Previously reported breakpoints. GNT, glioneuronal tumor; (continued on following page)

FIG 1. (Continued). mTOR, mammalian target of rapamycin; NOS, not otherwise specified; ODG, oligodendroglioma; PA, pilocytic astrocytoma; PI3K, phosphatidylinositol 3-kinase; PMA, pilomyxoid astrocytoma; p-RPS6, phosphorylated-ribosomal protein S6; PXA, pleomorphic xanthoastrocytoma; SNV, single nucleotide variant; WT, wild type.

Somatic alterations in glioma driver genes were studied in 59 (90.8%) patients with available tumor tissue. The majority of pLGGs (67.8%, n = 40) exhibited *BRAF* alterations (Fig 1A), including *BRAF* V600E mutations in nine patients (15.2%) and *BRAF* structural variants in 31 patients (52.5%). *KIAA1549::BRAF* fusion was confirmed in 21 patients (Fig 1B). For 10 patients, no additional information on *BRAF* fusion partners was available. Additional somatic alterations were identified in *CDKN2A* (8.5%, n = 5), *NF1* (3.4%, n = 2), *FGFR1* (3.4%, n = 2), *TP53* (3.4%, n = 2), and *IDH1* (3.4%, n = 2).

BRAF structural variants were most frequent in histologically defined PAs (71.0%, n = 31; Appendix Fig A1C), consistent with previous findings.[17] *BRAF* V600E mutations were identified in histologically defined PA (33.3%, n = 9) and glioneuronal tumors (33.3%, n = 9). Tumors with no identified *BRAF* alterations were most frequently histologically diagnosed as astrocytoma NOS (47.4%, n = 19).

Patients with *BRAF*-wildtype pLGG experienced faster tumor progression than those with altered *BRAF* (median PFS 5.5 *v* 16.7 months on the basis of site review; $P < .05$; FDR <0.05; n = 59; Fig 3A). Homozygous *CDKN2A* deletion, a known high-risk factor,[17] was present in five subjects and associated with rapid progression (median PFS, 4.4 [n = 5] *v* 12.5 months [n = 54]; $P < .001$; FDR<0.05;

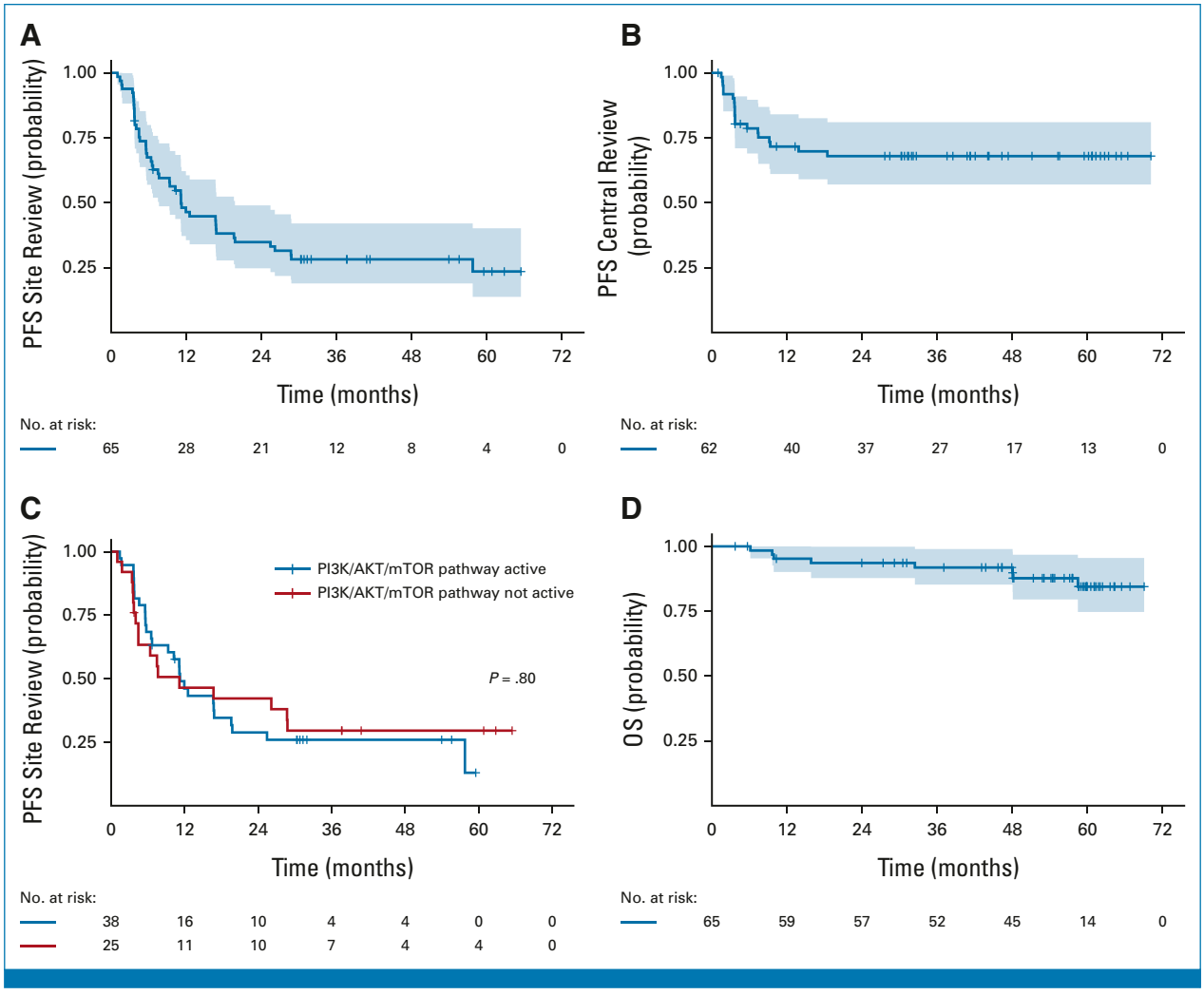

FIG 2. Clinical outcomes for patients receiving everolimus. (A) PFS of the PNOC001 cohort by site review. (B) PFS of the PNOC001 cohort by central imaging review. (C) PFS of the PNOC001 cohort stratified by active and nonactive pathway status using site imaging review. (D) OS of the PNOC001 cohort. mTOR, mammalian target of rapamycin; OS, overall survival; PFS, progression-free survival; PI3K, phosphatidylinositol 3-kinase.

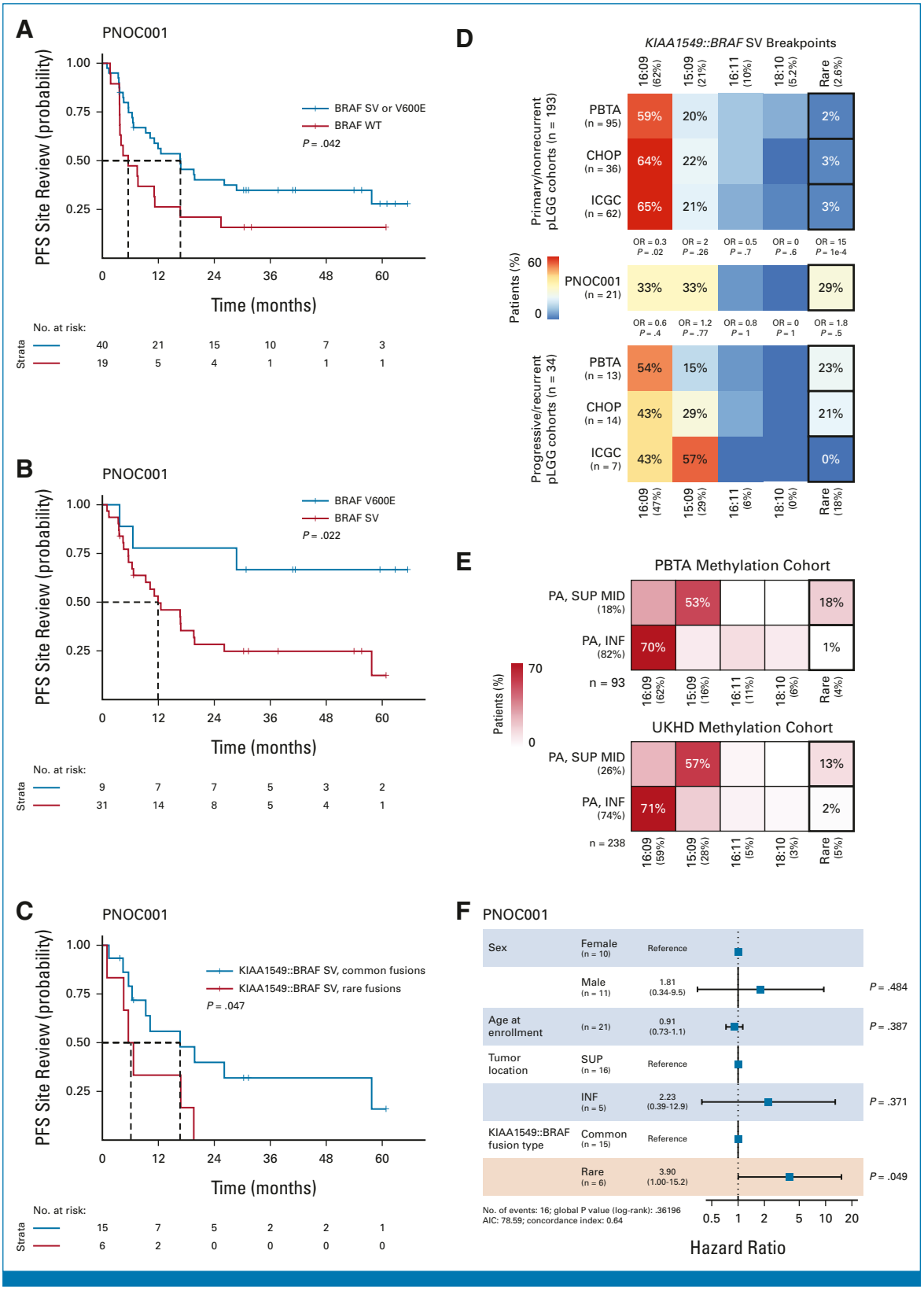

FIG 3. Association between somatic driver gene alterations and clinical outcomes. (A) Association between *BRAF* alterations and PFS by site review. (B) Association between *BRAF* V600E versus *BRAF* structural variants and PFS by site review. (C) Association between *KIAA1549::BRAF* fusion breakpoints and PFS by site review. (D) Replication between rare/novel *KIAA1549::BRAF* fusion breakpoints and progressive/recurrent pLGG in (continued on following page)

FIG 3. (Continued). retrospective cohort. (E) Association between rare/novel *KIAA1549::BRAF* fusion breakpoints and DNA methylation–based pilocytic astrocytoma subtypes. (F) Cox proportional hazards model for PFS (site review) in PNOC001 with demographic, anatomic, and molecular phenotypes. AIC, Akaike information criterion; CHOP, Children's Hospital of Philadelphia; ICGC, International Cancer Genome Consortium; OR, odds ratio; PA, INF: infratentorial pilocytic astrocytoma; PA, SUP MID, supratentorial midline pilocytic astrocytoma; PBTA, Pediatric Brain Tumor Atlas; PFS, progression-free survival; pLGG, pediatric low-grade glioma; SV, structural variant; UKHD, Universitätsklinikum Heidelberg; WT, wild type.

Appendix Fig A1D). Surprisingly, patients with *BRAF* structural variants (n = 31) experienced earlier tumor progression than patients with *BRAF* V600E mutations (n = 9; median PFS, 11.9 months *v* not reached; $P < .05$; FDR <0.05; Fig 3B; Appendix Fig A1E). This finding remained significant after excluding five patients with homozygous *CDKN2A* deletion (median PFS, 16.7 months *v* not reached; $P < .05$).

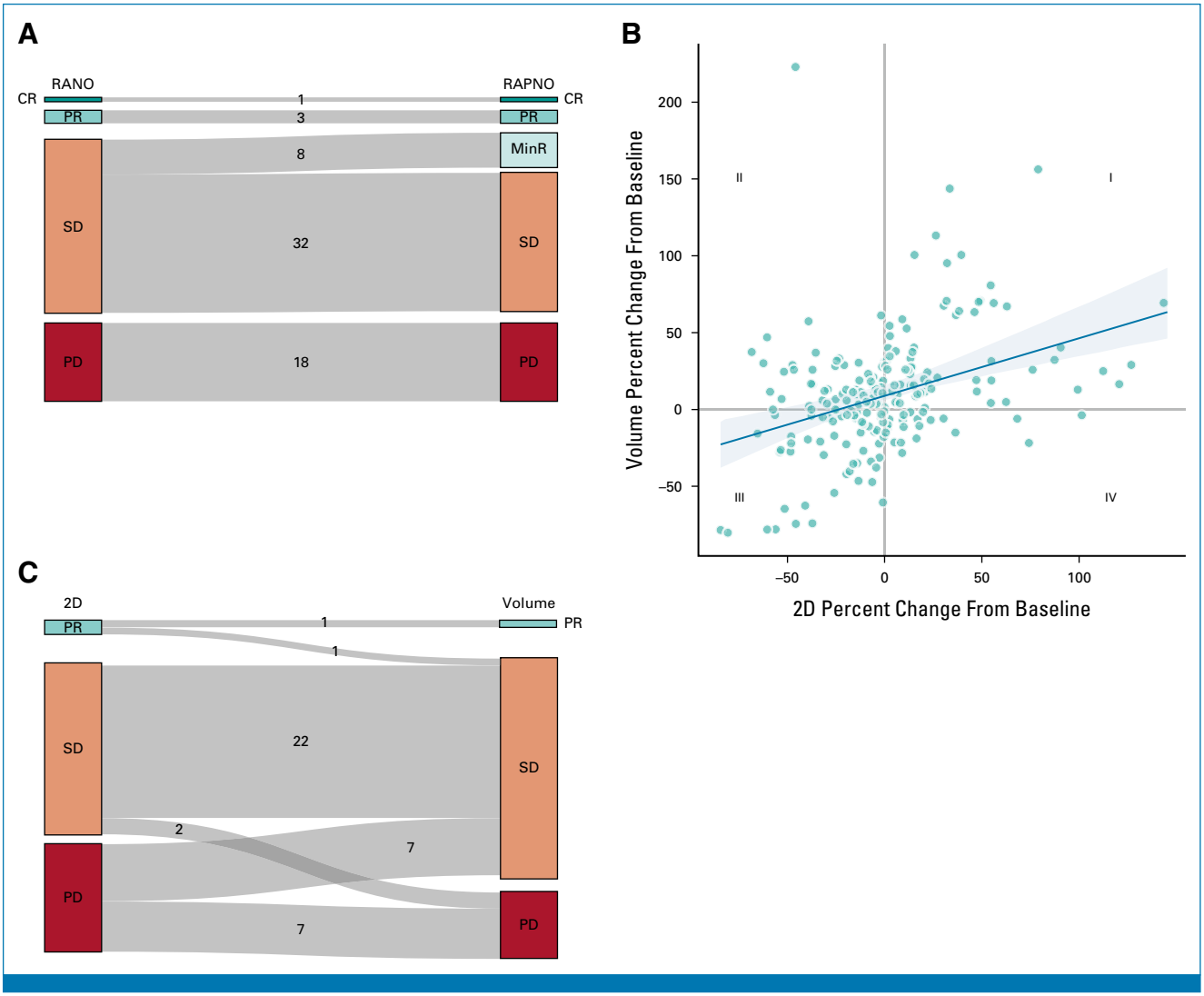

FIG 4. Comparison of volumetric and 2D response criteria. (A) Sankey diagram showing a comparison of response classification for 62 participants at the end of treatment on the basis of 2D RANO and RAPNO criteria. (B) Correlation between percent change in 2D and whole volume compared with baseline for 212 total follow-up scans from 40 participants with volumetric segmentations. Quadrants I and III show scans with percent change trends that agreed between 2D and volumetrics (128), while quadrants II and IV show scans with discordant percent change trends between 2D and volumetrics (84). (C) Sankey diagram comparing end-of-treatment response classification of 40 participants on the basis of 2D with standard RANO thresholds and whole volume with volume-extrapolated RANO thresholds. Note that one participant had a 12% increase in volume but was classified as PD by both 2D and volume criteria, given the appearance of a new lesion. CR, complete response; MinR, minor response; PD, progressive disease; PR, partial response; RANO, Response Assessment in Neuro-Oncology; RAPNO, Response Assessment in Pediatric Neuro-Oncology; SD, stable disease.

Rare and Novel *KIAA1549::BRAF* Fusion Breakpoints

A molecular analysis-based risk stratification system for pLGGs[17] reported rapid tumor progression among infants with rare/noncanonical *KIAA1549::BRAF* 15:11 fusion breakpoints. Analyzing 227 patients with pLGG from independent retrospective cohorts (Pediatric Brain Tumor Atlas [PBTA], Children's Hospital of Philadelphia [CHOP], and International Cancer Genome Consortium), we found that rare/novel *KIAA1549::BRAF* fusion breakpoints are rare (2.6%) in primary pLGGs (Fig 3D), but common (17.6%) in recurrent/progressive pLGGs (odds ratio [OR], 7.9; P = 2e-3; n = 227; Appendix Fig A1F). We also studied two retrospective molecular pLGG cohorts (n = 331) on the basis of DNA methylation profiling,[32] and found that rare/novel *KIAA1549::BRAF* fusion breakpoints were common (13% and 18%) in supratentorial midline PAs, yet rare (1% and 2%) in infratentorial PAs (Fig 3E).

In the prospective PNOC001 cohort, we also identified rare/novel *KIAA1549::BRAF* fusion breakpoints to be common in recurrent/progressive pLGGs (28.6%, n = 6) and supratentorial midline tumors (83.3%, n = 5; Figs 1B and 3D). Patients with rare/novel *KIAA1549::BRAF* fusion breakpoints were initially diagnosed as infants (median, 1.2 years; range, 0.5-8.4; n = 6), consistent with previous reports[17] and with our retrospective pLGG cohorts (PBTA/CHOP; median age, 3.0 years; range, 0.7-19.5; n = 9), and showed rapid progression on everolimus compared with patients with common *KIAA1549::BRAF* fusion breakpoints (median PFS rare/novel breakpoints 6.1 *v* common breakpoints 16.7 months; P < .05; FDR <0.05; n = 21; Fig 3C). Multivariate analysis that includes anatomic location demonstrated the independent predictive value of rare/novel *KIAA1549::BRAF* fusion breakpoints for early progression on everolimus (hazards ratio, 3.9; 95% CI, 1.0 to 15.2; P < .05; Fig 3F). In PBTA, rare/novel *KIAA1549::BRAF* fusion breakpoints were independently associated with recurrent/progressive disease (PD) when considering DNA methylation-based pLGG subtypes (P = .015; n = 93; Appendix Fig A1G).

Pathogenic Germline Variants

In PNOC001, 10% of patients (6/59) had pathogenic germline variants in cancer predisposition genes (*PALB2*, n = 2; *BRCA2*, n = 1; *BRCA1*, n = 1; *NF1*, n = 1; *SPRED1*, n = 1). Notably, variants in homologous recombination DNA repair genes were more common in patients with recurrent/progressive pLGG (6.8%, 4/59) than in a large prospective adult population cohort (0.65%; n = 394,656; OR, 11; 95% CI, 3 to 30; P = 6e-4; FDR <0.05).

Central Imaging Review and Volumetric Segmentation

Central imaging review assessed response using 2D measurements in 62 subjects (one subject was excluded due to intratumoral hemorrhage, two subjects lacked follow-up imaging). Using 2D RANO criteria and end-of-treatment classification, responses were complete response (2%, n = 1), partial response (PR; 5%, n = 3), SD (65%, n = 40), and PD (29%, n = 18; Fig 4A). RAPNO criteria reclassified eight SD patients as having a minor response (12.9%, 8/62). Volumetric segmentation for 40 subjects revealed a positive correlation (r = 0.38; P < .001) between 2D and whole volume percent change. Discordance in the overall trend (ie, increase or decrease from baseline) between 2D and volume-based response was observed in 39.6% (84/212) of follow-up scans (Fig 4B). A comparison of 2D and volume-based response using RANO and volume-extrapolated RANO thresholds found discordance in 25% (10/40) of participants at the end of treatment (Fig 4C).

DISCUSSION

Our study tested everolimus therapy in a biology-driven prospective trial that required tumor tissue for enrollment. We asked whether p-RPS6, a PI3K/AKT/mTOR activation marker, predicts everolimus response in pLGGs. We show that everolimus therapy is effective in recurrent/progressive pLGGs, regardless of p-RPS6 expression. To our knowledge, this is the first multi-institutional trial to integrate molecular biomarkers into assessment of targeted agent efficacy in pLGG. We also identify rare/novel *KIAA1549::BRAF* fusion breakpoints as biomarkers for PD in pLGG, confirmed in retrospective, independent cohorts.

Our study's safety and efficacy results for everolimus in recurrent/progressive pLGGs (PNOC001) align with a smaller phase II trial that found everolimus to be well tolerated.[23,33] The PFS rates in our study (35% at 24 months and 28% at 36 months by site review) are comparable with those reported by Wright et al[23] (39% at 24 months and 26% at 36 months).

We also assessed PFS through central imaging review, revealing notably higher PFS rates of 68% at 24 and 36 months. These disparities likely stem from clinical practice tendencies to alter therapy on the basis of less pronounced imaging changes compared with protocol-defined progression criteria. This might be especially relevant for pLGGs, for which clinicians have multiple treatment options, none clearly superior. Future trials should implement real-time central imaging review and consider volumetric assessments to mitigate such discrepancies. Our study included one patient with *NF1*-associated pLGG who displayed radiographic progression after 9.4 months by central review. Although *NF1*-associated pLGGs typically have a more favorable prognosis,[34] this single patient is unlikely to have significantly affected our cohort's clinical outcome results.

Our findings suggest that PI3K/AKT/mTOR activation does not predict everolimus response in recurrent/progressive pLGG. Although a previous study linked p-RPS6 expression to worse PFS in newly diagnosed pLGGs,[16] its predictive value in the recurrent/progressive setting remains uncertain, warranting further investigation. Conflicting data inform

outcomes for *BRAF* V600E-mutant pLGGs compared with *BRAF*-wildtype and those with *BRAF* structural variants.[35] In PNOC001, patients with recurrent/progressive pLGG harboring *BRAF* V600E mutations demonstrated improved PFS, necessitating future studies to clarify whether their prognosis differs from other *BRAF* alterations and whether this favorable prognosis results from an improved response to everolimus therapy.

pLGGs pose challenges in response assessment because of their diverse characteristics on imaging, including solid and cystic components, enhancing and nonenhancing portions, and complex configurations, making it difficult to establish clear response criteria.[29] We used various imaging criteria, including RANO and new LGG RAPNO criteria, and explored volumetric tumor assessments.[36,37] Although not our primary study focus, we examined these criteria, given their relevance in pLGG trials. Previous studies comparing 2D and volumetric measurements in pLGG had limited patient numbers.[30,38,39] In PNOC001, using 2D measurements, we found minimal differences between LGG RANO and RAPNO, with only 12.9% having discordant responses at the end of treatment, primarily involving minor response reclassification. By contrast, when applying standard RANO and volumetric RAPNO criteria, there were more significant disparities. A moderate correlation existed between 2D and whole volume percent change, with 40% of follow-up scans showing conflicting responses. Most participants with discordant responses were classified as SD by volumetric but PR or PD by 2D measurements. Importantly, volume-extrapolated thresholds were higher than 2D RANO thresholds for both PR and PD. Additionally, 2D and volumetric analyses focused on different tumor components, with 2D measurements on the solid portion and volumetric measurements encompassing the entire tumor with cystic and edematous components. This discrepancy can lead to different clinical decisions on the basis of volumetric versus 2D criteria. Thus, further research is needed to clarify the role of volumetric analysis in pLGG response assessment, validate existing volumetric thresholds, and establish their correlation with clinical outcomes in pLGG trials.

Mandated tumor tissue and blood collection enabled novel molecular findings. We identified a high rate of pathogenic germline variants in HR DNA repair pathway genes (*PALB2*, *BRCA1*, and *BRCA2*) in PNOC001, consistent with recent genetic studies in pLGG cohorts.[25,40-42] Previous studies noted *BRCA2* alterations in 1%-4% of pLGGs[25,40-43] but not in progressive/recurrent cohorts. The role of HR-deficiency in pLGG tumorigenesis and treatment strategies remains unclear.

Our molecular analyses highlight the clinical relevance of rare/novel *KIAA1549::BRAF* fusion breakpoints in pLGGs. Fusion breakpoints are currently underreported in clinical practice, yet our results indicate that they should be incorporated into routine diagnostic workflows. Ryall et al[17] described rare *KIAA1549::BRAF* fusion breakpoints (15:11) in two infants with disseminated pLGG who had poor outcomes. Our study builds on this by showing that rare/novel *KIAA1549::BRAF* fusion breakpoints, including 15:11, are highly enriched in recurrent/progressive pLGGs within PNOC001 and external cohorts[44,45] and predict worse clinical outcomes in PNOC001. We found these breakpoints enriched in young patients (younger than 3 years) and patients with a supratentorial midline PA. These factors are known to be associated with poorer prognosis,[6-11,24] which suggests that rare/novel *KIAA1549::BRAF* fusion breakpoints provide a novel genetic biomarker for high-risk pLGG. We recommend routine assessment of *KIAA1549::BRAF* fusion breakpoints in future trials to confirm this association and move toward integrated molecular diagnosis for optimal clinical decision making.

Despite its prospective design, relatively large size, and central imaging review, our study has limitations. New routine molecular testing, such as DNA methylation profiling,[46] has emerged since our study's inception, but we lacked sufficient tissue from all patients for some post hoc analyses. Molecular subgroup analyses were sometimes constrained by sample size. To address these limitations, we validated our findings in large, retrospective molecular pLGG cohorts.

In summary, to our knowledge, we report the results of the first multi-institutional clinical trial to use biomarkers in assessing the efficacy of a targeted agent in pLGGs and show that everolimus therapy is effective and well tolerated in recurrent/progressive pLGG. PI3K/AKT/mTOR pathway activation, indicated by p-RPS6 expression, did not correlate with clinical outcomes. Central imaging review is crucial for tracking disease progression in recurrent/progressive pLGG trials and volumetric analyses provide additional information compared with RANO and RAPNO criteria. Everolimus was less effective for pLGG patients with wildtype *BRAF*, rare/novel *KIAA1549::BRAF* fusions, or homozygous *CDKN2A* deletion. Molecular analyses revealed rare/novel *KIAA1549::BRAF* fusion breakpoints in supratentorial midline PAs and association with rapid disease progression. Future studies should confirm the poor prognosis of patients with novel biomarkers, study their biological impact on pLGG development, and determine their integration into clinical decision making and trial design.

AFFILIATIONS

[1]Department of Radiation Oncology, Brigham and Women's Hospital, Dana-Farber Cancer Institute, Boston Children's Hospital, Harvard Medical School, Boston, MA
[2]Department of Radiology and Biomedical Imaging, Yale School of Medicine, New Haven, CT
[3]Division of Oncology, Department of Pediatrics, Children's Hospital of Philadelphia, Philadelphia, PA
[4]Department of Pediatrics, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA
[5]Cancer and Blood Disorders Center, Seattle Children's Hospital, Seattle, WA

[6]Department of Pediatrics, University of Washington, Seattle, WA
[7]Ben Towne Center for Childhood Cancer Research, Seattle Children's Research Institute, Seattle, WA
[8]Department of Pediatrics, Washington University School of Medicine, St Louis, MO
[9]Phoenix Children's Hospital, Phoenix, AZ
[10]University of Arizona College of Medicine, Phoenix, AZ
[11]Division of Hematology Oncology, Department of Pediatrics, Rady Children's Hospital, University of California, San Diego, San Diego, CA
[12]Division of Neurosurgery, Center for Data-Driven Discovery in Biomedicine, Children's Hospital of Philadelphia, Philadelphia, PA
[13]Department of Pathology and Laboratory Medicine, Children's Hospital of Philadelphia, Philadelphia, PA
[14]University of Leipzig, Leipzig, Germany
[15]Department of Pathology, University of California, San Francisco, San Francisco, CA
[16]Department of Neurological Surgery, University of California, San Francisco, San Francisco, CA
[17]Department of Pediatrics, University of California, San Francisco, San Francisco, CA
[18]Laboratory of Computational Neuro-Oncology, Swiss Institute for Experimental Cancer Research, School of Life Sciences, École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland
[19]Department of Neurology, University of California, San Francisco, San Francisco, CA
[20]Centre for Molecular Medicine Norway (NCMM), Nordic EMBL Partnership, University of Oslo and Oslo University Hospital, Oslo, Norway
[21]Department of Pediatrics, University of Zurich, Zurich, Switzerland

CORRESPONDING AUTHOR

Sabine Mueller, MD, PhD, MAS, Departments of Neurology, Neurosurgery, and Pediatrics, University of California, San Francisco, 675 Nelson Rising Lane, San Francisco, CA 94158; e-mail: sabine.mueller@ucsf.edu.

EQUAL CONTRIBUTION

S.M.W. and S.M. contributed equally as senior authors.

PRIOR PRESENTATION

Presented in part at the 19th International Symposium on Pediatric Neuro-Oncology (ISPNO), Karuizawa, Japan, December 13-16, 2020; at the 25th Annual Scientific Meeting and Education Day of the Society for Neuro-Oncology (SNO), Austin, Texas, November 19-22, 2020; and at the 28th Annual Meeting and Education Day of the Society for Neuro-Oncology (SNO), Vancouver, Canada, November 16-19, 2023.

SUPPORT

Supported by the PNOC Foundation and the Pediatric Brain Tumor Foundation. Novartis provided everolimus for the trial. Data from Children's Brain Tumor Network were downloaded via the Gabriella Miller Kids First Data Resource Portal. D.A.S. was supported by the Morgan Adams Foundation, the Yuvaan Tiwari Foundation, and the Ross Family. J.J.P. was supported by The National Institutes of Health (U01 CA168878). D.A.H.-K. was supported by the William M. Wood Foundation, National Cancer Institute Specialized Programs of Research Excellence in Human Cancer grant 5P50CA165962-09, NINDS 1R01NS091620, and Pediatric Low-Grade Astrocytoma Program at PBTF. Resources were provided by the UCSF Brain Tumor Specialized Programs of Research Excellence Biorepository (National Institutes of Health P50 CA097257). S.M.W. was supported by the École Polytechnique Fédérale de Lausanne, the University of Oslo, The Research Council of Norway (187615), and the South-Eastern Norway Regional Health Authority. D.A.H.-K. and S.M. were supported by NIH1U54CA274516-01A1.

CLINICAL TRIAL INFORMATION

NCT01734512 (PNOC 001)

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at DOI https://doi.org/10.1200/JCO.23.01838.

DATA SHARING STATEMENT

Pacific Pediatric Neuro-Oncology Consortium (PNOC) is committed to sharing with qualified external researchers access to patient-level data and supporting clinical documents as available for eligible patients. Requests will be reviewed as per PNOC standard data sharing policy. All data are anonymized to keep patient confidentiality.

AUTHOR CONTRIBUTIONS

Conception and design: Daphne A. Haas-Kogan, Adam C. Resnick, Joanna J. Phillips, Michael Prados, Sebastian M. Waszak, Sabine Mueller, Annette M. Molinaro
Financial support: Daphne A. Haas-Kogan, Sabine Mueller
Administrative support: Daphne A. Haas-Kogan, Adam C. Resnick, Michael Prados
Provision of study materials or patients: Daphne A. Haas-Kogan, Jane E. Minturn, Jennifer D. Elster, Michael Prados, Sabine Mueller
Collection and assembly of data: Daphne A. Haas-Kogan, Jane E. Minturn, Sarah E.S. Leary, Stewart Goldman, Jennifer D. Elster, Matthew R. Lueder, Divya Ramakrishnan, Marc von Reppert, Jo Lynne Rokita, Adam C. Resnick, David A. Solomon, Joanna J. Phillips, Michael Prados, Sebastian M. Waszak, Sabine Mueller
Data analysis and interpretation: Daphne A. Haas-Kogan, Mariam S. Aboian, Mohamed S. Abdelbaki, Stewart Goldman, Adam Kraya, Divya Ramakrishnan, Kevin X. Liu, David A. Solomon, Joanna J. Phillips, Michael Prados, Sebastian M. Waszak, Sabine Mueller, Annette M. Molinaro
Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

ACKNOWLEDGMENT

The authors want to thank all Pacific Pediatric Neuro-Oncology Consortium (PNOC) staff, especially Aubrie Drechsler, Anny Shai from the UCSF Neurosurgery tissue bank, all the PNOC PIs, PNOC advanced nurse practitioners, PNOC clinical research coordinators and nurses, and PNOC patient coordinators for their contribution and involvement in the study. The authors also want to thank all families and patients who participated in this trial. The authors would also like to thank the UCSF DSMC for monitoring the study. The authors also acknowledge the Children's Brain Tumor Network for allowing us to use the Gabriella Miller Kids First Data Resource Portal.

REFERENCES

1. Bandopadhayay P, Bergthold G, London WB, et al: Long-term outcome of 4,040 children diagnosed with pediatric low-grade gliomas: An analysis of the Surveillance Epidemiology and End Results (SEER) database. Pediatr Blood Cancer 61:1173-1179, 2014
2. Hossain MJ, Xiao W, Tayeb M, et al: Epidemiology and prognostic factors of pediatric brain tumor survival in the US: Evidence from four decades of population data. Cancer Epidemiol 72:101942, 2021
3. Krishnatry R, Zhukova N, Guerreiro Stucklin AS, et al: Clinical and treatment factors determining long-term outcomes for adult survivors of childhood low-grade glioma: A population-based study. Cancer 122:1261-1269, 2016
4. Ostrom QT, De Blank PM, Kruchko C, et al: Alex's Lemonade Stand Foundation infant and childhood primary brain and central nervous system tumors diagnosed in the United States in 2007–2011. Neuro Oncol 16:x1-x36, 2015 (suppl 10)
5. Pollack IF: Multidisciplinary management of childhood brain tumors: A review of outcomes, recent advances, and challenges. J Neurosurg Pediatr 8:135-148, 2011
6. Goebel A-M, Gnekow AK, Kandels D, et al: Natural history of pediatric low-grade glioma disease—First multi-state model analysis. J Cancer 10:6314-6326, 2019
7. 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 14:1265-1284, 2012
8. Gnekow AK, Walker DA, Kandels D, et al: 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 Cancer 81:206-225, 2017
9. 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 30:2641-2647, 2012
10. 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 12:1257-1268, 2010
11. Armstrong GT, Conklin HM, Huang S, et al: Survival and long-term health and cognitive outcomes after low-grade glioma. Neuro Oncol 13:223-234, 2011
12. De Blank P, Bandopadhayay P, Haas-Kogan D, et al: Management of pediatric low-grade glioma. Curr Opin Pediatr 31:21-27, 2019
13. Indelicato DJ, Rotondo RL, Uezono H, et al: Outcomes following proton therapy for pediatric low-grade glioma. Int J Radiat Oncol Biol Phys 104:149-156, 2019
14. Merchant TE, Kun LE, Wu S, et al: Phase II trial of conformal radiation therapy for pediatric low-grade glioma. J Clin Oncol 27:3598-3604, 2009
15. 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 15:1604-1614, 2013
16. Mueller S, Phillips J, Onar-Thomas A, et al: PTEN promoter methylation and activation of the PI3K/Akt/mTOR pathway in pediatric gliomas and influence on clinical outcome. Neuro Oncol 14: 1146-1152, 2012
17. Ryall S, Zapotocky M, Fukuoka K, et al: Integrated molecular and clinical analysis of 1,000 pediatric low-grade gliomas. Cancer Cell 37:569-583.e5, 2020
18. Boulay A, Lane HA: The mammalian target of rapamycin kinase and tumor growth inhibition, in Groner B (ed): Targeted Interference With Signal Transduction Events. Berlin, Heidelberg, Springer Berlin Heidelberg, 2007, pp 99-124
19. Kaul A, Chen Y-H, Emnett RJ, et al: Pediatric glioma-associated *KIAA1549:BRAF* expression regulates neuroglial cell growth in a cell type-specific and mTOR-dependent manner. Genes Dev 26: 2561-2566, 2012
20. Sandsmark DK, Pelletier C, Weber JD, et al: Mammalian target of rapamycin: Master regulator of cell growth in the nervous system. Histol Histopathol 22:895-903, 2007
21. 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 22:1527-1535, 2020
22. 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 123:4631-4639, 2017
23. Wright KD, Yao X, London WB, et al: A POETIC phase II study of continuous oral everolimus in recurrent, radiographically progressive pediatric low-grade glioma. Pediatr Blood Cancer 68:e28787, 2021
24. Brossier NM, Strahle JM, Cler SJ, et al: Children with supratentorial midline pilocytic astrocytomas exhibit multiple progressions and acquisition of neurologic deficits over time. Neuro Oncol Adv 4: vdab187, 2022
25. Gröbner SN, Worst BC, Weischenfeldt J, et al: The landscape of genomic alterations across childhood cancers. Nature 555:321-327, 2018
26. Sturm D, Pfister SM, Jones DTW: Pediatric gliomas: Current concepts on diagnosis, biology, and clinical management. J Clin Oncol 35:2370-2377, 2017
27. Van Den Bent M, Wefel J, Schiff D, et al: Response assessment in neuro-oncology (a report of the RANO group): Assessment of outcome in trials of diffuse low-grade gliomas. Lancet Oncol 12: 583-593, 2011
28. Greuter L, Guzman R, Soleman J: Pediatric and adult low-grade gliomas: Where do the differences lie? Children 8:1075, 2021
29. D'Arco F, O'Hare P, Dashti F, et al: Volumetric assessment of tumor size changes in pediatric low-grade gliomas: Feasibility and comparison with linear measurements. Neuroradiology 60:427-436, 2018
30. Mohamed E, Kumar A, Zhang Y, et al: PI3K/AKT/mTOR signaling pathway activity in IDH-mutant diffuse glioma and clinical implications. Neuro Oncol 24:1471-1481, 2022
31. Karczewski KJ, Solomonson M, Chao KR, et al: Systematic single-variant and gene-based association testing of thousands of phenotypes in 394,841 UK Biobank exomes. Cell Genom 2:100168, 2022
32. Capper D, Jones DTW, Sill M, et al: DNA methylation-based classification of central nervous system tumours. Nature 555:469-474, 2018
33. Cacchione A, Lodi M, Carai A, et al: Upfront treatment with mTOR inhibitor everolimus in pediatric low-grade gliomas: A single-center experience. Intl J Cancer 148:2522-2534, 2021
34. Ryall S, Tabori U, Hawkins C: Pediatric low-grade glioma in the era of molecular diagnostics. Acta Neuropathol Commun 8:30, 2020
35. Lassaletta A, Zapotocky M, Mistry M, et al: Therapeutic and prognostic implications of BRAF V600E in pediatric low-grade gliomas. J Clin Oncol 35:2934-2941, 2017
36. Fangusaro J, Witt O, Hernáiz Driever P, et al: Response assessment in paediatric low-grade glioma: Recommendations from the Response Assessment in Pediatric Neuro-Oncology (RAPNO) working group. Lancet Oncol 21:e305-e316, 2020
37. Leao DJ, Craig PG, Godoy LF, et al: Response assessment in neuro-oncology criteria for gliomas: Practical approach using conventional and advanced techniques. AJNR Am J Neuroradiol 41: 10-20, 2020
38. Kilday J-P, Branson H, Rockel C, et al: Tumor volumetric measurements in surgically inaccessible pediatric low-grade glioma. J Pediatr Hematol Oncol 37:e31-e36, 2015
39. Warren KE, Patronas N, Aikin AA, et al: Comparison of one-two-and three-dimensional measurements of childhood brain tumors. J Natl Cancer Inst 93:1401-1405, 2001
40. Fiala EM, Jayakumaran G, Mauguen A, et al: Prospective pan-cancer germline testing using MSK-IMPACT informs clinical translation in 751 patients with pediatric solid tumors. Nat Cancer 2: 357-365, 2021
41. Kratz CP, Smirnov D, Autry R, et al: Heterozygous *BRCA1* and *BRCA2* and mismatch repair gene pathogenic variants in children and adolescents with cancer. J Natl Cancer Inst 114:1523-1532, 2022
42. Von Stedingk K, Stjernfelt K-J, Kvist A, et al: Prevalence of germline pathogenic variants in 22 cancer susceptibility genes in Swedish pediatric cancer patients. Sci Rep 11:5307, 2021
43. ICGC/TCGA Pan-Cancer Analysis of Whole Genomes Consortium: Pan-cancer analysis of whole genomes. Nature 578:82-93, 2020
44. Lilly JV, Rokita JL, Mason JL, et al: The children's brain tumor network (CBTN)—Accelerating research in pediatric central nervous system tumors through collaboration and open science. Neoplasia 35:100846, 2023
45. Petralia F, Tignor N, Reva B, et al: Integrated proteogenomic characterization across major histological types of pediatric brain cancer. Cell 183:1962-1985.e31, 2020
46. Fukuoka K, Mamatjan Y, Tatevossian R, et al: Clinical impact of combined epigenetic and molecular analysis of pediatric low-grade gliomas. Neuro Oncol 22:1474-1483, 2020
47. Jin R, Zhang Y, Rokita JL, et al: PediatricOpenTargets/OpenPedCan-analysis: Release v1.1.0 (v1.1.0). Zenodo. https://doi.org/10.5281/zenodo.7383318

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Everolimus for Children With Recurrent or Progressive Low-Grade Glioma: Results From the Phase II PNOC001 Trial

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated unless otherwise noted. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO's conflict of interest policy, please refer to www.asco.org/rwc or ascopubs.org/jco/authors/author-center.

Open Payments is a public database containing information reported by companies about payments made to US-licensed physicians (Open Payments).

Daphne A. Haas-Kogan
Employment: Dana Farber Cancer Hospital, Brigham and Women's Hospital
Leadership: TeleRad Oncology, EmpNia, Graegis Pharmaceuticals SAB
Honoraria: St Jude Children's Research Hospital, Cincinnati Children's Hospital DSMC, General Dynamic Information Tech, Inc Reviewer, Defense Finance Service Agent DHHS
Consulting or Advisory Role: Gerson Lehrman Group, Guidepoint Global
Research Funding: Artios
Expert Testimony: Bendin, Sumrall @ Ladner, LLC, Bowman and Brooke, LLP, The EMMES Company

Mariam S. Aboian
Research Funding: Blue Earth Diagnostics (Inst)
Uncompensated Relationships: Visage Imaging

Mohamed S. Abdelbaki
Consulting or Advisory Role: Day One pharmaceuticals, Bexion
Speakers' Bureau: Day One Biopharmaceuticals
Research Funding: Novartis

Stewart Goldman
Honoraria: AstraZeneca
Speakers' Bureau: AstraZeneca

Adam Kraya
Employment: Children's Hospital of Philadelphia
Research Funding: Children's Hospital of Philadelphia

Matthew R. Lueder
Employment: Children's Hospital of Philadelphia, Corewell Health

Kevin X. Liu
Employment: Moderna Therapeutics

Michael Prados
Stock and Other Ownership Interests: Quadriga Biosciences
Research Funding: Genentech/Roche (Inst), Novartis (Inst), Bristol Myers Squibb (Inst), Day One Therapeutics (Inst), Regeneron (Inst), Oncoceutics (Inst)

Sebastian M. Waszak
Consulting or Advisory Role: BioMed X

Sabine Mueller
Consulting or Advisory Role: Roche
Research Funding: Regeneron (Inst), DayOne Pharmaceuticals (Inst), Curis Pharamceuticals (Inst), Bristol Meyer Scribbs (Inst), SonaCare Medical (Inst), Chimerix (Inst), Blueprint Medicines (Inst), Pfizer (Inst)

No other potential conflicts of interest were reported.

APPENDIX 1. TRIAL ELIGIBILITY

Patients must have a progressive or recurrent histologically confirmed low-grade glioma (WHO grade I or II). Genetic driver alterations such as *BRAF V600E, KIAA1549::BRAF* fusion, or *CDKN2A* homozygous deletion status were not included in trial eligibility criteria. All participants or their parents provided verbal and written informed consent, with assent for appropriately aged patients. Patients must have failed at least one previous therapy (radiation or systemic therapy, with no maximum number of previous treatments) and have measurable progressive or recurrent disease (defined as at least one lesion with measurements in at least two dimensions on magnetic resonance imaging). Tissue availability from initial diagnosis or recurrence was mandatory for trial enrollment. Outcome data were collected until the cutoff date of May 31, 2023. Subjects must have recovered from acute side effects of previous therapies and have adequate bone marrow function (absolute neutrophil count >1,000 cells/μL, hemoglobin >9 g/dL, and platelets >100,000/μL), renal function within normal limits for age, appropriate liver function (total bilirubin <1.5 $\times$ upper limit of normal [ULN] for age and ALT <2.5 $\times$ ULN for age), and normal pulse oximetry for age. Before starting therapy, patients must have cholesterol levels <350 mg/dL and triglycerides <400 mg/dL. Patients agreed to use contraception if they were of childbearing potential, and female patients of childbearing potential had a negative pregnancy test. Patients were excluded if they had primary spinal cord tumors, a history of HIV seropositivity, hepatitis C antibody positivity, hepatitis B antigen positivity, previous treatment with an mammalian target of rapamycin inhibitor, a known hypersensitivity to everolimus or rapamycin GI disease or impairment of GI function that would impair absorption of everolimus, and a history of other cancers unless in complete remission and off therapy for at least 3 years. Patients were also excluded if they were taking concomitant medications that may interfere with everolimus metabolism or function, chronic immunosuppressive therapy (including corticosteroids), other concurrent anticancer or investigational therapy, or radiation. Initially, the protocol allowed patients with spinal cord tumors to enroll; however, we amended the protocol starting protocol version v11.1, which was approved on January 5, 2018. We enrolled a total of four patients with spinal cord tumors on PNOC001.

Treatment

Everolimus tablets were swallowed whole or entirely dissolved in approximately 30 mL of water for patients unable to swallow them.

Definition of Dose Modification

Dose modifications were based on toxicities graded based on the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0. Dose level 0 was the starting dose (5 mg/m^2 once daily), level -1 was 2.5 mg/m^2 daily, and level -2 was 2.5 mg/m^2 every other day. For grade 2 nonhematologic toxicity (except pneumonitis), patients maintained the same dose if tolerable to the patient and dose interruption until recovery to grade ≤1 if intolerable to the patient, but everolimus could be reintroduced at the same dose. If second grade 2 nonhematologic toxicity (except pneumonitis) after reintroduction, everolimus was held until recovery to grade ≤1, and everolimus was reintroduced at the lower dose level. For grade 3 nonhematologic toxicity (except hyperlipidemia and pneumonitis), everolimus was held until recovery to grade ≤1, and reintroduced at the lower dose level. For grade 2 pneumonitis, the everolimus dose was reduced until recovery to grade ≤1 or interrupted if symptoms were concerning, along with a course of corticosteroids. Patients who failed to recover to grade ≤1 within 3 weeks were withdrawn from the study. For grade 3 pneumonitis, treatment was held until recovery to grade ≤1 with a plan to restart at a reduced dose level within 2 weeks if there was evidence of clinical benefit. Patients who failed to recover to grade ≤1 within 2 weeks were withdrawn from the study. For grade 3 hyperlipidemia, medical therapies were used for management without changing the dose of everolimus. For grade 4 nonhematologic toxicity, everolimus was discontinued, and patients were withdrawn from the study. The dose was interrupted for grade 2 thrombocytopenia or grade 3 neutropenia until recovery to grade ≤1 with the resumption of everolimus at the initial dose. For grade 3 thrombocytopenia, grade 4 neutropenia, the second episode of grade 2 thrombocytopenia, or the second episode of grade 3 neutropenia, everolimus was held until recovery to grade ≤1 with resumption at a lower dose level. For grade 3 febrile neutropenia, everolimus was held until resolution of fever and recovery to grade ≤1 with resumption at a lower dose level. Patients were withdrawn from the study after grade 4 thrombocytopenia, a second episode of grade 3 thrombocytopenia, grade 4 febrile neutropenia, a second episode of grade 3 febrile neutropenia, a third episode of grade 3 neutropenia, or a second episode of grade ≥3 neutropenia after grade 4 neutropenia. Everolimus was also discontinued for patients with toxicity requiring dose interruption for ≥3 weeks.

Imaging Review and Definition of Response

Images were obtained on either 3 or 1.5 Tesla scanners. Standard clinical sequences included 3 plane localizer, axial T2-weighted imaging, 3D fluid-attenuated inversion recovery (FLAIR), and T1-weighted imaging without and with intravenous gadolinium. Tumor measurements included the solid portion of the tumor in lesions where the solid portion could be measured in isolation. The cystic component was excluded if peripherally located. If the cystic portion was centrally located in the tumor and represented <25% of the tumor, the entire tumor was measured. Tumors with only cystic components were deemed not measurable. Complete response was defined as the complete disappearance of the target lesion and no new lesions; following Response Assessment in Neuro-Oncology (RANO) criteria, partial response (PR) was defined as 50% reduction in the sum of bidimensional orthogonal diameters of all solid lesions. Progressive disease (PD) was defined as ≥25% increase or the appearance of a new lesion, and stable disease (SD) as <25% increase or <50% decrease. Response categorization was also evaluated for all participants at the end of treatment using Response Assessment in Pediatric Neuro-Oncology (RAPNO) criteria. Minor response was defined as ≥25% but <50% decrease, and SD was defined as <25% increase or decrease. PR and PD RAPNO definitions use the same thresholds as in RANO criteria.

Volumetric analysis used research PACS (Visage AI Accelerator, Visage Imaging, Inc, San Diego, CA)[30] and segmentation contours of the whole tumor, including solid and cystic components, were performed on FLAIR sequences. The neuroradiologist (M.S.A.) checked all segmentations, blinded to pathologic results. Volumetric response was defined based on the percent change in whole volume compared with baseline using volume-extrapolated RANO thresholds: PR (≥65% tumor volume reduction), PD (≥40% increase or the appearance of a new lesion), and SD (<40% increase or <65% decrease in tumor volume). These thresholds are based on mathematically derived values after extrapolating 2D thresholds to a perfect spherical volume. Although they have not been empirically validated, they are currently the volumetric thresholds most commonly used in the literature when comparing 2D and volumetric assessment methods.

Imaging Response Evaluation

All images were anonymized before a retrospective, central review by a study-assigned neuroradiologist (M.S.A.). Measurable disease was defined as lesions that can be accurately measured in two dimensions with a minimum size of no less than double the slice thickness. T2 FLAIR sequences were used for disease assessment. All tumor measurements were recorded in millimeters or decimal fractions of centimeters and expressed as the sum of products of the largest diameter and perpendicular diameter. Tumor measurements over time were performed side by side in a single session to maintain the corresponding plane of view.

Molecular Tumor Characterization

Briefly, tumor sections (5 μm) were received or prepared from submitted blocks and stored at −20°C before use. All immunostainings were performed using a Benchmark Ultra autostainer (Ventana Medical Systems, Inc, Tucson, AZ). Phosphorylated-ribosomal protein S6 (p-RPS6) was detected, after antigen retrieval with CC1 (Ventana Medical Systems, Inc, Tucson, AZ) for 32 minutes, using anti–p-RPS6 (Ser 240/244; 1:100, Cat # 2215S, Cell Signaling Technology, Inc, Danvers, MA) incubated for 32 min at 37°C, as reported previously (Mohamed et al[30]).

Independent Pediatric Low-Grade Glioma Cohorts

KIAA1549::BRAF fusion breakpoints were validated and assessed in four independent retrospective and molecular cohorts of pediatric low-grade glioma (pLGG): Pediatric Brain Tumor Atlas (PBTA)[32]; Children's Hospital of Philadelphia (CHOP) OpenPedCan Project and clinical sequencing data via the CHOP Division of Genomic Diagnostics (DGD)[47]; International Cancer Genome Consortium (ICGC)[33]; and Heidelberg University Hospital (UKHD).[34] PBTA included whole genome sequencing (WGS), RNA-seq, and DNA methylation data for newly diagnosed and progressive/recurrent pLGGs; CHOP DGD included mRNA sequencing data for newly diagnosed and progressive/recurrent pLGGs; ICGC included WGS data for newly diagnosed and progressive/recurrent pLGGs. UKHD included mRNA sequencing and DNA methylation array data for pLGGs without further information about primary or progressive/recurrent status. PBTA, CHOP OpenPedCan, and ICGC cohorts were used to assess the frequency of rare/novel *KIAA1549::BRAF* fusion breakpoints in newly diagnosed versus progressive/recurrent pLGGs. PBTA and UKHD cohorts were used to evaluate the association between *KIAA1549::BRAF* fusion breakpoints and DNA methylation subtypes.

TABLE A1. PNOC001 Patient Characteristics and Therapy Details

Characteristic	N = 65 Patients
Age, years, median (range)	9.6 (3.1-19.9)
Sex, No. (%)	
Female	30 (46.2)
Male	35 (53.8)
Race, No. (%)	
White	47 (72.3)
African American	1 (1.5)
Asian	2 (3.1)
Other	1 (1.5)
Declined	14 (21.5)
Ethnicity, No. (%)	
Non-Hispanic	47 (72.3)
Hispanic	15 (23.1)
Not available/declined	3 (4.6)
Tumor location on the basis of central imaging review, No. (%)	
Supratentorial midline	38 (58.5)
Supratentorial cortex	9 (13.8)
Brainstem	6 (9.2)
Spinal cord	6 (9.2)
Posterior fossa	4 (6.2)
Tumor histology (>5% frequency), No. (%)	
Pilocytic astrocytoma	38 (58.5)
Astrocytoma, NOS	13 (20.0)
Pilomyxoid astrocytoma	7 (10.8)
Glioneuronal tumor	4 (6.2)
No. of treatment cycles, median (range)	8 (1-24)

Abbreviation: NOS, not otherwise specified.

TABLE A2. Treatment-Related Grade 3 or Higher Adverse Events Observed in PNOC001

Adverse Event	N = 65 Patients, No. (%)
Blood and lymphatic system disorders	2 (3.1)
Anemia	2 (3.1)
Ear and labyrinth disorders	1 (1.5)
Hearing impaired	1 (1.5)
GI disorders	10 (15.4)
Diarrhea	4 (6.2)
Mucositis oral	5 (7.7)
Nausea	1 (1.5)
General disorders and administration site conditions	4 (6.2)
Fatigue	1 (1.5)
Fever	2 (3.1)
Weight loss	1 (1.5)
Infections and infestations	5 (7.7)
Infections and infestations—other, specify	1 (1.5)
Lung infection	2 (3.1)
Sepsis	1 (1.5)
Skin infection	1 (1.5)
Investigations	8 (12.3)
ALT increased	2 (3.1)
AST increased	1 (1.5)
Lymphocyte count decreased	2 (3.1)
Neutrophil count decreased	3 (4.6)
Metabolism and nutrition disorders	14 (21.5)
Hyperglycemia	1 (1.5)
Hypertriglyceridemia	11 (16.9)
Hyperuricemia	1 (1.5)
Hypophosphatemia	1 (1.5)
Respiratory, thoracic, and mediastinal disorders	8 (12.3)
Cough	1 (1.5)
Dyspnea	2 (3.1)
Hypoxia	2 (3.1)
Respiratory, thoracic, and mediastinal disorders—other, specify	3 (4.6)

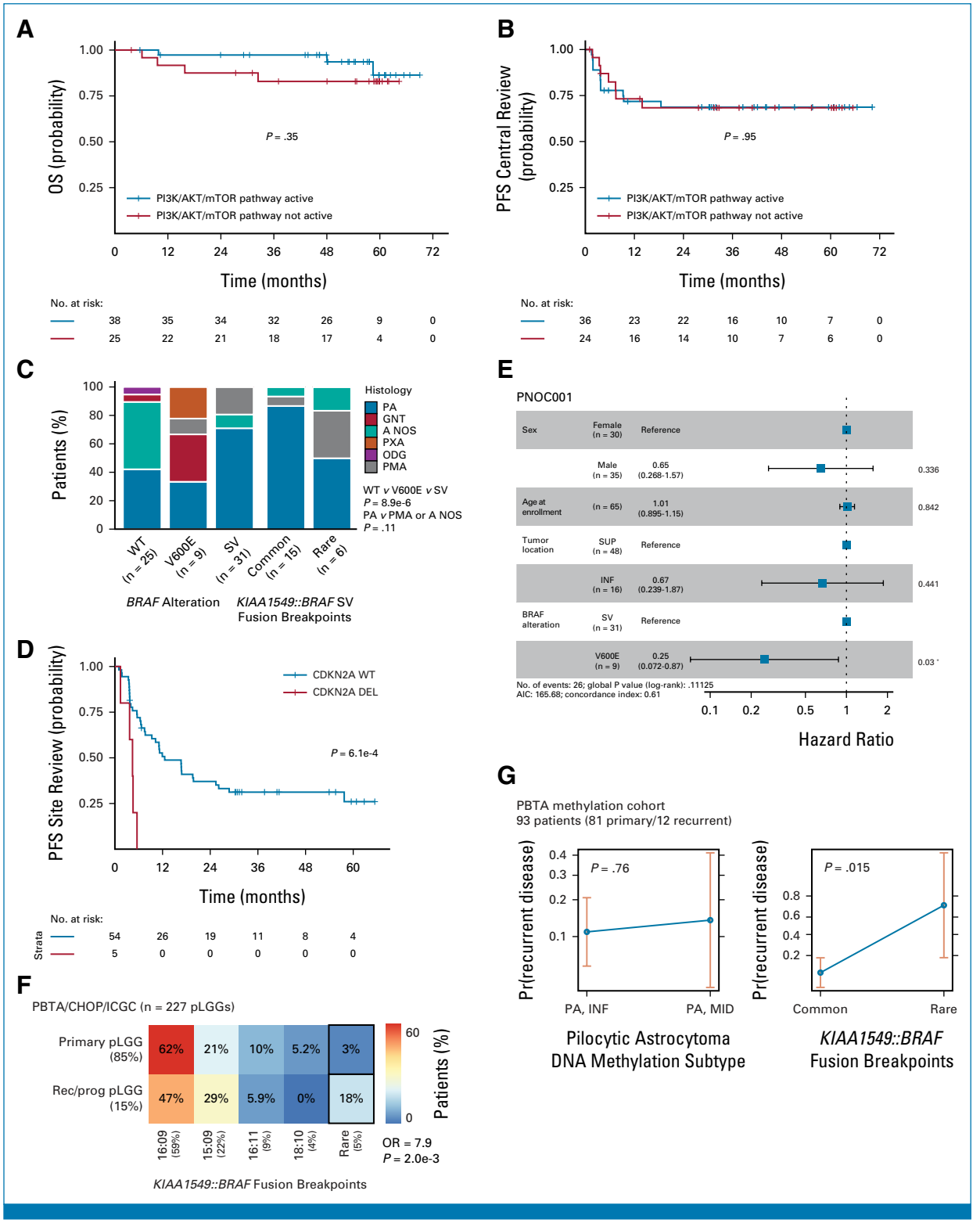

FIG A1. (A) Kaplan-Meier curve of OS by PI3K/AKT/mTOR pathway activation status. (B) Kaplan-Meier curve of central review PFS by PI3K/AKT/mTOR pathway activation status. (C) Frequency of *BRAF* alterations by histology and frequency of rare/novel and common *KIAA1549::BRAF* fusion breakpoints by histology. (D) Kaplan-Meier curve of site-review PFS by homozygous *CDKN2A* deletion status. (E) Cox proportional hazards model of site-review PFS by patient characteristics, anatomic location, and *BRAF* alteration status (V600E *v* SV). (F) Frequency of *KIAA1549::BRAF* fusion breakpoints in primary and progressive/recurrent PAs in PBTA, CHOP, and ICGC cohorts. (G) Association between rare/novel *KIAA1549::BRAF* fusion breakpoints, (continued on following page)

FIG A1. (Continued). DNA methylation subtypes, and recurrent/progressive PA. AIC, Akaike information criterion; CHOP, Children's Hospital of Philadelphia; GNT, glioneuronal tumor; ICGC, International Cancer Genome Consortium; INF, infratentorial; mTOR, mammalian target of rapamycin; NOS, not otherwise specified; ODG, oligodendroglioma; OS, overall survival; PA, pilocytic astrocytoma; PBTA, Pediatric Brain Tumor Atlas; PFS, progression-free survival; PI3K, phosphatidylinositol 3-kinase; pLGG, pediatric low-grade glioma; PMA, pilomyxoid astrocytoma; PXA, pleomorphic xanthoastrocytoma; SNV, single nucleotide variant; SUP, supratentorial; SV, structural variant; WT, wild type.