original reports

Comprehensive Genomic Analysis in NRG Oncology/RTOG 9802: A Phase III Trial of Radiation Versus Radiation Plus Procarbazine, Lomustine (CCNU), and Vincristine in High-Risk Low-Grade Glioma

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abstract

PURPOSE NRG Oncology/RTOG 9802 (ClinicalTrials.gov Identifier: NCT00003375) is a practice-changing study for patients with WHO low-grade glioma (LGG, grade II), as it was the first to demonstrate a survival benefit of adjuvant chemoradiotherapy over radiotherapy. This post hoc study sought to determine the prognostic and predictive impact of the WHO-defined molecular subgroups and corresponding molecular alterations within NRG Oncology/RTOG 9802.

METHODS *IDH1/2* mutations were determined by immunohistochemistry and/or deep sequencing. A custom Ion AmpliSeq panel was used for mutation analysis. 1p/19q codeletion and *MGMT* promoter methylation were determined by copy-number arrays and/or Illumina 450K array, respectively. Progression-free survival (PFS) and overall survival (OS) were estimated using the Kaplan-Meier method. Hazard ratios (HRs) were calculated using the Cox proportional hazard model and tested using the log-rank test. Multivariable analyses (MVAs) were performed incorporating treatment and common prognostic factors as covariates.

RESULTS Of the eligible patients successfully profiled for the WHO-defined molecular groups (n = 106/251), 26 (24%) were *IDH*-wild type, 43 (41%) were *IDH*-mutant/non-codeleted, and 37(35%) were *IDH*-mutant/codeleted. MVAs demonstrated that WHO subgroup was a significant predictor of PFS after adjustment for clinical variables and treatment. Notably, treatment with postradiation chemotherapy (PCV; procarbazine, lomustine (CCNU), and vincristine) was associated with longer PFS (HR, 0.32; $P = .003$; HR, 0.13; $P < .001$) and OS (HR, 0.38; $P = .013$; HR, 0.21; $P = .029$) in the *IDH*-mutant/non-codeleted and *IDH*-mutant/codeleted subgroups, respectively. In contrast, no significant difference in either PFS or OS was observed with the addition of PCV in the *IDH*-wild-type subgroup.

CONCLUSION This study is the first to report the predictive value of the WHO-defined diagnostic classification in a set of uniformly treated patients with LGG in a clinical trial. Importantly, this post hoc analysis supports the notion that patients with *IDH*-mutant high-risk LGG regardless of codeletion status receive benefit from the addition of PCV.

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ASSOCIATED CONTENT

Data Supplement
Protocol

Author affiliations and support information (if applicable) appear at the end of this article.

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INTRODUCTION

Presently, there are a number of controversial and unresolved issues in the management of patients with diffuse low-grade glioma (LGG; grade II),[1] primarily accurate predictive biomarker classification and treatment selection. Clinical trial correlative data are limited because of tumor rarity, requirements for long-term follow-up, and lack of mandatory tissue collection requirements. Notably, NRG Oncology/RTOG 9802 (ClinicalTrials.gov Identifier: NCT00003375)[2] demonstrated for the first time an increase in overall survival (OS) with the addition of postradiation chemotherapy (PCV; procarbazine, lomustine (CCNU), and vincristine) in high-risk LGG, where high-risk is defined as age ≥ 40 years or subtotal resection/biopsy. The current report, with extensive follow-up, is a continuation of the

CONTEXT

Key Objective

High-risk low-grade glioma patients display highly variable survival outcomes depending on molecular subgroup. This analysis examined whether the WHO-defined molecular subgroups demonstrated prognostic and predictive value when patients received postradiation chemotherapy (PCV) versus radiation alone on NRG Oncology/RTOG 9802.

Knowledge Generated

The analyses demonstrated that both *IDH*-mutant subgroups regardless of codeletion status receive benefit with the addition of postradiation PCV. This is the first phase III study to our knowledge to demonstrate this benefit.

Relevance

Consideration should be given for adjuvant PCV in the setting of high-risk low-grade glioma patients harboring *IDH* mutations regardless of co-deletion status until more effective strategies are validated in large randomized studies. High-risk low-grade glioma patients harboring *IDH* wild-type tumors require more aggressive regimens and should be considered for clinical trials.

correlative analysis from the primary report,[2] which initially only included IDH1 R132H immunohistochemistry (IHC) data because of limited tissue availability. Since the initial analysis, additional tissues have been retrieved retrospectively to enable comprehensive genomic analyses, although sample sizes are still limited because tissue was not prospectively mandated.

Although a substantial number of comprehensive large studies[3-5] have established the molecular-based prognostic classification for LGGs that led to the WHO 2016 reclassification,[6] the majority of these previous studies were primarily done retrospectively using multiple institutional cohorts and comprised a range of WHO-defined grades, histologies, treatment modalities, and limited follow-up, compromising predictive interpretation. Only the recent report of the prognostic analysis of EORTC 22033-26033 (ClinicalTrials.gov Identifier: NCT00182819)[7] used tissue that was prospectively collected from patients with grade II glioma; however, this study only assessed different adjuvant monotherapies and was not able to assess predictive values. Most importantly, there have been no reports to date on the specific molecular classes that are predictive of adjuvant PCV in high-risk LGG, specifically utilizing prospective clinical trial data.

NRG Oncology/RTOG 9802[2,8] provides a unique opportunity for correlative research, as the study was practice-changing, having established radiation (RT) plus PCV as the new standard of care for patients with high-risk LGGs. The well-annotated demographic and clinical data with long-term follow-up have enabled rigorous examination of the prognostic and predictive significance of these genetic biomarkers. There has been substantial focus on *IDH1/2* mutations and 1p/19q status,[3-6,9-12] as these markers are required for glioma classification within the revised 2016 WHO CNS guidelines.[6] Importantly, additional mutations (eg, *ATRX*, *TP53*, and *TERT* promoter) are also associated with the 3 diagnostic subgroups.[3-5,12-14] Herein, we report the validation of the prognostic values and for the first time, to the best of our knowledge, the predictive values of the WHO-defined molecular subgroups within the context of a prospective high-risk LGG phase III trial.

METHODS

Tissue Cohort

A total of 116/251(46%) enrolled "high-risk" patients with LGG from the 2 treatment arms of NRG Oncology/RTOG 9802 had adequate tissues available for genomic analyses using multiple platforms as indicated. After neuropathology review, representative areas (> 70% tumor) were selected for DNA isolation.

Mutation and Codeletion Analysis

IHC with the mutation-specific monoclonal antibody *IDH1*-R132H (Dianova, Hamburg, Germany) was used to assess for the canonical IDH1-R132H mutation. To assess for noncanonical *IDH1/2* mutations and mutations in *ATRX*, *CIC*, and *FUBP1*, a customized Ion AmpliSeq (Thermo Fisher Scientific, Waltham, MA) DNA panel was designed and used. Sequence alignment and variant calling were performed using the Ion Suite and Reporter software. *TERT* promoter mutations were assessed by Sanger sequencing. Codeletion of chromosomes 1p and 19q was determined by Affymetrix Oncoscan FFPE Assay and/or Illumina 450K methylation arrays, and *MGMT* promoter methylation was determined by using the MGMT-STP27 model.[15] Additional methods can be found in the Data Supplement (online only).

Statistical Analysis

Pretreatment characteristics were compared between patients with adequate tissue included in this analysis and those without tissue, to ensure that the selected and evaluated cohort was truly representative of the entire trial (Table 1). Each biomarker was analyzed individually for its prognostic effect on survival outcomes, with OS being the

TABLE 1. Pretreatment Characteristics by Analysis Inclusion

	In Analysis		Not in Analysis		Total	
Characteristic	**No.**	**%**	**No.**	**%**	**No.**	**%**
Age, years					$P = .93$	
≤ 29	18	15.5	25	18.5	43	17.1
30-39	35	30.2	38	28.1	73	29.1
40-49	32	27.6	36	26.7	68	27.1
≥ 50	31	26.7	36	26.7	67	26.7
Sex					$P = .39$	
Male	69	59.5	73	54.1	142	56.6
Female	47	40.5	62	45.9	109	43.4
Race, White *v* other					$P = .21$	
White	102	87.9	125	92.6	227	90.4
Hispanic or Latino	7	6.0	4	3.0	11	4.4
Black or African American	5	4.3	5	3.7	10	4.0
Asian	0	0.0	1	0.7	1	0.4
American Indian or Alaskan Native	2	1.7	0	0.0	2	0.8
Karnofsky performance status					$P = .68$	
60-80	31	26.7	33	24.4	64	25.5
90-100	85	73.3	102	75.6	187	74.5
Prior surgery					$P = .009$	
Biopsy	43	37.1	76	56.3	119	47.4
Partial resection	58	50.0	49	36.3	107	42.6
Total resection	15	12.9	10	7.4	25	10.0
Neurologic function, no *v* minor *v* moderate					$P = .20$	
No symptoms	45	38.8	66	48.9	111	44.2
Minor symptoms	57	49.1	59	43.7	116	46.2
Moderate (fully active)	11	9.5	4	3.0	15	6.0
Moderate (not fully active)	3	2.6	6	4.4	9	3.6
Histology, astrocytoma *v* oligodendroglioma *v* oligoastrocytoma					$P = .36$	
Astrocytoma	26	22.4	39	28.9	65	25.9
Oligodendroglioma	49	42.2	58	43.0	107	42.6
Oligoastrocytoma, astro dominant	18	15.5	20	14.8	38	15.1
Oligoastrocytoma, astro = oligo	3	2.6	3	2.2	6	2.4
Oligoastrocytoma, oligo dominant	20	17.2	15	11.1	35	13.9
Assigned treatment					$P = .85$	
RT alone	59	50.9	67	49.6	126	50.2
RT + PCV	57	49.1	68	50.4	125	49.8
Total	116	100.0	135	100.0	251	100.0
Median survival time, years	10.7		9.5		9.9	

NOTE. *P* value by χ^2 test.
Abbreviations: PCV, procarbazine, lomustine (CCNU), and vincristine; RT, radiation therapy.

primary end point, followed by PFS. The prognostic effect of the combination of *IDH1/2* mutation and 1p/19q codeletion was analyzed with the 3 WHO diagnostic subgroups: *IDH*wt, *IDH*mut/non-codel, and *IDH*mut/codel. OS was defined as time from randomization to death or the last follow-up when patients were reported alive; PFS was defined as time from randomization to progression or death, whichever occured first, or the last follow-up when patients

were reported alive without having experienced disease progression. OS and PFS were estimated using the Kaplan-Meier method.[16] Hazard ratios (HRs) were calculated using the Cox proportional hazard model[17] and tested using the log-rank test. MVAs were performed, including age, sex, surgery, performance status, neurologic function, histology, and treatment assignment, as covariates, using the stepwise method for variable selection. The proportional hazards assumption was examined by testing the association between the scaled Schoenfeld residuals and the Kaplan-Meier transformed survival times. For the predictive effects of each biomarker on OS and PFS, only univariable analyses were performed for each marker group, and the log-rank test was used to test the difference in treatment effects. All predictive analyses were considered exploratory because of small sample sizes for patients with specific biomarker features in the majority of the cases. A standard 5% significance level was used for all analyses.

RESULTS

Molecular Analyses

IDH1/2 *mutation analysis.* Of the 116 patients with adequate tissue for *IDH* analysis, 115 had *IDH1/2* mutation information from IHC and/or sequencing (Figs 1, 2 and 3A-B). Of the 115 samples, 89 (77%) had *IDH1/2* mutations and 26 (23%) were classified as IDH1/2-wild type. Regarding IHC, 112 patients had IDH1R132H IHC data (43 negative and 69 positive). Sequencing data were obtained for *IDH1* on 103 patients, 80 (78%) of whom were positive, and 23 (22%) negative for the R132 mutation. Of the 43 negative cases assessed by IHC, 2 had the IDH2R172K mutation, 10 had noncanonical IDH1R132 mutations, and 7 were not sequenced. Of the 80 patients with an *IDH1*R132 mutation, 70 (88%) had the classic R132H alteration, 4 (5%) had R132S, 3 had R132G (4%), and 3 (4%) had R132C. *IDH2* mutation status was available for 103 patients, and only 2 (2%) patients had the *IDH2*R172K mutation.

Other mutations and* MGMT *promoter methylation. Patient samples (n = 105) were subjected to a custom Ion Torrent sequencing panel targeting the coding regions of *IDH1*, *IDH2*, *CIC*, *FUBP1*, and *ATRX*, of which mutation calls could not be determined in 3 samples because of low coverage in ≥ 1 genes (Data Supplement). In summary, mutations were identified within the *ATRX* gene in 25% (26/103), *CIC* in 23% (23/102), and *FUBP1* in 9% (9/103) of analyzed cases (Figs 1 and 2). Each individual mutation was then analyzed by deleterious predictive algorithms to

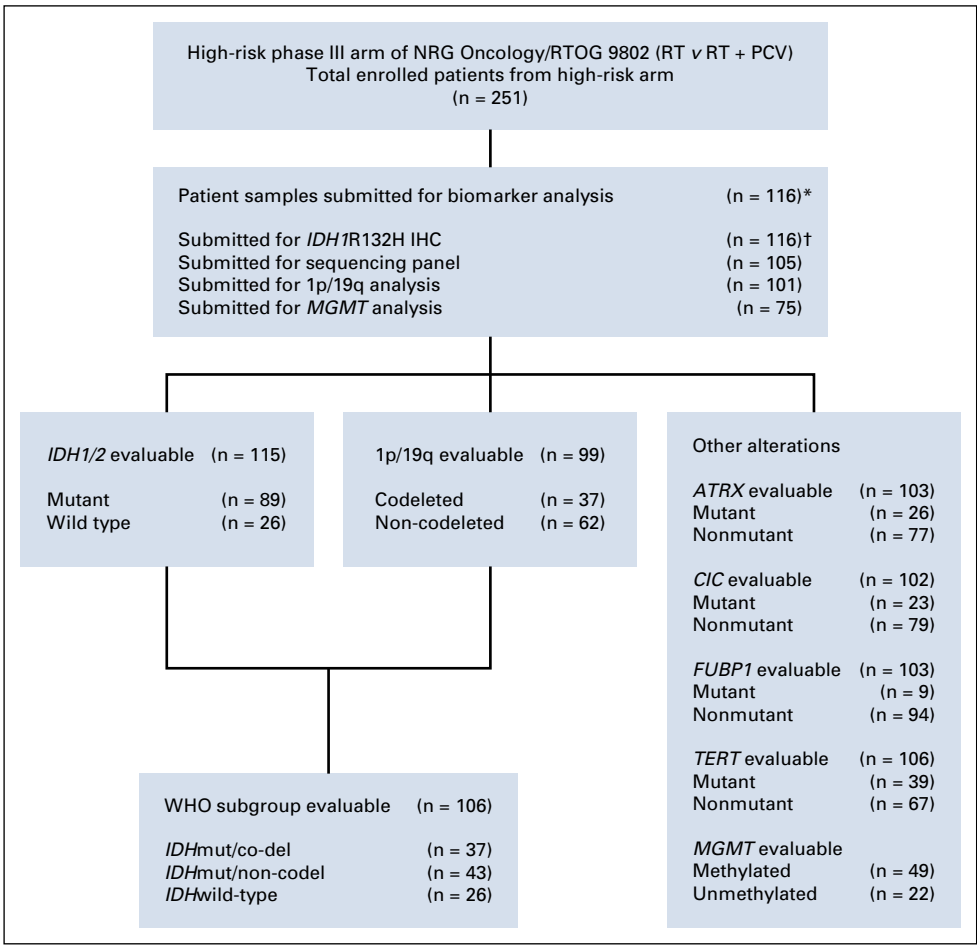

FIG 1. Biomarker analysis for NRG oncology/RTOG 9802. (*) Tissue collection was not mandatory for this trial. (†) Patient samples were prioritized for each platform accordingly: (1) IDHR132H IHC, (2) sequencing panel, (3) 1p/19q analysis, (4) *MGMT* promoter methylation analysis. IHC, immunohistochemistry; PCV, procarbazine, lomustine (CCNU), and vincristine; RT, radiation therapy.

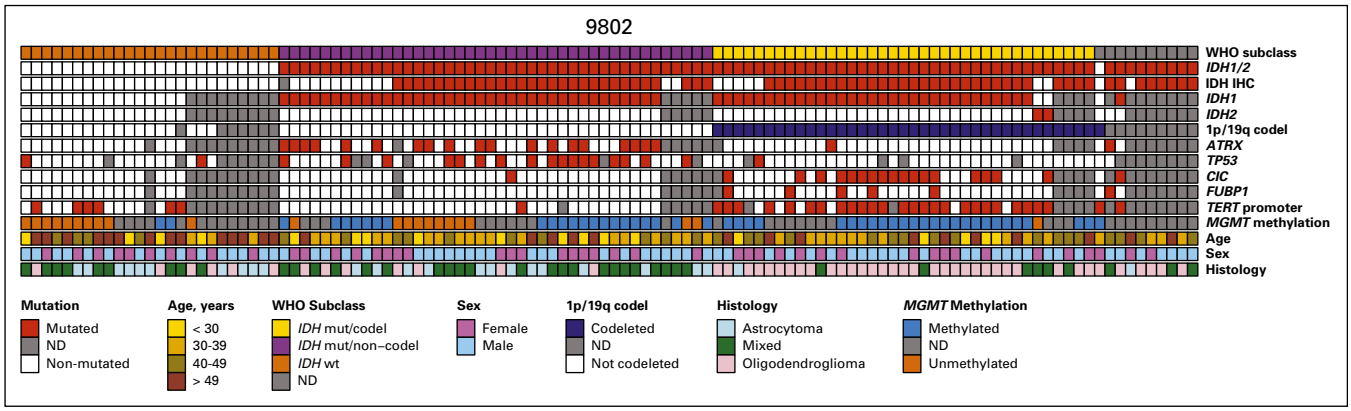

FIG 2. Mutational landscape in NRG Oncology/RTOG 9802. A summary of the molecular findings in 115 RTOG/NRG 9802 cases along with select clinical data including age, sex, and histology. The top row shows the classification of patients into the 3 newly established molecular subgroups (*IDH*mutant/codeleted (*IDH*mut/codel) *IDH*mutant/non-codeleted (*IDH*mut/non-codel), and *IDH*wild-type (*IDH*wt), along with a fourth group, *IDH*mut/not determined (ND), because of the lack of available information on 1p19q status within these patients. The second row is a final summary of patients with *IDH1/2* mutations acquire by either sequencing or immunohistochemistry (IHC). Below are the individual results of IDH1 IHC and *IDH1* and *IDH2* sequencing, respectively.

determine the mutation assessor score and the likely functional significance of each mutation and/or alteration.[18] The Data Supplement contains the compilation tables of mutations and deleterious predictions. Moreover, specific mutations in the *TERT* promoter were identified in 37% (39/106) of analyzed cases, in which 79% (31/39) of those mutated had mutations at the C228T-124nt site and 21% (8/39) of patients had mutations at the C250T-146nt site (Data Supplement). *TP53* mutation data at specific sites were available from Affymetrix Oncoscan data on 91 patient samples. Of these, 21 patients had *TP53* mutations (Data Supplement). Other mutations included on the Affymetrix Oncoscan array were *BRAF*, *EGFR*, *KRAS*, *NRAS*, *PIK3CA*, and *PTEN*; however, these mutations had very low frequencies and were not used for additional correlative analyses.

A total of 101 patients had available copy number variation (CNV) from Affymetrix Oncoscan arrays (n = 89) and/or the Illumina DNA Methylation 450K arrays (n = 69), and 59 patients' data were available from both platforms (Fig 1). Ninety-nine patient samples (98%) had good quality CNV to determine 1p and 19q codeletion status. Of these 99 patients, 37 (37%) were 1p/19q codeleted and 62 (63%) 1p/19q non-codeleted. Using the methylation arrays, 71 patients had good-quality data to assess the *MGMT* promoter methylation status: 49 (69%) were methylated, and 22 (31%) were unmethylated (Fig 1; Data Supplement).

WHO classification. To group the patients from NRG Oncology/RTOG 9802 into the newly defined 3 WHO prognostic classes *IDH*mut/codel (oligodendroglioma), *IDH*mut/non-codel (astrocytoma *IDH*mt), and *IDH*wt (astrocytoma *IDH*wt), we combined the *IDH1/2* mutation data with 1p/19q codeletion status. Of the 106 patients with adequate data and analyzed, 37 (35%) were *IDH*mut/codeleted, 43 (41%) *IDH*mut/non-codeleted, and 26 (24%) *IDH*wt. One unique patient was *IDH* wild type (by sequencing and immunohistochemistry) and 1p/19q codeleted. However, this unique patient (originally classified as an oligodendroglioma) in deeper analysis had a previously unknown noncanonical *IDH2* alteration with 2 amino acid changes at positions 172-173 and was not included within the WHO classification and survival analyses.

Survival Analyses

For all patients included in this study, median follow-up time was 9.0 (95% CI, 0.2-14.8) years. For all patients alive at the time of the analyses, median follow-up was 12.0 (95% CI, 5.3-14.8) years. Patients included in the analysis were not significantly different from the nonincluded cohort in terms of pretreatment characteristics or survival (Table 1; Data Supplement). Patient characteristics by molecular subgroup are shown in Table 2. For the analyses of the prognostic effects of some biomarkers, there was some evidence of nonproportional effects between marker groups. However, given the small sample sizes in the majority of cases, and that the Kaplan-Meier curves do not strongly converge or cross, HRs still provide a useful summary of the relative failure risk between groups and are presented.

Prognostic analyses. **Univariable analyses.** For OS, the 3 molecular subgroup analyses significantly associated with OS for all 3 comparisons (Table 3; Fig 3A). The median survival times (MSTs) were 13.9 years (95% CI, 11.4 to not reached [NR]; *IDH*mut/codel), 6.9 years (95% CI, 4.2 to 11.4; *IDH*mut/non-codel), and 1.9 years (95% CI, 1.1 to 4.2; *IDH*wt), respectively. As individual biomarkers, *IDH1/2* mutations, 1p/19q codeletion, and *TERT* promoter mutations were significantly associated with better OS (Data Supplement).

TABLE 2. Pretreatment Characteristics by *IDH*-1p/19q Subgroup for Patients With High-Risk Low-Grade Glioma

	***IDH* Wild Type**		***IDH* Mutant With 1p19q Non-codeleted**		***IDH* Mutant with 1p19q Codeleted**		**Total**	
Characteristic	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Age, years							*P* = .002	
≤ 29	4	15.4	9	20.9	5	13.5	18	17.0
30-39	3	11.5	18	41.9	11	29.7	32	30.2
40-49	4	15.4	11	25.6	13	35.1	28	26.4
≥ 50	15	57.7	5	11.6	8	21.6	28	26.4
Sex							*P* = .99	
Male	15	57.7	25	58.1	22	59.5	62	58.5
Female	11	42.3	18	41.9	15	40.5	44	41.5
Race, White *v* other							*P* = .36	
White	25	96.2	37	86.0	31	83.8	93	87.7
Hispanic or Latino	0	0.0	4	9.3	2	5.4	6	5.7
Black or African American	1	3.8	2	4.7	2	5.4	5	4.7
American Indian or Alaskan Native	0	0.0	0	0.0	2	5.4	2	1.9
Karnofsky performance status							*P* = .41	
60-80	9	34.6	9	20.9	8	21.6	26	24.5
90-100	17	65.4	34	79.1	29	78.4	80	75.5
Prior surgery							*P* < .001	
Biopsy	18	69.2	6	14.0	12	32.4	36	34.0
Partial resection	6	23.1	32	74.4	17	45.9	55	51.9
Total resection	2	7.7	5	11.6	8	21.6	15	14.2
Neurologic function, no *v* minor *v* moderate							*P* = .47	
No symptoms	9	34.6	17	39.5	16	43.2	42	39.6
Minor symptoms	13	50.0	17	39.5	20	54.1	50	47.2
Moderate (fully active)	3	11.5	8	18.6	0	0.0	11	10.4
Moderate (not fully active)	1	3.8	1	2.3	1	2.7	3	2.8
Histology, astrocytoma *v* oligodendroglioma *v* oligoastrocytoma							*P* < .001	
Astrocytoma	11	42.3	13	30.2	1	2.7	25	23.6
Oligodendroglioma	6	23.1	7	16.3	30	81.1	43	40.6
Oligoastrocytoma, astro dominant	4	15.4	13	30.2	0	0.0	17	16.0
Oligoastrocytoma, astro = oligo	0	0.0	3	7.0	0	0.0	3	2.8
Oligoastrocytoma, oligo dominant	5	19.2	7	16.3	6	16.2	18	17.0
Assigned treatment							*P* = .99	
RT alone	14	53.8	22	51.2	19	51.4	55	51.9
RT + PCV	12	46.2	21	48.8	18	48.6	51	48.1
Total	26	100.0	43	100.0	37	100.0	106	100.0

NOTE. *P* value by Fisher's exact test.

Abbreviations: PCV, procarbazine, lomustine (CCNU), and vincristine; RT, radiation therapy.

For PFS, molecular subgroup was associated with PFS for all 3 comparisons (Table 3; Fig 3B). The median PFS times were 10.2 years (95% CI, 7.6 to NR; *IDH*mut/codel), 3.9 years (95% CI, 2.4 to 6.0; *IDH*mut/non-codel), and 0.7 years (95% CI, 0.5 to 0.9; *IDH*wt), respectively. As individual biomarkers, *IDH1/2* mutations and 1p/19q codeletions correlated with better outcomes, whereas *TERT* promoter mutations only trended toward better PFS (Data Supplement).

TABLE 3. Univariable and Multivariable Cox Proportional Hazards Models for WHO Classification and Survival Outcomes

Variable	*P*	HR (95% CI)
Overall survival		
Combined *IDH1/2*-1p/19q status model		
Univariable analysis		
IDH-1p/19q (***IDH* mutant with 1p/19q codeleted** *v IDH* wild type)	< .001	0.20 (0.09 to 0.41)
(***IDH* mutant with 1p/19q codeleted** *v IDH* mutant with 1p/19q non-codeleted)	.001	0.31 (0.16 to 0.63)
(***IDH* mutant with 1p/19q non-codeleted** *v IDH* wild type)	.02	0.51 (0.29 to 0.91)
Multivariable analysis		
Assigned treatment (**RT + PCV** *v* RT alone)	.008	0.48 (0.28 to 0.83)
IDH-1p/19q (***IDH* mutant with 1p/19q codeleted** *v IDH* wild type)	< .001	0.18 (0.09 to 0.40)
(***IDH* mutant with 1p/19q non-codeleted** *v IDH* wild type)	.048	0.56 (0.31 to 0.99)
Progression-free survival		
Combined *IDH1/2*-1p/19q status model		
Univariable analysis		
IDH-1p/19q (***IDH* mutant with 1p/19q codeleted** *v IDH* wild type)	< .001	0.23 (0.12 to 0.43)
(***IDH* mutant with 1p/19q codeleted** *v IDH* mutant with 1p/19q non-codeleted)	.01	0.46 (0.26 to 0.82)
(***IDH* mutant with 1/19q non-codeleted** *v IDH* wild type)	.002	0.43 (0.25 to 0.73)
Multivariable analysis		
Assigned treatment (**RT + PCV** *v* RT alone)	< .001	0.37 (0.22 to 0.61)
IDH-1p/19q (***IDH* mutant with 1p/19q codeleted** *v IDH* wild type)	< .001	0.22 (0.11 to 0.40)
(***IDH* mutant with 1p/19q non-codeleted** *v IDH* wild type)	.005	0.46 (0.27 to 0.80)

NOTE. Model is derived from stepwise selection. Treatment, histology, age, neurologic function, sex, surgery, and Karnofsky Performance Status were considered as covariates in variable selection. Those not listed dropped out of the selection process. Bolded value has favorable outcome.

Abbreviations: HR, hazard ratio; PCV, procarbazine, lomustine (CCNU), and vincristine; RT, radiation therapy.

CIC alterations did show correlation with OS, although they concomitantly occur with 1p/19q codeletions. All other alterations (*ATRX*, *FUBP1*, *TP53*, and *MGMT*) did not reach statistical significance for OS or PFS on univariable analysis (Data Supplement). Additional subset analyses within the 3 subgroups also did not reach statistical significance.

Multivariable analyses. On MVAs for OS (Table 3), the 3 molecular subgroups were also significantly different for both comparisons on OS (HR, 0.18; 95% CI, 0.09 to 0.40; $P < .001$; *IDH*mut/codel *v IDH*wt; and HR, 0.56; 95% CI, 0.31 to 0.99; $P = .048$; *IDH*mut/non-codel *v IDH*wt). Individually, the statistical significance for favorable OS was maintained for *IDH1/2* mutations and 1p/19q codeletions (Data Supplement) but not for the *TERT* promoter.

Regarding PFS (Table 3), the 3 molecular subgroups were statistically significant on MVA for both of the comparisons (HR, 0.22; 95% CI, 0.11 to 0.40; $P < .001$; *IDH*mut/codel *v IDH*wt; and HR, 0.46; 95% CI, 0.27 to 0.80; $P = .005$; *IDH*mut/non-codel *v IDH*wt). Individually, the statistical significance with better outcomes was maintained for *IDH1/2* mutations as well as for 1p/19q codeletions (Data Supplement), but the effect of *TERT* promoter mutations remained insignificant. *MGMT* promoter methylation trended toward significance for OS and PFS incorporating clinical variables but did not retain this trend when incorporating *IDH* (Data Supplement). Sample sizes for other mutations (*ATRX*, *FUBP1*, and *TP53*) were too small, especially for less-frequent mutations, to consider them for additional investigation.

Predictive analyses. **Univariable analyses.** Treatment effects on OS and PFS within each WHO-defined molecular subgroup were analyzed. For the *IDH*mut/codel subgroup, patients treated with RT + PCV experienced longer OS and PFS times, compared with patients treated with RT alone (Figs 4A and 4B; OS: HR, 0.21; $P = .029$; MST, 13.9 years [RT] *v* NR [RT + PCV]; and PFS: HR, 0.13; $P < .001$; MST, 5.8 years [RT] *v* NR [RT + PCV]). For the *IDH*mut/non-codel subgroup, patients treated with RT + PCV experienced longer OS and PFS times compared with patients treated with RT alone (Figs 4C and 4D; OS: HR, 0.38; $P = .013$; MST, 4.3 years [RT] *v* 11.4 years [RT + PCV]; and PFS: HR, 0.32; $P = .003$; MST, 3.3 years [RT] *v* 10.4 years [RT + PCV]). For the group of *IDH*wt patients, OS and PFS were comparable between the 2 treatment arms (Figs 4E and 4F), implying no clinical benefit from the addition of PCV. Furthermore, this cohort had the worst outcomes, with

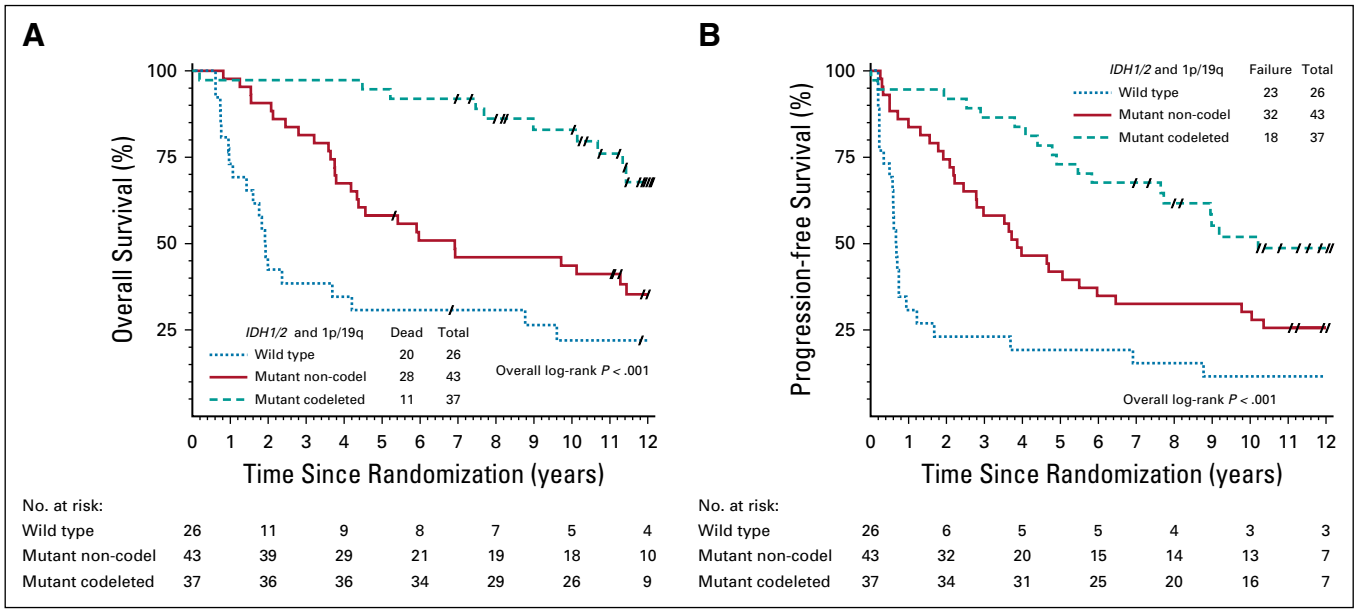

FIG 3. Molecular subgroup prognostic survival analyses. Kaplan-Meier survival plots show that the 3 WHO-defined molecular subgroups (*IDH*mut/codel, *IDH*mut/non-codel, and *IDH*wt) significantly stratified patients for both (A) overall survival, and (B) progression-free survival.

median OS and PFS of 1.9 and 0.7 years, respectively, values that approach those observed for glioblastoma. Because of the constraint on sample sizes, multivariable statistical tests were not performed for any of the predictive analyses.

DISCUSSION

Most notably, our study is the first to our knowledge to demonstrate the predictive value of the WHO-defined molecular subgroups in a practice-changing phase III clinical trial (NRG Oncology/RTOG 9802) of high-risk grade II glioma in correlation to OS with long-term follow-up data. Importantly, this study (although limited in sample size) demonstrates that both *IDH*mut subgroups regardless of codeletion status received benefit from the addition of adjuvant PCV to RT in the subset of patients examined in NRG Oncology/RTOG 9802. Our predictive results are consistent with previous studies that have comprehensively examined *IDH1/2* mutations and 1p/19q codeletions in phase III trials of grade III anaplastic oligodendrogliomas treated with RT plus PCV (RTOG 9402 [ClinicalTrials.gov identifier: NCT00002569], EORTC 26951 [ClinicalTrials.gov identifier: NCT00002840.]).[13,19-21] Thus, before the current study, the predictive value of the WHO-defined molecular subgroups for high-risk grade II gliomas was expected to reflect what was observed for high-risk grade III gliomas but had not yet been demonstrated in a clinical trial. Our evidence suggests that *IDH* mutation status could serve as the primary predictor of response to PCV in addition to RT in high-risk LGGs and is a more accurate predictor of response than historical histopathological classifications (Data Supplement). Nevertheless, patients with the 1p/19q codeletion did experience the greatest benefit in risk reduction to PFS and OS on treatment with adjuvant PCV plus RT, similar to previous reports in grade III patients.[19,21] Interestingly, the *IDH*mut/non-codel group had an unexpectedly poor median OS time of 6.9 years (95% CI, 4.2 to 11.4) relative to previous reports[5,22] and highlights the selection of high-risk patients with LGG in this trial. Conversely, the *IDH*wt subgroup experienced no demonstrable survival benefit from the addition of PCV.

Previously, other larger retrospective studies (eg, The Cancer Genome Atlas, Mayo/University of California San Francisco) have comprehensively established the prognostic classification of the combined IDH-1p19q subgroups; however, many of these were composed of heterogeneously treated grade II gliomas[3-5] and/or lacked long-term overall survival data.[3-5,7] Importantly, the results of this study also validated the prognostic significance of the molecular-based WHO subgroups in a phase III clinical trial independent of known clinical confounders. All other alterations (including *MGMT* promoter methylation) did not reach statistical significance, nor was significance maintained on MVA for PFS and OS in this study using RT plus PCV. These mutations likely did not hold statistical significance because they are associated with histology and the WHO-defined molecular subgroups in addition to the sample size being too small to determine their significance in each subgroup. For *MGMT*, it is crucial to interpret this in the context that the chemotherapy backbone was PCV, and not temozolomide. Particularly, it remains to be determined in a large cohort whether *TERT* promoter mutations,[4] *ATRX* mutations,[12,23] and *MGMT* promoter methylation[24] are prognostic within individual LGG molecular subgroups, as

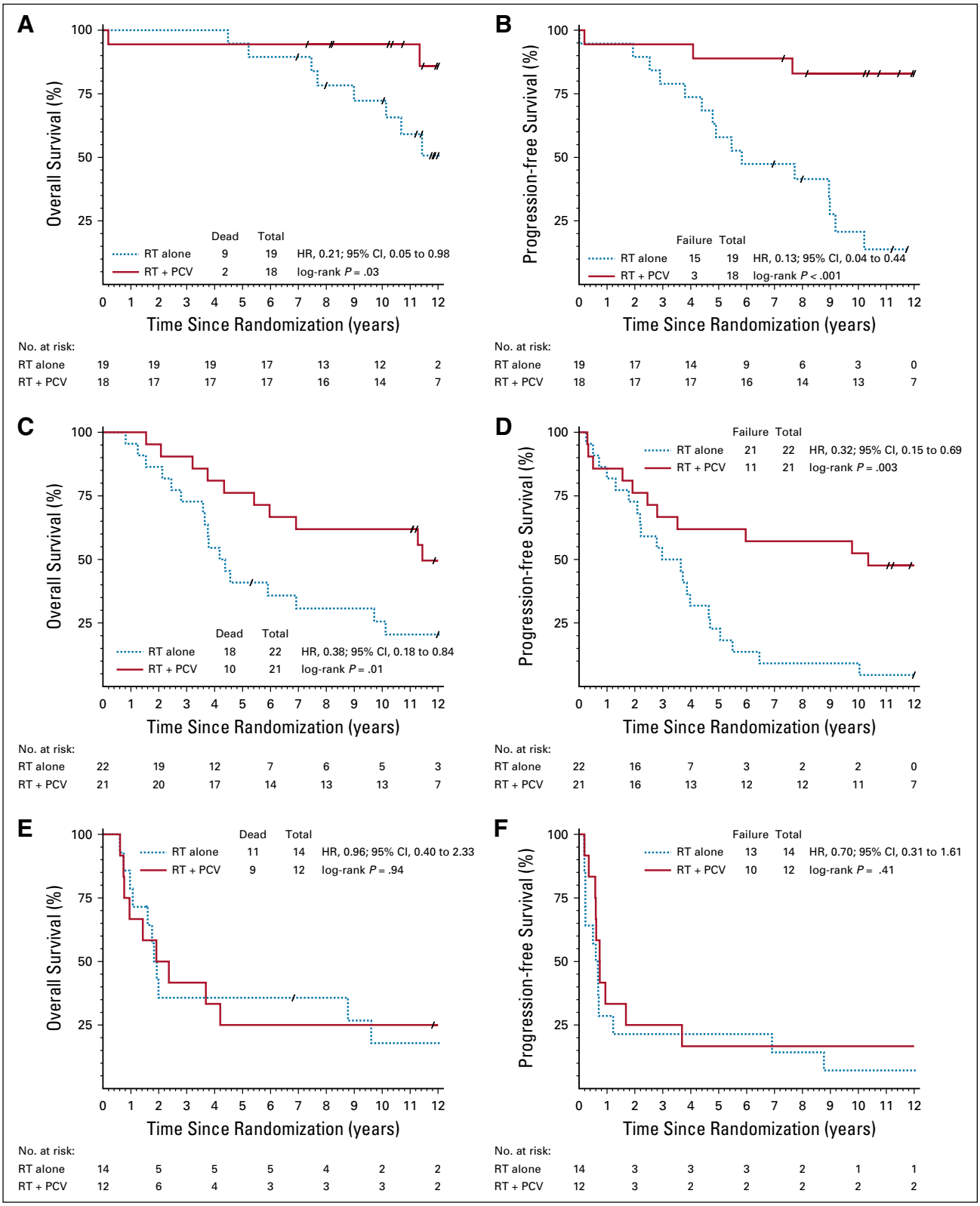

FIG 4. Survival by treatment and WHO-defined molecular subgroup. Kaplan-Meier survival plots show that patients with (A, B) *IDH*mut/codel and (C, D) *IDH*mut/non-codel demonstrated significantly improved overall survival and progression-free survival rates when treated with radiation therapy (RT) plus PCV (procarbazine, lomustine (CCNU), and vincristine) versus RT alone. (E, F) *IDH*wt tumors had no significant survival difference by treatment.

suggested in previous reports. However, interpretation of *TERT* promoter mutations is not straightforward, as they occur in both the glioblastoma-like (*IDH*wt) and oligodendroglioma (*IDH*mut/codel) tumors.

In addition, this study examined *IDH1/2* status on the basis of multiple platforms, and the differences observed between sequencing and IHC were primarily due to noncanonical mutations for which antibodies were not available,[25] thus reinforcing the approach of sequencing mutations in IDH1 IHC-negative patients. In some cases (7/112), IHC was negative for the R132H mutation and sequencing data were not available, although 2 patients were confirmed to be non-codeleted. Because these cases could have a noncanonical mutation (of which frequency is typically $< 10\%$[9]), our results may marginally underestimate the *IDH1/2* mutation frequency as well as survival differences between the *IDH* mutant and wild-type subgroups.

This correlative analysis demonstrates the necessity of upfront tissue collection, because future molecular and technological developments at the time of trial development are hard to predict. Unfortunately, specimens were not prospectively collected for molecular analyses on NRG Oncology/RTOG 9802, which limited our sample size. Despite these limitations, this study demonstrated a significant survival advantage with the addition of adjuvant PCV to RT for patients harboring either an *IDH*mut/codel or *IDH*mut/non-codel tumor. An ongoing clinical trial (ClinicalTrials.gov identifier: NCT00887146) will help determine the role of the effectiveness of PCV versus temozolomide and the predictive significance of specific WHO-defined molecular subgroups in this context. This study, importantly, can now help clinicians interpret the results of NRG Oncology/RTOG 9802 within the context of the altered molecular landscape and serve as a basis for survival times for the design of future high-risk LGG clinical trials.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Comprehensive Genomic Analysis in NRG Oncology/RTOG 9802: A Phase III Trial of Radiation Versus Radiation Plus Procarbazine, Lomustine (CCNU), and Vincristine in High-Risk Low-Grade Glioma

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