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Letter to the Editor

Hemizygous *CDKN2A* deletion confers worse survival outcomes in IDHmut-noncodel gliomas

The tumor suppressor gene *CDKN2A* encodes $p16^{INK4a}$ and $p14^{ARF}$ which regulate cell cycle progression, and is frequently deleted in cancer. Homozygous deletion (homdel) of *CDKN2A* has been incorporated as a Grade 4 defining criterion for IDH-mutant gliomas without 1p-/19q-codeletion (IDHmut-noncodel) in the WHO CNS5 classification.[1] However, the prognostic value of hemizygous *CDKN2A* deletions (hemidel) is unclear. We assessed copy number variation profiles of initial (n = 1256) and recurrent (n = 494) IDHmut gliomas to evaluate the impact of *CDKN2A* hemidel on overall survival (OS) outcomes (Figure 1).

Using the GLASS primary/recurrent glioma dataset,[2,3] we inferred *CDKN2A* status using DNAseq (n = 240) and DNA methylation array data (n = 100). Longitudinal analyses of IDH-mutant gliomas without 1p-/19q-codeletion (IDHmut-noncodels) revealed a significant increase in *CDKN2A* homdel at recurrence, as previously described,[4] from n = 8 to n = 25 (6% to 20%, P = .0002, Fisher's-exact test), but also in *CDKN2A* hemidel from n = 22 to n = 37 (17% to 29%, P = .0037). Overlapping DNAseq and DNA methylation profiles, available for n = 29 cases, illustrated strong concordance of log2-*CDKN2A* values between these platforms (R = 0.82, P = 4.4e-13, Pearson correlation).

We inferred tumor purity from DNAseq and DNA methylation array data. Neither the *CDKN2A* status derived from DNA methylation array (P = .27) nor from DNAseq (P = .61) was affected by tumor purity, indicating a robust assessment of *CDKN2A* status independent of platform or purity. Importantly, *CDKN2A* hemidels were present in a large proportion of cancer cells (median cancer cell fraction = 0.98), indicating a high degree of clonality. These data reflect the observation that *CDKN2A* hemidels are not misclassifications or merely subcloncal homozygous deletions. Similar distribution analyses of IDHmut-codels demonstrated that the increase in *CDKN2A* homdel and hemidel at recurrence is specific to IDHmut-noncodels.

These findings prompted us to characterize the impact of *CDKN2A* hemidel on overall survival, defined as time from diagnosis to death or last follow-up, in IDHmut-noncodel gliomas. The presence of *CDKN2A* hemidel was significantly associated with poor OS at initial diagnosis (P = .00019, log-rank test) and recurrence (P = 00018). This association remained significant in a multivariable Cox regression model adjusting for age and treatment with radiotherapy and/or alkylating agents: *CDKN2A* hemidel HR 2.15 (95% CI: 1.15–4.03, P = .02) and homdel HR 3.99 (95% CI: 1.83–8.70, P < .001).

We then sought to validate our observations using the DFCI,[5] MSKCC,[6] and TCGA[7] datasets, which were combined to form a discovery cohort. For the TCGA dataset,[7] cases included in GLASS were excluded, and only initial cases were included. In the DFCI dataset,[5] we noted a higher rate of *CDKN2A* hemidel among IDHmut-noncodels recurrences compared to non-matched primaries (14% to 23%, P = .025, Fishers' exact test). Analyses of the MSKCC dataset[6] also revealed a similar distribution of *CDKN2A* status and an increase of *CDKN2A* homdel/hemidel at recurrence, particularly in IDHmut-noncodels. Clinical outcome analyses of the discovery cohort demonstrated worse OS in initial (P < .0001) and recurrent (P < .0034) *CDKN2A* deleted IDHmut-noncodel gliomas. This association remained significant in a multivariable Cox regression model that accounted for age, treatment and center: *CDKN2A* hemidel HR 2.08 (95% CI: 1.41–3.08, P < .001) and homdel HR 2.45 (95% CI: 1.54–3.88, P < .001).

Taken together, we have confirmed in four independent datasets[4–7] that *CDKN2A* hemidel was associated with worse OS in IDHmut-noncodel gliomas independent of time point. These results highlight the clinical relevance of assessing *CDKN2A* status at initial diagnosis and at recurrence.

CDKN2A status can be accurately assessed by DNAseq, including the panel sequencing used in the DFCI and MSKCC datasets. DNA methylation array profiling is also increasingly being integrated into clinical workflows[8] and can be used to derive DNA copy number profiles, enabling assessment of the *CDKN2A* status in gliomas. We analyzed the DKFZ DNA methylation dataset[8] and found that the frequency of *CDKN2A* hemidels more than doubled in recurrent cases (P = .016), suggesting that DNA methylation profiles are amenable to detection of *CDKN2A* hemidel.

As a technical note, the *CDKN2A* copy number status was obtained from previous publications when available.[2,4,5,7] For the MSKCC[6] and the DNA methylation array[8] datasets, the distinction between homozygous and hemizygous deletions was not available, and we applied conservative cutoffs defining a log2 copy ratio ≤ –1-1 as *homozygous deletion* and –1.1 < log2 copy ratio ≤ –0.4 as *hemizygous deletion*, consistent with the stringent criteria of the other datasets.[2,4,5,7]

In conclusion, our analysis shows that the presence of *CDKN2A* hemidel at initial diagnosis and recurrence is associated with significantly worse OS in IDHmut-noncodel gliomas. Similar to *CDKN2A* homdel,[4] *CDKN2A* hemidel is enriched in post-treatment, recurrent IDHmut-noncodel gliomas, confirming the value of *CDKN2A* status (re-) assessment in recurrences. Our results highlight the importance of incorporating *CDKN2A* status into the diagnostic work-up to inform prognosis and treatment strategies.

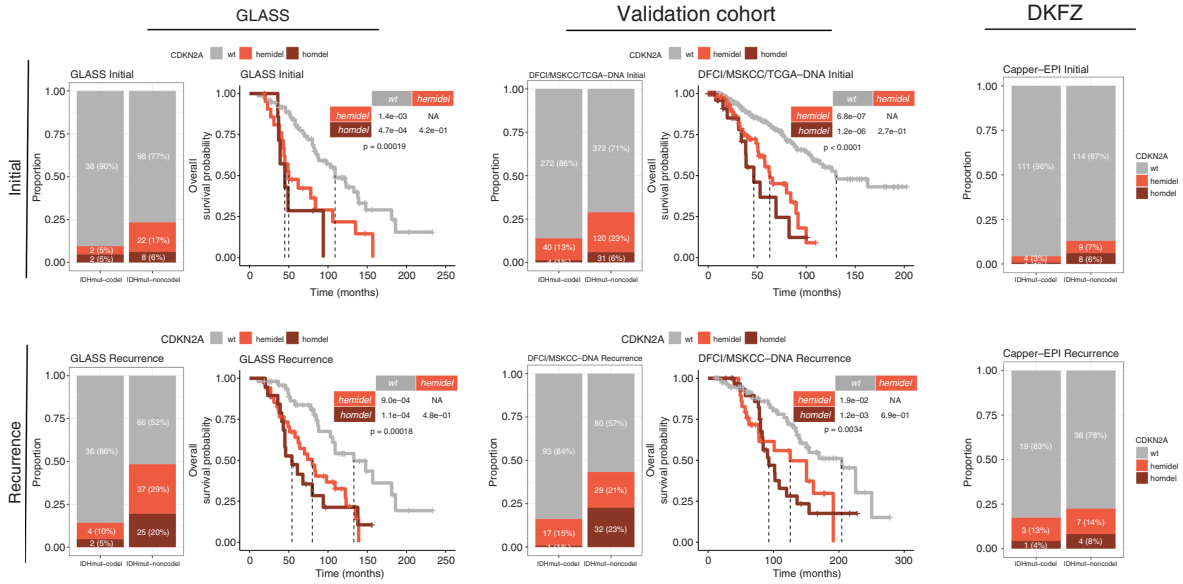

Figure 1. Distribution of *CDKN2A* homozygous deletion, hemizygous deletion and wild type (wt) across six IDHmut glioma cohorts. The discovery cohort consists of the GLASS DNAseq and DNA methylation profiling datasets. The validation cohort consists of the DFCI, MSKCC, and TCGA datasets, with further validation in DKFZ methylation profiling dataset. Survival analyses are specific to IDHmut-noncodel gliomas. Upper panel depicts initial cases; lower panel depicts recurrent cases. Stacked bar plots showing the total number and relative proportion of cases with CDKN2A homozygous deletion, hemizygous deletion and wt, separated by molecular subtype into columns representing IDHmut-codel and IDHmut-noncodel. Fisher's exact test was applied as a statistical test to compare initial and recurrent gliomas. Kaplan–Meier survival plots depict overall survival probability (y-axis) and survival time (x-axis). Overall survival indicates the time from diagnosis to death or last date of follow-up as censoring. Global log-rank test was applied for comparison of 3 groups, and pairwise log-rank test was applied for comparison of 2 groups. Note that the TCGA cohort included only initial glioma cases. No survival data was available for the DKFZ cohort. GLASS, Glioma Longitudinal Analysis Consortium; DFCI, Dana-Farber Cancer Institute; MSKCC, Memorial Sloan Kettering Cancer Center; TCGA, The Cancer Genome Atlas; DKFZ, Deutsches Krebsforschungszentrum.

Keywords

astrocytoma | *CDKN2A* | glioma | hemizygous deletion | IDH-mutant

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Conflict of interests statement

RGWV is a co-founder of Boundless Bio.

Emre Kocakavuk, Kevin C. Johnson, Thais S. Sabedot, Hans Christian Reinhardt, Houtan Noushmehr, Roel G.W. Verhaak

Department of Neurosurgery, Yale School of Medicine, New Haven, Connecticut, USA(E.K., K.C.J., R.G.W.V.); Department of Hematology and Stem Cell Transplantation, West German Cancer Center (WTZ), National Center for Tumor Diseases (NCT) West, University Hospital Essen, University of Duisburg-Essen, Essen, Germany(E.K., H.C.R.); Department of Neurosurgery, Hermelin Brain Tumor Center, Henry Ford Hospital, Detroit, Michigan, USA(T.S.S., H.N.); Department of Neurosurgery, Amsterdam University Medical Center, Amsterdam, The Netherlands(R.G.W.V.)

Corresponding Authors: Emre Kocakavuk, MD, Department of Hematology and Stem Cell Transplantation, University Hospital Essen, Hufelandstr. 55, 45147 Essen, Germany (emre.kocakavuk@uk-essen.de) or Roel G.W. Verhaak, MD, Department of Neurosurgery, Yale School of Medicine, 333 Cedar St, New Haven, CT 06510, USA (roel.verhaak@yale.edu).

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