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Clinical utility of whole-genome DNA methylation profiling as a primary molecular diagnostic assay for central nervous system tumors—A prospective study and guidelines for clinical testing

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Abstract

Background. Central nervous system (CNS) cancer is the 10th leading cause of cancer-associated deaths for adults, but the leading cause in pediatric patients and young adults. The variety and complexity of histologic subtypes can lead to diagnostic errors. DNA methylation is an epigenetic modification that provides a tumor type-specific signature that can be used for diagnosis.

Methods. We performed a prospective study using DNA methylation analysis as a primary diagnostic method for 1921 brain tumors. All tumors received a pathology diagnosis and profiling by whole genome DNA methylation, followed by next-generation DNA and RNA sequencing. Results were stratified by concordance between DNA methylation and histopathology, establishing diagnostic utility.

Results. Of the 1602 cases with a World Health Organization histologic diagnosis, DNA methylation identified a diagnostic mismatch in 225 cases (14%), 78 cases (5%) did not classify with any class, and in an additional 110 (7%) cases DNA methylation confirmed the diagnosis and provided prognostic information. Of 319 cases carrying 195 different descriptive histologic diagnoses, DNA methylation provided a definitive diagnosis in 273 (86%) cases, separated them into 55 methylation classes, and changed the grading in 58 (18%) cases.

Conclusions. DNA methylation analysis is a robust method to diagnose primary CNS tumors, improving diagnostic accuracy, decreasing diagnostic errors and inconclusive diagnoses, and providing prognostic subclassification. This study provides a framework for inclusion of DNA methylation profiling as a primary molecular diagnostic test into professional guidelines for CNS tumors. The benefits include increased diagnostic accuracy, improved patient management, and refinements in clinical trial design.

Key Points

- Diagnostic interobserver variability is high based on histology alone, leading to a discordant diagnosis in 12% of academic institutions and 26% of community hospitals.
- The DNA methylation has emerged as a diagnostic tool that improves diagnostic accuracy, decreases inconclusive diagnoses, and provides prognostic subclassification.
- Guidelines do not exist for incorporation of DNA methylation into clinical practice.

Importance of the Study

Central nervous system (CNS) tumors are a diverse group of tumors with the recent World Health Organization (WHO) classification recognizing more than 100 unique entities. Diagnostic interobserver variability is high based on histology alone. DNA methylation has emerged as a diagnostic tool aiding in the precision of CNS tumor diagnosis; however, guidelines do not exist for its use. We performed DNA methylation on 1921 brain tumors and analyzed the concordance of diagnoses by histology and by DNA methylation in an effort to determine guidelines for when DNA methylation is the most clinically useful. While studies have shown that DNA methylation has an impact on tumor diagnosis, these studies do not provide guidance as to when it is the most clinically useful. Given the importance of the tumor diagnosis on patient treatment, eligibility for clinical trials, and success of clinical trials, the precision, and accuracy of the diagnosis of CNS tumors are paramount.

Although central nervous system (CNS) tumors represent 1% of all cancer diagnoses in the United States, they are the 10th leading cause of death for adults and the leading cause of cancer-associated deaths in pediatric patients, men under the age of 40 years, and women under the age of 20 years.[1] CNS tumors are a highly diverse, heterogenous group of tumors with the most recent World Health Organization (WHO) Classification recognizing over 100 unique entities.[2]

Diagnosis of brain tumors is primarily based on histopathologic analysis, followed by ancillary studies. Diagnostic interobserver variability is high due to overlapping histological features of the different brain tumors.[3] One study found that 26% of cases from community

hospitals and 12% of cases from academic hospitals showed discordant diagnoses between a primary and secondary review by different pathologists.[4] Previous studies have also shown that poor accuracy of histologic diagnosis leads to clinical trial failures.[3,5]

Recently, DNA methylation profiling has emerged as a potential pan-CNS tumor diagnostic assay. DNA methylation is an epigenetic mechanism involving the transfer of a methyl group onto the C5 position of cytosine to form 5-methylcytosine. DNA methylation provides an epigenetic "fingerprint" that reflects cell lineage and development, as well as acquired methylation changes due to mutations, environment, and aging.[6–8] The whole genome DNA methylation classifier utilizes tumor type-specific signatures and Random Forest machine learning to determine tumor diagnosis independent of histology.[9] Retrospective studies have shown that implementation of DNA methylation identified potential errors in histologic diagnosis in 12%–17% of cases and changes in WHO grading in 71% of cases.[9,10] In addition, the use of DNA methylation in clinical practice has resulted in the discovery of new entities and subclasses, decreasing the uncertainty in CNS tumor diagnostics.

Studies evaluating the diagnostic utility of the DNA methylation classifier were performed at tertiary institutions with a potential referral bias towards difficult and undiagnosable cases.[10] DNA methylation has been incorporated into the most recent WHO Classification of CNS Tumors, fifth edition as recommended testing for multiple tumor entities; however, it has yet to be incorporated into clinical guidelines. The first National Comprehensive Cancer Network (NCCN) guidelines for pediatric CNS cancers (version 1.2023) currently include a comment that DNA methylation may offer objective and more precise tumor classification but do not endorse it as a first-line molecular test and only recommend it when the tissue sample is limited, despite a study advocating for its inclusion into the NCCN guidelines for routine use in the diagnosis and subclassification of medulloblastoma.[11] The NCCN guidelines for adult CNS tumors only recommend DNA methylation as an ancillary method for tumor classification for equivocal cases.

In this study, we aimed to determine the clinical utility of DNA methylation for primary diagnosis of brain tumors and propose standardized criteria for the use of DNA methylation in clinical practice.

Methods

Study Design and Pathology Review

We performed a prospective DNA methylation analysis of 1921 primary CNS tumors diagnosed at NYU Langone Health between 2014 and 2022. All tumors received the standard pathology diagnosis, molecular testing recommended by WHO at the time of diagnosis, and were simultaneously profiled by whole genome DNA methylation profiling at the time of initial pathology diagnosis (see Figure 1). DNA and RNA next-generation sequencing were performed to confirm the DNA methylation diagnosis

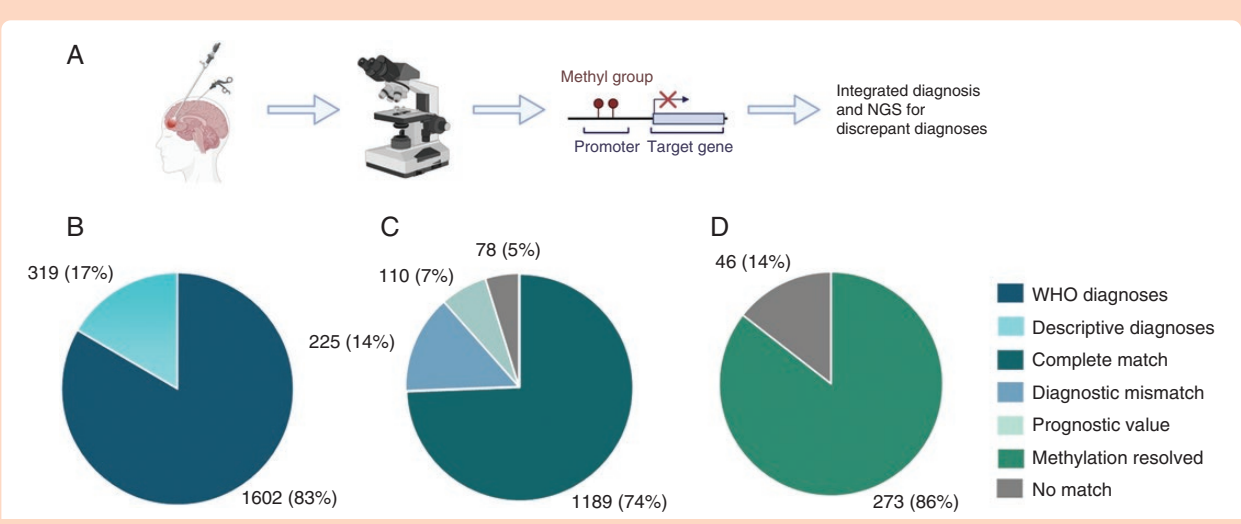

Figure 1. **(A)** This prospective study started with surgical resection of the brain tumor and tissue processing for a pathologist. All tumors received the standard of care pathology diagnosis as judged appropriate at the time of initial review, and simultaneous whole genome DNA methylation profiling. The histologic diagnosis and the DNA methylation diagnosis were compared and additional molecular studies including DNA and RNA NGS studies were performed as required to resolve discrepant cases. **(B)** Our cohort included 1921 primary central nervous system tumors, of which 1602 (83%) had World Health Organization (WHO) recognized diagnoses and 319 (17%) had descriptive diagnoses. **(C)** Of the 1602 WHO diagnoses, 1189 (74%) tumors showed concordance between histopathology and DNA methylation and were considered a complete diagnostic match, 225 (14%) tumors were a diagnostic mismatch with discrepant tumor type and/or grade, 110 (7%) tumors DNA methylation was able to add additional prognostic information, and 78 (5%) tumors did not classify by DNA methylation (referred to as "no match"). **(D)** Of the 319 tumors carrying descriptive diagnoses, DNA methylation provided a conclusive diagnosis in 273 (86%), 46 (14%) tumors did not classify and were therefore considered "no match."

and resolve discrepant cases. Histologic diagnoses were updated according to the fifth edition of the CNS WHO. Institutional review board approval was obtained (IRB# S14-00948).

DNA Methylation Profiling

DNA was extracted from archival formalin-fixed paraffin-embedded tissue using the Maxwell Promega. DNA methylation was performed at the NYU Department of Molecular Pathology CLIA-certified laboratory, using the Illumina Infinium Human Methylation 450 Bead-Chip (450 K array) or Illumina EPIC array as described previously[12] and analyzed using the Heidelberg (DKFZ)-developed and NYU-clinically validated DNA methylation classifier[9] in a CLIA certified laboratory. As we previously described in Capper et al., cases with a score > 0.9 were considered positive, cases with an indeterminant score of 0.3 to 0.9 were evaluated with additional molecular testing to confirm diagnosis, and cases with a score of < 0.3 were considered negative.[9] Cases that failed profiling due to low tissue amount, low tumor cell content, and poor DNA quality/quantity were excluded from further analysis.

DNA and RNA Next-Generation Sequencing

Mutational and copy number analyses were performed by the clinically validated NYU Oncomine Focus panel or NYU Langone Genome PACT, a 510(k) FDA-cleared (K202304) matched tumor-normal 607 gene panel. Fusion detection was performed using clinically validated NYU Fusion SEQer, as described previously.[13]

Integration of Pathology and Molecular Data

Cases were stratified based on histologic and DNA methylation concordance into the following categories: Complete match, defined as concordance between histologic diagnosis and DNA methylation class, and diagnostic mismatch defined as a change in histologic diagnosis and/or WHO grade. In addition, we reviewed whether DNA methylation further stratified tumors into prognostically relevant subtypes. Cases with descriptive diagnoses were separated for further analysis. Descriptive diagnoses were defined as any diagnosis not represented in the CNS WHO fifth edition classification of tumors.

Results

Cohort Description

We analyzed 1921 primary brain tumors (Table 1). Our cohort included 67 WHO-recognized histopathologic diagnoses and we detected 88 methylation classes (Figure 1, Supplementary Table 1, and Supplementary Table 2). The majority of our cohort were NYU cases (internal $N = 1514$, 79% and referral $N = 407$, 21%).

Table 1. Clinical Characteristics of the Cohort

	N (%)
All tumors	1921
WHO recognized histologic diagnoses	67
Descriptive diagnoses	195
Methylation classes identified	88
Male	1006 (52%)
Female	915 (48%)
Adult	1303 (68%)
Pediatric	545 (28%)
Incomplete clinical data	73 (4%)

The most frequent original histopathologic diagnoses in adults included GBM ($N = 314$, 16%), meningioma ($N = 274$, 14%), pituitary adenoma ($N = 59$, 3%), and schwannoma ($N = 51$, 3%). The most frequent pediatric diagnoses included medulloblastoma ($N = 96$, 5%), pilocytic astrocytoma ($N = 65$, 3%), ependymoma ($N = 56$, 3%), and GBM ($N = 47$, 2%), see Supplementary Figure 1.

Of all 1921 cases, 319 (17%) cases carried a descriptive diagnosis. Of the 1602 cases with a recognized WHO histologic diagnosis, 1189 were a complete match (74%), 225 were a diagnostic mismatch (14%), 78 (5%) did not match with any methylation class, and in 110 cases (7%) histopathologic diagnosis was concordant, but DNA methylation added prognostic value by additional subclassification (Figure 1). The complete list of tumors, histologic diagnoses, and DNA methylation results are listed in Supplementary Table 1.

Tumor Entities With the Highest Diagnostic Mismatch

GBM IDH wild-type CNS WHO grade 4 is the most common adult brain tumor and is invariably fatal despite aggressive therapy. Our cohort included 390 tumors diagnosed as GBM, 72 (18%) of which were histologically classified as another entity by DNA methylation (Figure 2A). DNA methylation downgraded the diagnosis to a lower-grade tumor type in 14 (19%) of these cases, potentially changing the prognosis and treatment.

Ependymomas represented 6% of our cohort (109 cases). DNA methylation reclassified 25 (23%) cases diagnosed as ependymoma while 9 tumors (8%) histologically considered ependymoma showed no matching DNA methylation class (Figure 2B).

Low-grade glial and glioneuronal tumors are the most common low-grade tumors of the CNS and of childhood and include a broad range of histologic subtypes. Our cohort included 160 (8%) low-grade glial and glioneuronal tumors with a diagnostic mismatch rate of 27%. (Figure 2C).

Oligodendroglioma is an IDH mutant infiltrating glioma, defined by the codeletion of chromosomal arms 1p and 19q. Oligodendroglioma represented 3% of our cohort ($N = 66$). DNA methylation analysis found that 15% of

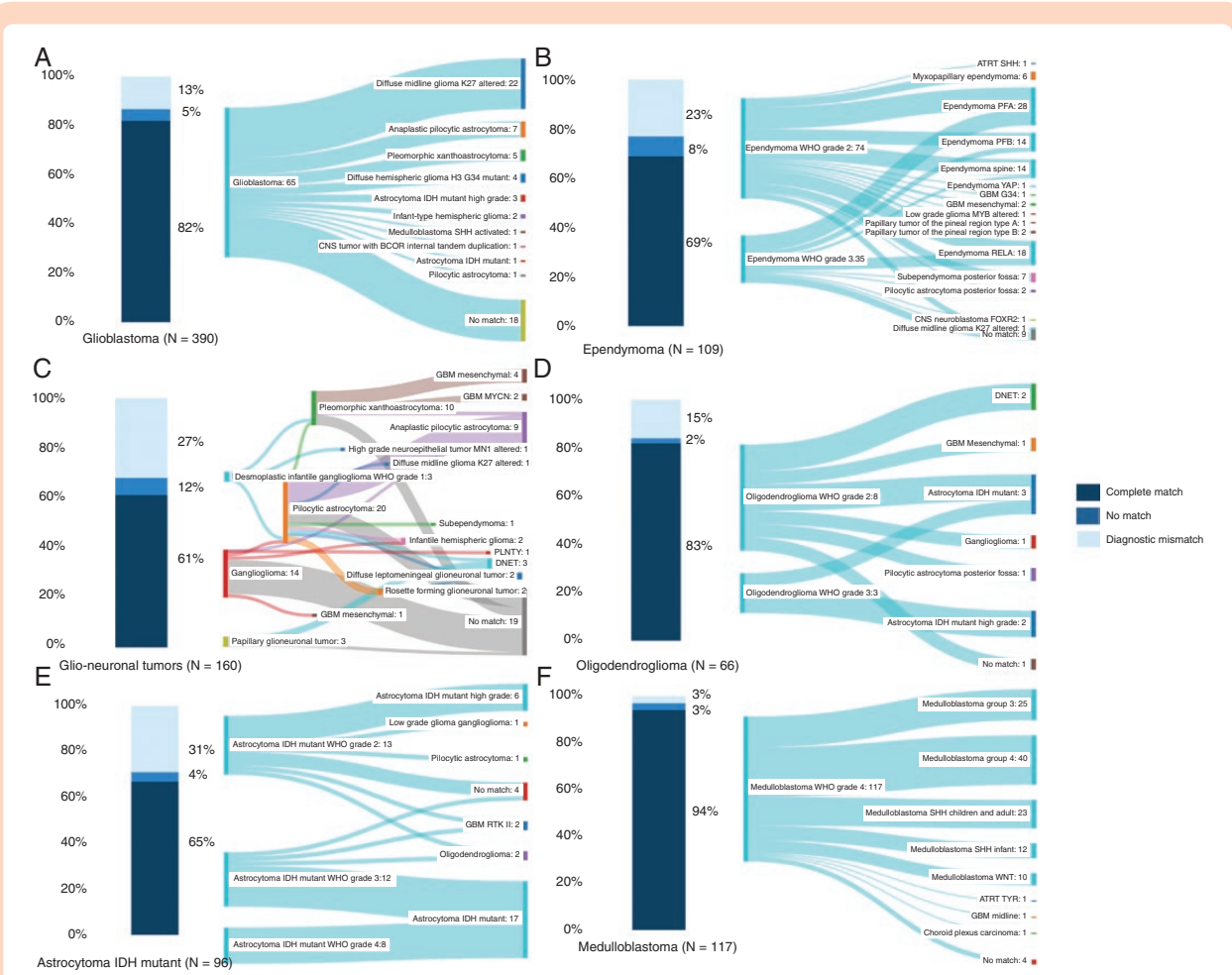

Figure 2. Diagnostic utility for accurate diagnosis and prognostic stratification. Six tumor groups with the highest yield of DNA methylation included GBM, ependymoma, glioneuronal tumors, oligodendroglioma, astrocytoma IDH mutant, and medulloblastoma. **(A)** GBM (*N* = 390) were a complete match in 82% of cases, a diagnostic mismatch in 13% of cases, and did not classify with any entity by DNA methylation in 5% of cases (no match). Most misdiagnosed GBMs were reclassified as diffuse midline glioma K27 altered (31%), anaplastic pilocytic astrocytoma (10%), and pleomorphic xanthoastrocytoma (7%). **(B)** Ependymoma (N = 109) had a complete match rate of 69%, a diagnostic mismatch rate of 23%, and a no-match rate of 8%. Ependymomas were most commonly reclassified as myxopapillary ependymoma (24%) and subependymoma (28%), **(C)** Glio-neuronal tumors (*N* = 160) had a diagnostic complete match rate of 61%, a diagnostic mismatch rate of 27%, and a no match rate of 12%. Pilocytic astrocytoma (*N* = 80) were reclassified by DNA methylation in 20 cases (28%) and DNA methylation upgraded the diagnosis in 11% of these cases. Ganglioglioma (*N* = 32) had a diagnostic mismatch rate of 31% and DNA methylation upgraded the diagnosis in 15% of cases. **(D)** Oligodendroglioma (*N* = 66) had a complete match rate of 83%, a diagnostic mismatch rate of 15%, and a no-match rate of 2%. Tumors diagnosed histologically as oligodendroglioma are most often reclassified as astrocytoma (10%), glioblastoma (2%), and DNET (3%). **(E)** Astrocytoma IDH mutant (*N* = 96) were a complete match in 65% of cases, a diagnostic mismatch in 31% of cases, and a no match in 4% of cases. Astrocytoma IDH mutant World Health Organization (WHO) grade 2 was most reclassified as a higher-grade IDH mutant astrocytoma (11%), astrocytoma IDH mutant WHO grade 3 was most commonly reclassified as a lower-grade IDH mutant glioma in 48% of cases, and astrocytoma IDH mutant WHO grade 4 most commonly reclassified as a lower grade IDH mutant astrocytoma in 28% of cases. **(F)** While medulloblastoma is rarely misdiagnosed (3% of cases) DNA methylation provides prognostic information by stratifying tumors into established molecular subgroups including Shh, Wnt, group 3, and group 4.

tumors histologically diagnosed as oligodendroglioma were reclassified as a different tumor (N = 10) (Figure 2D). On further analysis, 7 out of 10 cases were tested for the 1p/19q codeletion, 1 case was not tested, and 2 cases did not have this information available. Of the 7 tested cases, 4 were co-deleted, 2 were negative, and 1 showed loss of chromosome 1p. The discrepancy between DNA methylation and 1p/19q codeletion testing highlights the technical limitations of FISH and PCR LOH in analysis of 1p19q codeletion status, which can lead to false positive results and misdiagnosis.[14]

DNA Methylation Resolves the Majority of Descriptive Diagnoses

A definitive histopathologic diagnosis could not always be achieved. Our cohort contained a total of 319 (17%) cases

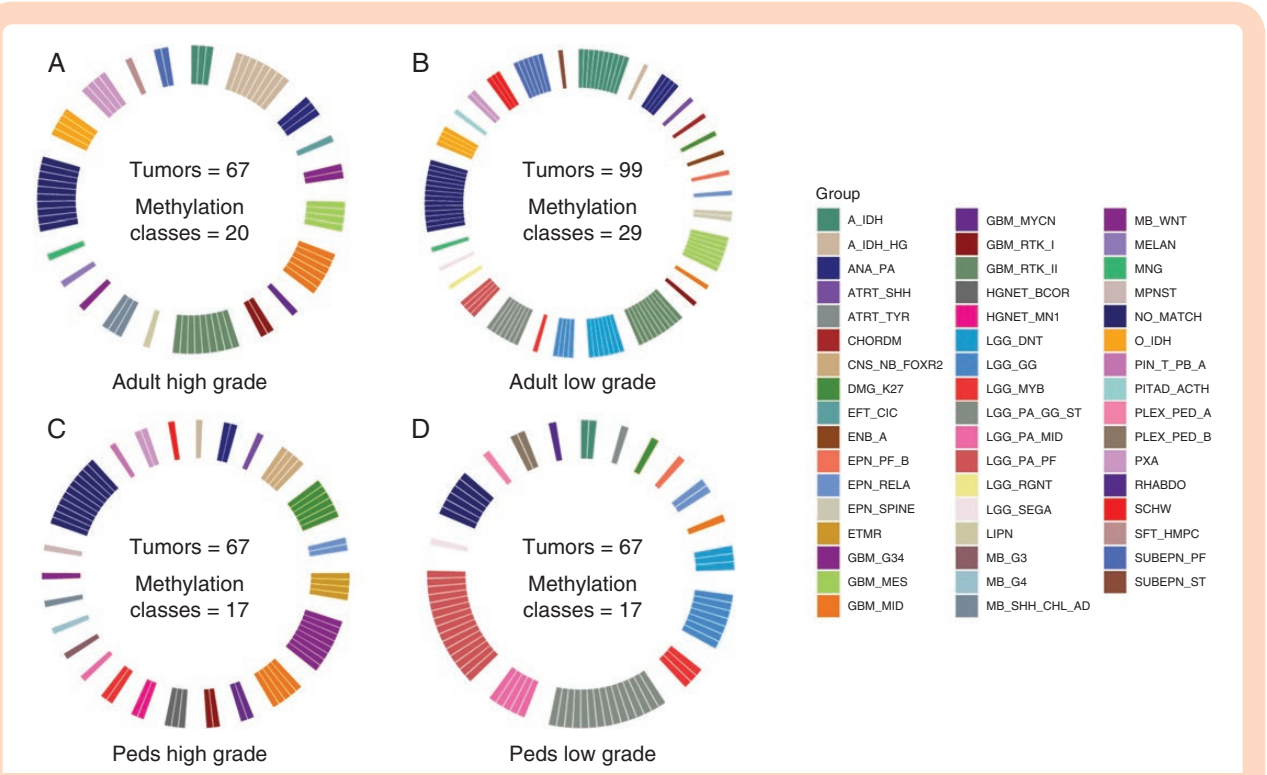

Figure 3. Of the entire 1921 cohort, 319 (17%) brain tumors were diagnosed descriptively and carried 195 different descriptive diagnoses. For the analysis, tumors were stratified into adult high-grade, adult low-grade, pediatric high-grade, and pediatric low-grade. In the adult high-grade group, there were 67 tumors and 49 unique descriptive diagnoses for which DNA methylation was able to provide a diagnosis in 94% of cases resulting in 20 different methylation classes **(A)**. In the adult low-grade group, there were 99 tumors and 62 unique descriptive diagnoses for which DNA methylation was able to provide a diagnosis in 86% of cases resulting in 29 different methylation classes **(B)**. In the pediatric high-grade group, there were 65 tumors and 24 unique descriptive diagnoses for which DNA methylation was able to provide a diagnosis in 92% of cases resulting in 17 different methylation classes **(C)**. In the pediatric low-grade group, there were 69 tumors and unique descriptive diagnoses for which DNA methylation was able to provide a diagnosis in 91% of cases resulting in 17 different methylation classes **(D)**. For the full list of descriptive diagnoses and DNA methylation classes see Supplementary Table 1. For the list of abbreviations see Supplementary Table 3.

carrying in total of 195 unique descriptive diagnoses. DNA methylation was able to resolve 273 cases (86%), which were then confirmed by targeted DNA and RNA NGS, (Figures 1 and 3). To further estimate the impact on management, 195 descriptive diagnoses were divided into 4 categories, high- and low-grade adult cases and high- and low-grade pediatric cases. DNA methylation classified 49 descriptive adult high-grade diagnoses into 20 distinct methylation classes (Figure 3) downgrading 18 (27%) tumors. Adult tumors with 62 descriptive low-grade diagnoses were reclassified into 29 methylation classes (Figure 3) and 29 (28%) tumors were upgraded. In the pediatric population, 44 descriptive high-grade diagnoses, were classified into 24 distinct methylation classes (Figure 3) and 6 (9%) tumors were downgraded. Out of 48 descriptive low-grade diagnoses in pediatric patients, DNA methylation identified 17 distinct diagnoses (Figure 3) and upgraded 5 (7%) tumors.

In total, DNA methylation analysis of 319 tumors with 195 descriptive diagnoses accurately classified 272 (86%) cases, resulting in 51 diagnostic DNA methylation classes, providing a definitive diagnosis for clinical management and change in grade for 66 (21%) cases (Figure 4).

Tumors With Minimal Impact of DNA Methylation on Diagnosis

Tumor types in which DNA methylation provided minimal impact were meningioma (97% complete match), pituitary adenoma (98% complete match), and schwannoma (100% complete match) (Supplementary Figure 1). Recent studies also suggested that methylation profiling may have prognostic utility in meningioma.[15,16]

DNA Methylation as a Prognostic Biomarker

DNA methylation provides prognostic value in some CNS tumors. Our study included 117 (6%) cases of medulloblastoma, the most common malignant brain tumor of children, 110 (94%) of which were concordant and accurately classified into 4 prognostically relevant subgroups (Figure 2F),.[11,17]

IDH mutant astrocytoma is defined as a diffusely infiltrating astrocytic neoplasm with mutations in either the *IDH1* or *IDH2* genes. Tumors are stratified into WHO grade 2, 3, or 4 and histopathologic criteria have consistently been poor predictors of prognosis [26]. Astrocytoma IDH mutant WHO grade 2 can be followed

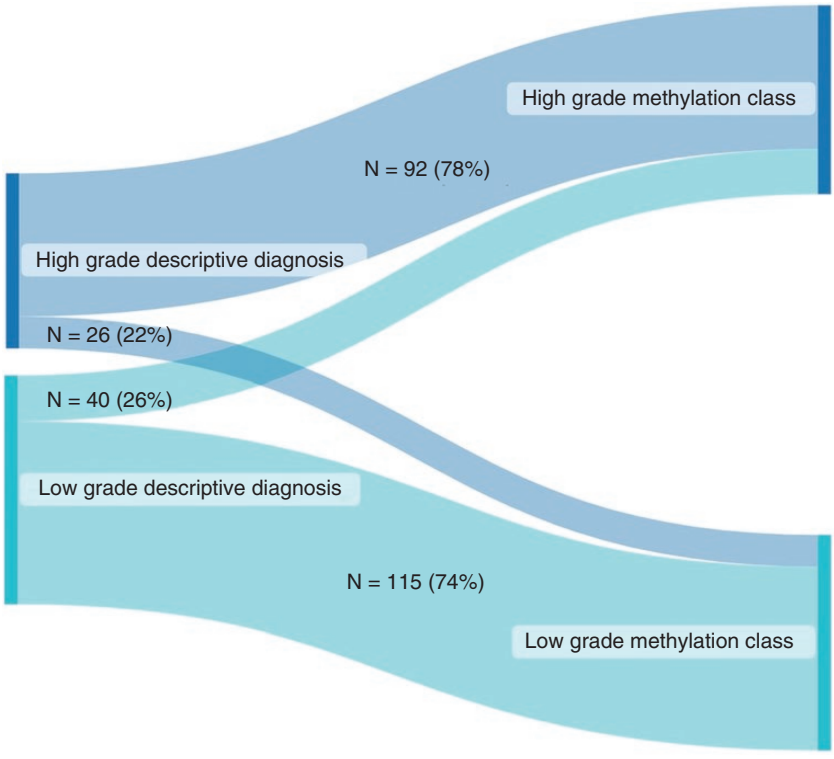

Figure 4. DNA methylation and clinical re-stratification of descriptive cases: In addition to providing accurate diagnosis, DNA methylation changed grading of the tumors. In total, 40 (26%) out of 155 descriptive low-grade tumors were upgraded to a higher-grade tumor type by DNA methylation, and 26 (22%) out of 118 were downgraded to a lower-grade tumor by DNA methylation.

radiologically if gross totally resected, in fact, previous studies have shown that temozolomide and radiation can be detrimental, inducing a hypermutant phenotype that drives aggressive behavior and progression.[18,19] IDH mutant astrocytomas accounted for 5% of our cohort (N = 96) and DNA methylation modified the diagnosis in 31% of cases (*N* = 29) (Figure 2E). DNA methylation, upgraded histologic astrocytoma IDH mutant WHO grade 2 to the DNA methylation class high-grade IDH mutated astrocytoma in 13% of cases, and downgraded astrocytoma IDH mutant WHO grade 3 and 4 to a low-grade IDH astrocytoma in 43% and 28% of cases, respectively (Figure 2E).

Studies have shown the prognostic implications of ependymoma subgroups and the utility of DNA methylation in diagnosing them.[20,21] In addition, the fifth edition of the CNS WHO considers DNA methylation to be the standard method to classify PFA and PFB ependymomas.[2] In our cohort, all 75 tumors classified as ependymoma were successfully subclassified by DNA methylation into established prognostic subgroups (Figure 2B).

Tumors With no Matching DNA Methylation Class and False Positive Results

Out of 1602 tumors with WHO diagnoses, DNA methylation was unable to match a tumor to any established DNA methylation class in 78 (5%) cases and out of 319 tumors with descriptive diagnoses, DNA methylation was unable to match 46 (14%) cases. The reasons may include low tumor cell content, which is a known preanalytical variable, novel or rare driver mutations, or novel entities not yet characterized.[22–24] In 6 cases, there was a diagnostic mismatch in which additional molecular studies confirmed that the DNA methylation result was misleading representing a false positive rate of 0.3%.

Criteria for Utilization of DNA Methylation in Clinical Practice

Based on our cohort, we propose the following criteria for the use of DNA methylation in clinical practice. We identify tumors and scenarios with high yield, intermediate yield, and low yield for DNA methylation analysis at the time of initial diagnosis. The high-yield category includes all descriptive diagnoses, tumors with a high chance of diagnostic mismatch on histology and immunohistochemistry alone, tumors with inconclusive immunohistochemical or molecular results, and tumors with established prognostic subclassification. The intermediate yield category includes tumors in which DNA methylation could guide further molecular testing, tumors with moderate chance of diagnostic error in the absence of other molecular studies, and tumors in which multiple immunohistochemical or molecular tests may be required or were insufficient for diagnosis. The low yield category includes tumors in which

DNA methylation provides little diagnostic benefit, tumors with a minimal chance of diagnostic error, and tumors in which other molecular tests have sufficiently established tumor type and DNA methylation has no established prognostic value (Table 2).

Discussion

DNA methylation analysis has emerged as a robust method to diagnose primary CNS tumors, improving diagnostic accuracy, and providing molecular subclassification for prognosis. We show that DNA methylation can avert 14% of potential diagnostic errors in tumors with a WHO diagnosis and resolve diagnostic uncertainty in 86% of tumors with a descriptive diagnosis. The improved DNA methylation-based diagnoses can have an impact on clinical management by down- or up-grading 18% and 20% of tumors, respectively.

Studies have suggested the value in clinical diagnostics of identifying new tumor entities, reevaluating clinical trials, and diagnosing histologically challenging tumors.[5,25,26] In our study we have prospectively profiled primary CNS tumors across all histopathologic subtypes, rather than limiting its application to subjectively identified diagnostically challenging cases, to determine clinical utility. This unbiased prospective approach demonstrated the utility of DNA methylation not only for challenging cases but also for seemingly straightforward cases with potential impact on clinical management.

Our data demonstrate that approximately 75% of cases are concordant between histopathology and DNA methylation; however, diagnostic accuracy is improved in approximately 24% of cases, consistent with previous studies.[9,26] Our study spanned 7 years of unprecedented development in brain tumor classification. While some of the complete mismatch cases, such as diffuse midline glioma, K27 altered, may be avoided using new in situ techniques, like the K27M mutation-specific antibody, the majority would require extensive molecular testing. DNA methylation can decrease the financial cost and tissue waste associated with numerous immunohistochemical stains, and help provide guidance as to which type of molecular test is of the highest yield, for example, DNA versus RNA NGS, or single gene FISH if tissue is limited. Furthermore, DNA methylation analysis results in newly discovered entities that can be incorporated by simply retraining the classifier, without the need to validate a new assay.

Tumors with descriptive diagnoses represent a particular challenge for clinical management. In our cohort, 17% of brain tumor cases did not have a WHO diagnosis or grade. DNA methylation was informative in 86% of descriptive cases, improving patients' ability to receive appropriate clinical treatment and potential enrollment in clinical trials. DNA methylation classifiers continue to evolve and the remaining ~20% of unclassifiable cases likely represent previously undescribed entities or underlying molecular drivers.[6,25,27]

Clinical trials are dependent on enrollment of the correct tumor types. Previous studies have shown high interobserver variability among pathologists[3,4] and clinical trials have failed due to histopathologic diagnostic inaccuracy.[5] While NGS may still be required to find a target that enables enrollment into a clinical trial, DNA methylation provides an accurate and unbiased assessment of primary CNS tumors ensuring that only intended tumor subtypes are enrolled and compared. With the NYU DNA methylation profiling criteria, we propose to stratify the utility of DNA methylation based on the initial histopathologic assessment, likelihood of diagnostic error, and clinical value (Table 2).

It is important to note, that many diagnostic discrepancies could potentially be resolved by a combination of other methods, such as DNA and RNA NGS panels, copy number analysis, or a panel of immunohistochemical stains. DNA and RNA NGS panels that would be required to cover all CNS tumor entities are significantly more expensive and tissue-consuming than DNA methylation and do not provide a diagnosis but a molecular driver that may or may not be specific for a certain tumor entity. For example, BRAF V600E mutation is a driver both

Table 2. NYU Criteria for the Use of DNA Methylation in Clinical Practice

High Yield
• CNS tumors defined by DNA methylation signatures • All CNS tumors with descriptive diagnoses • Tumor entities with a high chance of diagnostic error in the absence of other molecular studies • Tumors with inconclusive or contradictory immunohistochemical or molecular results • Tumors where subclassification may affect clinical management or provides prognostic information
Intermediate yield
• Tumors in which DNA methylation could triage further molecular testing • Tumors with moderate chance of diagnostic error in the absence of other molecular studies • Tumors in which > 10 immunohistochemical stains and/or multiple molecular tests may be required for diagnosis (tissue preservation/cost efficiency)
Low yield
• Tumors with low chance of diagnostic error when using recommended techniques according to WHO required criteria) • Tumors in which other molecular tests have sufficiently established molecular drivers and tumor classification • No established prognostic value of molecular subclassification

in pilocytic astrocytoma WHO Grade 1, as well as glioblastoma, WHO grade 4, as well as in a metastatic melanoma. While all these tumors can show similar histologic features, they have specific DNA methylation signatures. Therefore, DNA methylation is the only pan-CNS tumor assay, that incorporates detection of all other biomarkers, decreasing the cost, turnaround time, and tissue waste associated with sequential molecular testing, and diagnostic errors. Furthermore, certain subtypes of brain tumors such as high-grade astrocytoma with pilocytic features (HGAP) and diffuse glioneuronal tumors with oligodendroglioma-like features and nuclear clusters (DGONC), are exclusively defined by their DNA methylation signature.[6]

DNA methylation does not show utility in all primary CNS tumors. Our data suggest that in cases with the diagnosis of meningioma, schwannoma, and pituitary adenoma, DNA methylation is of limited diagnostic or prognostic value, although studies indicate this may change in the future.[16,28]

Lastly, while the total cost may vary between institutions and NGS panels, DNA methylation has a significantly lower cost than large DNA and RNA NGS panels. Therefore, DNA methylation profiling provides an opportunity to decrease costs associated with molecular testing. Further cost-effectiveness studies are necessary to establish the financial impact.

Our proposed criteria for incorporating DNA methylation in clinical practice recognize that different clinical practices have different access to molecular tests. While in some practices, DNA methylation might be used as a first-tier diagnostic method, others may utilize DNA methylation in cases unclassifiable by other available methods. Results of our study provide a framework for inclusion of DNA methylation profiling into professional guidelines for management of primary CNS tumors, which has the potential to increase diagnostic accuracy and improve patient management as well as the design of clinical trials.

Supplementary material

Supplementary material is available online at *Neuro-Oncology Advances* online.

Keywords

central nervous system tumors | DNA methylation | guidelines | molecular | tumor classification

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Conflict of interest statement

M.S. is scientific advisor and shareholder of C2i Genomics, Heidelberg Epignostix and Halo Dx, and a scientific advisor of Arima Genomics, and received research funding from Lilly USA. Other authors declare no conflict of interest.

Authorship statement

Study concept and design: KG, MS; Acquisition of cases and data collection: KG, MS, SP, MM, AF, MS, LGR, EM, SAD, BLL, DP, OK, MR, SS, DB, CD, WM, MS, PN, AH, AS, JJ, LP, MDJ, BM, WS, DM, PPG, PDC, CH, JMF, MDF, MG, NRW, TER, MU, NMT, JCD, CS, DGP, DP, JHW, ETH, DH, CMW, CC, SCK, MB, DAO, MAK, EPS, SLG, DZ, JCA, JGG; Experiments: IT, GS, NA, MW; Analysis of data: VV, JS, AT, KG, MS; Manuscript Review: KG, MS, VV, JS, GS, IT, NA, MW, SP, MM, AF, MS, LGR, EM, SAD, BLL, DP, OK, MR, SS, DB, CD, WM, MS, PN, AH, AS, JJ, LP, MDJ, BM, WS, DM, PPG, PDC, CH, JMF, MDF, MG, NRW, TER, MU, NMT, JCD, CS, DGP, DP, JHW, ETH, DH, CMW, CC, SCK, MB, DAO, MAK, EPS, SLG, DZ, AT, JCA, JGG; Wrote Manuscript: KG, MS; All authors read and approved the final paper.

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