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The Cognitive Framework Behind Modern Neuropathology

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• ***Context.***—**In 2021 the World Health Organization distributed a new classification of central nervous system tumors that incorporated modern testing modalities in the diagnosis. Although universally accepted as a scientifically superior system, this schema has created controversy because its deployment globally is challenging in the best of circumstances and impossible in resource-poor health care ecosystems. Compounding this problem is the significant challenge that neuropathologists with expertise in central nervous system tumors are rare.**

Objective.—**To demonstrate diagnostic use of simple unsupervised machine learning techniques using publicly available data sets. I also discuss some potential solutions to the deployment of neuropathology classification in health care ecosystems burdened by this classification schema.**

Data Sources.—**The Cancer Genome Atlas RNA sequencing data from low-grade and high-grade gliomas.**

Conclusions.—**Methylation-based classification will be unable to solve all diagnostic problems in neuropathology. Information theory quantifications generate focused workflows in pathology, resulting in prevention of ordering unnecessary tests and identifying biomarkers that facilitate diagnosis.**

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The neuropathology community has overtly failed to deliver a "deployable by general surgical pathologist" workflow for the diagnosis of primary central nervous system (CNS) tumors. This outcome is obviously unintentional, but nevertheless represents a significant failure that results in substandard neuro-oncologic treatment planning and intraoperative neurosurgical management for our patients. First, one must understand that at the foundation of this failure lies the cold reality that brain tumor classification suffers a high cognitive burden. Compounding this challenge is the fact that in the United States, only an estimated approximately 450 neuropathologists are boarded by the American Board of Pathology.[1] Despite the fact that there is a massive shortage of trained neuropathologists throughout the country, the neuropathology community has maintained the requirement for a 2-year fellowship. In my own training program, the second year of the research was a full-blown postdoctoral fellowship tailored for a career in academic neuropathology. Although this option may have made sense for me, continuing to focus our training on efforts to generate academic neuropathologists results in about one-third of neuropathology fellows focusing on a research-intensive career, be it in academics, government, or biotechnology. One may thus consider continued insistence on this path to be incongruent with society's needs and a contribution to the perpetuation of multiple inequities across our various health care ecosystems.

The presence of a significant philosophical tension within the neuropathology community further complicates the upskilling of general surgical pathologists to meet this challenge. In traditional perspectives, a neuropathologic diagnosis indicated a unique clinical-pathologic syndrome, where diagnoses/disease categories were segregated by epidemiology and patient outcomes. For instance, a myxopapillary ependymoma is the neoplasm associated with a clinical-pathologic entity with unique distribution of neuroanatomical location, morphology, and clinical outcome. However, the notion of what a diagnosis encompasses has progressed over time. In a highly progressive view, a diagnosis represents a subtype of a neoplastic process, and such subtypes can be categorized by objective and quantitative analysis of genomic, epigenomic, transcriptomic, epitranscriptomic, lipidomic, and/or metabolomic pathways, with diagnoses often aided by machine learning tools such as dimensionality reduction and clustering analyses. It is critical for the general surgical pathologist to understand that these 2 philosophical viewpoints represent extremes of a spectrum, and that all neuropathologists incorporate both philosophies when they integrate diagnoses. Most neuropathologists will find themselves naturally on one side of the spectrum, and autocorrection to an appropriately nuanced center is required in surgical neuropathology practice.

The lack of successful advocacy for neuropathology compensation also represents a significant challenge. Despite the key role that intraoperative consultation plays in tissue triaging, current reimbursement rates for neuropathologic intraoperative consultation are only $63.17 per consult (Current Procedural Terminology code = 88331), and charges for the cytologic

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preparation are not allowed if a frozen section is performed on the same part. These issues render it financially unfeasible to staff neuropathologists on call in community cancer center clinical settings, ultimately leading to nonneuropathologists fielding most neurosurgical intraoperative consultations throughout the United States. Neuropathology staffing problems are even worse worldwide. As stereotactic targeting significantly improves with intraoperative consultation, patients without neuropathologists reading intraoperative consults receive inferior care. Furthermore, treatment planning by neuro-oncologists and radiation oncologists requires time-sensitive integrated histopathologic/molecular pathologic reports. As primary brain cancers are rare, glioma-specific biomarkers are not commonly stocked in community and academic pathology laboratories. In summary, we routinely lose the opportunity to commence brain biopsy/resection tissue triaging at the point of intraoperative consultation, at least in part because the public policy framework surrounding neuropathology does not facilitate neuropathology staffing.

HOW DID WE GET TO WHERE WE ARE NOW?

Throughout the world, the clinical practice guidelines that set the neuropathology diagnostic standards arise from the World Health Organization (WHO), which in 2021 published a revised classification of CNS tumors. This publication further emphasized the need for molecular pathology integration with diagnosis. It has been demonstrated through numerous studies that integration of molecular data with histology improves diagnosis and prognostication for patients[2–7] and represents a key component within the neuro-oncologist's toolbox. Unfortunately, in resource-starved ecosystems, which represent approximately 60% of the world's cancer burden,[8] molecular pathology workflows are nonexistent. Even within the United States, neuropathology staffing of non–comprehensive cancer center tertiary care facilities rarely exists. As National Cancer Institute–designated comprehensive cancer centers cover only about 20% of US cancer patients, one can estimate that the majority of primary brain cancer patients in the United States do not obtain a first look at their pathology by trained neuropathologists. This results in nonneuropathologists fielding complex intraoperative consultation questions and general surgical pathologists sending cases to external experts, leading to increased turnaround times (TATs) that ultimately delay treatment planning. Note that although these excessive TATs may remain in line with chemoradiation therapy target of 4 weeks,[9] scheduling logistics of oncologic and radiation therapies are highly complex and represent a key logistical challenge (see discussion in Petrovic et al[10]). In addition to the TAT challenges, efficiency is hindered by the well-known problem that diagnostic tests change their predictive values when disease incidence decreases in the sampling population. This phenomenon most commonly occurs when increasing the number of subjects being tested without regard to pretest probability thresholds. For instance, 1p19q fluorescence in situ hybridization, a standard test for oligodendroglioma workup, suffers a 5% false-positivity rate, and ordering this test on all gliomas without consideration of pretest probabilities is ill-advised. Of note, in some clinical paradigms, next-generation sequencing (NGS) has shown to be cost-effective as a diagnostic modality only at specific pretest probability thresholds.[11] The net result of these challenges is delayed treatment plan generation by neuro-oncologists and radiation oncologists.

To understand how we arrived at this, let us consider a thought experiment where we return to the ignorant state of neuropathology circa the early 2000s. In this thought experiment, we will consider the toolbox that neuropathology possessed at that time and demonstrate how implementation of applied mathematics and machine learning resulted in the promise that objective quantification of genomic or transcriptomic findings may be capable of providing solutions to the shortage of trained neuropathologists.

WHAT DID WE KNOW BEFORE MOLECULAR CLASSIFICATION?

In the early 2000s, our knowledge of clinical outcomes for infiltrating glioma patients was based on some morphologic biomarkers, chromosomal biomarkers, and epidemiologic outcomes data. For instance, the morphologic markers of high cellularity, nuclear pleomorphism, and infiltration patterns across Scherer secondary structures were reported in the neuropathology literature more than 90 years ago and undergo extraction with a hematoxylin-eosin (H&E) stain.[12] Currently the sine qua non for oligodendroglioma, 1p19q codeletion was reported more than 20 years ago.[13] Using The Cancer Genome Atlas (TCGA),[14] one can download data on patient demographics such as sex, age, and clinical outcome. As illustrated in Figure 1, A through C, by the early 2000s neuropathology knew (1) that morphologic biomarkers such as necrosis and microvascular proliferation and proliferative biomarkers such as Ki-67, proliferating cell nuclear antigen (PCNA), or PHH3 were elevated in tumors of patients with poor prognosis, and (2) that younger age was highly associated with low-grade morphologic biomarkers, lower expression of proliferative genes, and improved survival. However, as shown in Figure 1, A and B, a significant overlap exists between these conditions. In 2010, the TCGA group and others discovered through gene promoter methylation profiling that a subset of patients in this group showed a concerted hypermethylation in several genomic loci, a patient cluster initially referred to as glioma–CpG island methylator phenotype, and that these patients possessed mutations in *IDH1*, or less commonly in *IDH2*.[15]

Discovering IDH mutation in astrocytoma and oligodendroglioma represented a near-quantum shift in diagnostic neuropathology, which culminated in the release in 2016 of updated diagnostic guidelines for the classification of CNS tumors. In this edition, the focus of the diagnostic nosology was to emphasize objectivity through departing from subjective morphologic biomarkers. To illustrate how machine learning approaches could be implemented in this modern neuropathology construct, consider a potential workflow that could identify through objective quantification a series of cancer biomarkers that could segregate infiltrating gliomas. To illustrate this, I downloaded the RNA sequencing data from TCGA, which constituted 703 patients, with each bulk RNA sequencing data set containing more than 22 000 genes. These 703 patients were distributed among 3 diagnoses: (1) astrocytoma, IDH mutated, WHO grade 2; (2) oligodendroglioma, IDH mutated, WHO grade 2; and (3) glioblastoma, IDH wild type, WHO grade 4. This data set's dimensionality was reduced through principal components (PCs) analysis from about 22 000 dimensions (or genes) to 412 dimensions (or PCs), with these 412 PCs capturing 91% of the data. Figure 2, A and B, shows a plot where these 412 PCs are

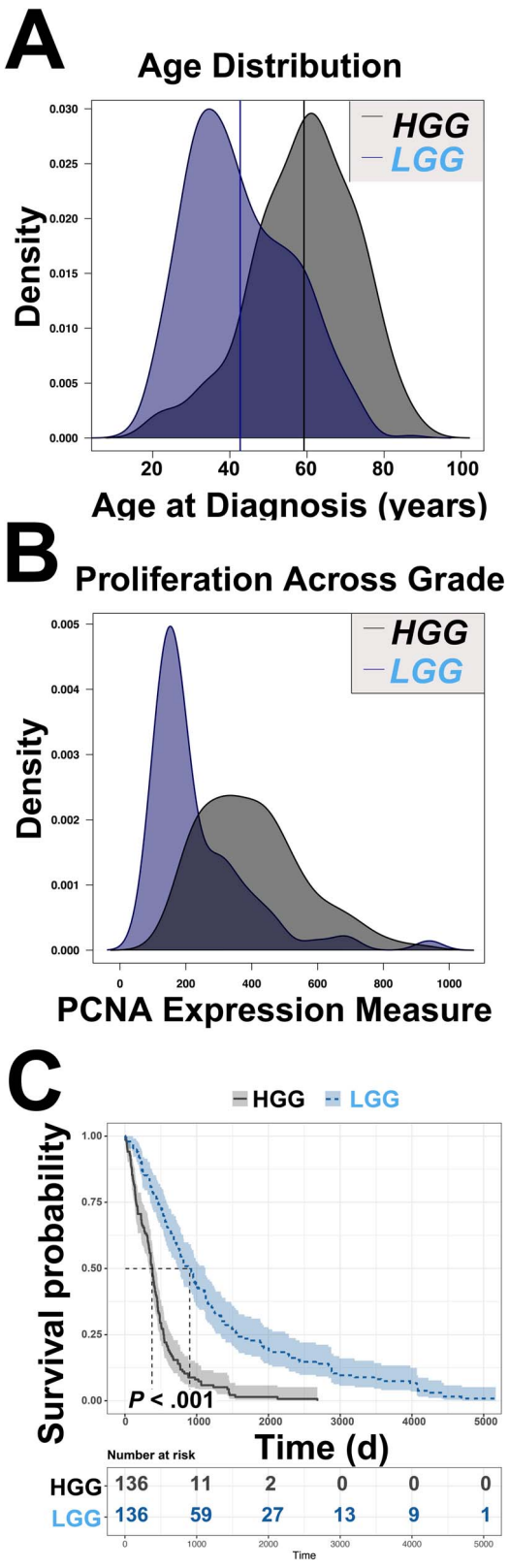

Figure 1. *Extent of knowledge regarding patient outcomes with biomarkers commonly available in the year 2000. Data from The Cancer Genome Atlas are downloaded and plotted as density curves for distribution between low grade (blue) and high grade (black and gray) for age (A), distribution of proliferating cell nuclear antigen (PCNA) expression (B), and a Kaplan-Meier survival curve of low- versus high-grade infiltrating glioma (C). Abbreviations: LGG, low-grade glioma; HGG, high-grade glioma.*

projected onto 2 dimensions using the uniform manifold approximation and projection (UMAP) algorithm, with each closed dot representing a biopsy from one patient. Note that this scatterplot easily shows 3 broad groups that roughly correspond to 3 patient clusters (Figure 2, A). Each point is then pseudocolored based on diagnosis, which nearly perfectly separate into clusters of oligodendroglioma; astrocytoma, IDH mutated; and glioblastoma, IDH wild type (Figure 2, B). What has been achieved by this approach is a major shift in how we can think of diagnostics. Specifically, an algorithm, which has never dealt with data from these patients, has clustered patients into clinically significant diagnostic categories through only the following code in Rstudio using libraries dplyr and UMAP.

```
#data importation from TCGA and data wrangling
hgg <- read.csv("~TCGA_GBM_TPM_counts.csv")
lgg <- read.csv("~TCGA_LGG_TPM_counts.csv")
df <- left_join(hgg, lgg)
df2 <- t(df) %>% as.data.frame
#identifying which genes low variance to exclude from downstream analysis
df3 <- apply(df2, 2, var)
#principal components analysis
my_pca <- prcomp(df2[, which(df3>0)], scale = TRUE)
#UMAP analysis
my_umap <- umap(df_pca[, 1:412])
#Plotting the umap analysis
plot(my_umap$layout)
```

The key take-home message of this exercise is that machine learning, and potentially artificial intelligence, are tools that now are available in the neuropathologist's toolbox. The obvious next question would be, what is our best candidate to perform such an objective, data-driven approach to diagnostic neuropathology?

WHOLE-METHYLOME SEQUENCING AS A CANDIDATE FOR MACHINE LEARNING–BASED NEUROPATHOLOGY SIGN-OUT

The technical scope of methylation profiling is beyond this review, and I refer the reader to excellent reviews on this topic by others.[16,17] Regardless of whether or not the methylome is acquired via NGS or methylation-specific microarrays, whole-methylome–based sequencing has shown significant promise in CNS tumor classification.[18] Specifically, the potential to cluster patients into disease categories using unsupervised hierarchical clustering represents a strong potential method of obtaining diagnoses directly from biochemical inputs (ie, genomic DNA extraction). Here, I present 3 examples of methylation profiling from my practice at Ohio State University (Columbus) that were performed at the National Institutes of Health (NIH) that demonstrate the promise and pitfalls of this methodology.

The first example comes from a patient with a recurrent cerebellar lesion that was diagnosed in the electronic health record as recurrent pilocytic astrocytoma (Figure 3, A through D). The tissue sections demonstrated a glial proliferation admixed with hemorrhage. Mitoses were noted, as were microvascular hyperplasia. Diffuse expression for OLIG2 was noted in the tumor cells, and p53 and IDH1R132H were negative. ATRX was negative by immunohistochemistry, indicating a mutated ATRX locus. The Ki-67 labeling index was estimated at 2% in the tumor cells. IDH1/2 and *BRAF* V600E testing was negative. NGS did not show any mutations

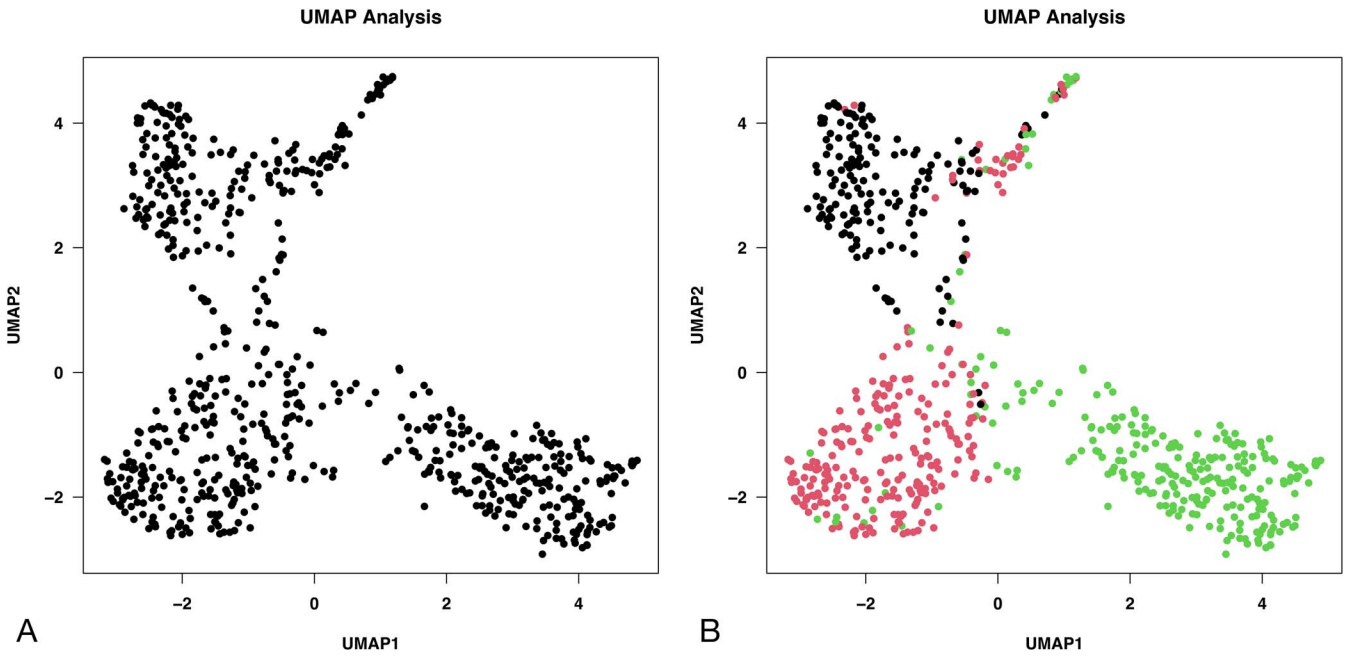

Figure 2. *Unsupervised machine learning approaches capable of identifying glioma subtypes from bulk ribonucleic acid sequencing (RNAseq). A, Uniform manifold approximation and projection (UMAP) of only bulk RNAseq data from The Cancer Genome Atlas without pseudocoloring shows 3 clusters. B, Pseudocoloring based on diagnosis shows clear association to visual clusters for the diagnoses tested. Black is oligodendroglioma isocitrate dehydrogenase (IDH) mutant 1p19q codeleted; red, astrocytoma IDH mutant 1p19q noncodeleted; and green, glioblastoma, IDH wild type.*

indicative of a high-grade glioma. The patient had received a prior diagnosis of pilocytic astrocytoma 3 years previously from another pathologist. We reviewed that original specimen, and it showed several classic features of pilocytic astrocytoma, such as the lack of infiltration and the presence of Rosenthal fibers. The presence of proliferation and microvascular hyperplasia was concerning, and we decided to obtain methylation profiling. Methylation profiling demonstrated a high-grade astrocytoma with piloid features, a diagnosis that provided crucial information resulting in a change in radiation planning. Time from initial specimen submission to NIH to result was 24 business days.

In example 2, we performed methylation sequencing on a resection specimen with a right frontal lobe lesion (Figure 4, A through C). The H&E-stained sections demonstrated abundant eosinophilic granular bodies, Rosenthal fibers, and occasional ganglion cells, some of which were binucleated. Multiple synaptophysin-positive ganglion cells, some of which were binucleated, were noted in the tissue sections. CD34 immunoreactive cells showing a complex dendritic arborization, a finding commonly associated with ganglioglioma, were identified. The Ki-67 labeling index was estimated at less than 1% in all tissue sections examined. Evaluation demonstrated a *BRAF* V600E mutation. IDH1/2 sequencing was performed and was negative. We rendered a diagnosis of ganglioglioma, WHO grade 1.

Although we did not have a diagnostic conundrum in this case, we opted to submit for methylation profiling to the NIH as we considered this to be such a classical case that it might be of interest to the NIH program. When classical cases are submitted to the NIH, the NIH methylation classifier improves and benefits all users. Methylation profile revealed this specimen to pertain to the pilocytic astrocytoma class. We were surprised that the NIH methylation profiler could not distinguish pilocytic astrocytoma from gangliogliomа, and this case underscores some of the limitations of whole-methylome profiling. The TAT from ordering the methylation study to rendering the report was 26 business days.

My last example is of a right ventricular mass with a challenging lesion (Figure 5, A through I). The H&E-stained tissue sections demonstrated a fragment of tissue composed principally of clusters of cells admixed with highly fibrillar areas of low cellularity. This morphology was most consistent with that of subependymoma. At the edge of the specimen, however, cytologically atypical cells with elongate morphology were noted, which were admixed with Rosenthal fibers. One mitosis was identified. These cytologically atypical cells showed permeation throughout the tissue section. No necrosis and no microvascular hyperplasia were noted. The cells within the well-defined clusters showed occasional dotlike EMA immunoreactivity, IDH1 R132H was negative, and ATRX was positive by immunohistochemistry (indicating an intact ATRX locus). Throughout the tissue, elongate OLIG2 immunoreactive cells were noted, and the Ki-67 labeling index was estimated at 2% by manual assessment, with the majority of the Ki-67 immunoreactive cells present in cytologically abnormal glia; p53 showed intense immunoreactivity in an estimated 2% of the tumor cell nuclei, and GFAP showed diffuse expression. Neurofilament demonstrated background axons, and NEUN was negative. Additional testing for H3K27M and H3K27ME3 was negative for H3K27 mutation, with intact H3K27ME3 staining. Molecular analyses demonstrated that the specimen showed no amplification of EGFR, no mutation in IDH1/2, and no mutation in *BRAF* V600E. Overall, the majority of the tissue section showed a morphology consistent with subependymoma. The presence of bipolar elongate glial cells at the periphery admixed with Rosenthal fibers also raised a pilocytic astrocytoma within the differential diagnosis. We felt that this did not fit any one

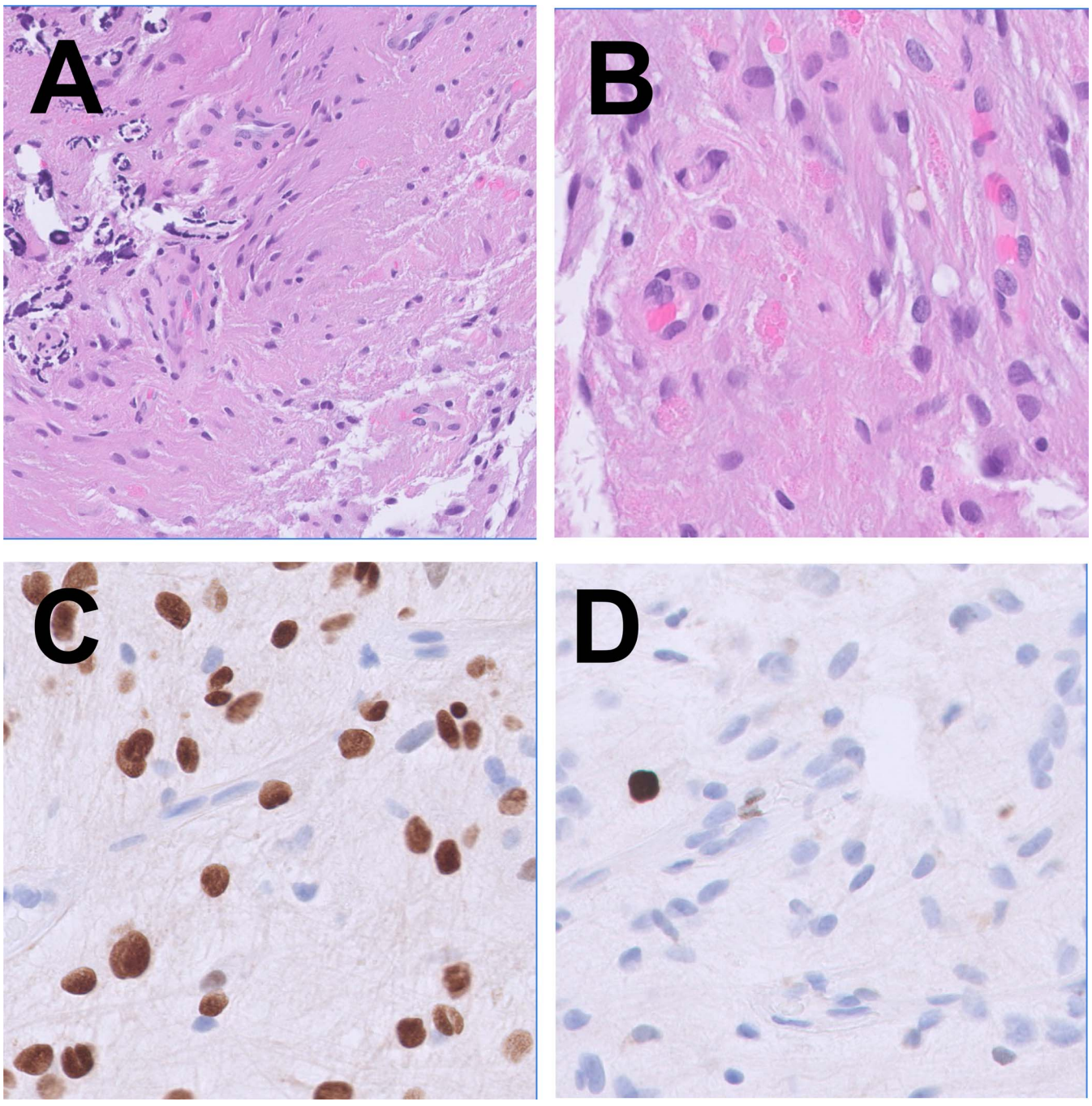

Figure 3. *Pertinent histologic images of example 1. A and B, Hematoxylin-eosin–stained images; A is captured from a whole slide image and selected by Philips IMS region of interest. C, Olig2 immunohistochemistry. D, Ki-67 (original magnifications ×20 [A] and ×40 [B through D]).*

tumor entity, and so we rendered the following diagnosis: primary CNS glial neoplasm.

Methylation profiling at the NIH indicated no match. In this example, the unusual histologic architecture and biomarker workup demonstrated a biphasic tumor, and based on our interpretation, this neoplastic process was not classifiable in the current grading system. For this reason, we used principally a descriptive diagnosis. This tumor may represent a unique tumor class, as the NIH methylation classifier was unable to identify a cluster to which it could designate its methylome. TAT from ordering to receiving the methylation was 34 business days.

In these 3 examples we can conclude that (1) methylation profiling has the potential to identify tumors with poor outcomes when traditional histology and genomic sequencing cannot, as seen in the example of the high-grade astrocytoma with piloid features; (2) methylation profiling is incapable of differentiating all tumor types, as shown in the example of ganglioglioma; (3) the methylation classifier is only as good as its training data, and (4) the NIH system that is performing the bulk of the methylation classifications suffers from long TAT and is not reliable for rapid clinical decision-making. In the defense of the NIH program, it is

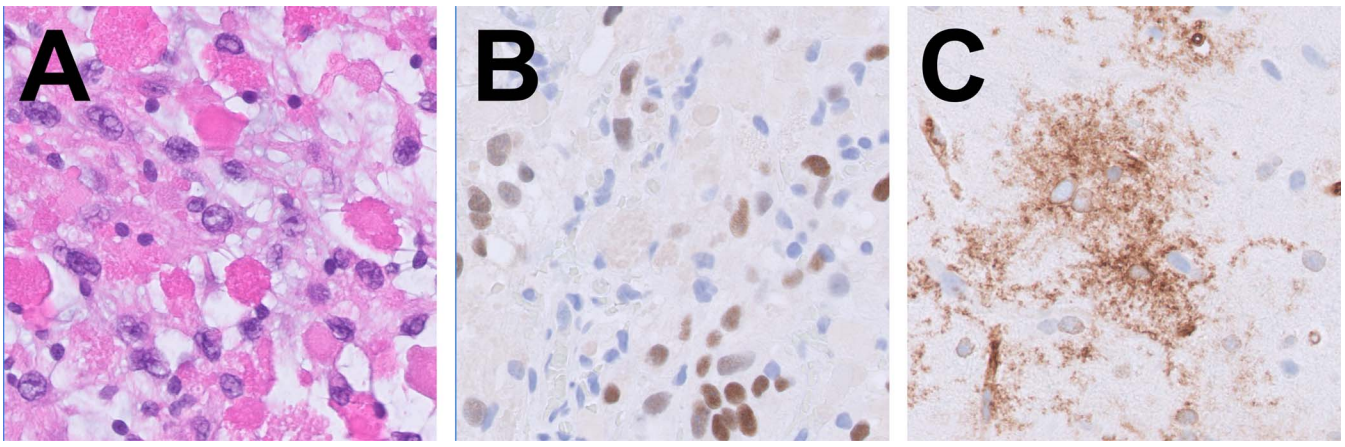

Figure 4. *Pertinent histologic images from example 2. A, Hematoxylin-eosin–stained image with eosinophilic granular bodies. B, Olig2 immunohistochemistry. C, CD34 immunohistochemistry. All images captured by Philips IMS region of interest (original magnification ×40 [A through C]).*

performing these services free of charge and is focused on developing the methylation classifier rather than serving as the preferred site for methylation, and when its performance is evaluated in the context of this scope it has performed in a stellar fashion, making a real difference to the patients in our practice. Nevertheless, distribution of this classifier throughout the United States for computational modeling should represent a tangible objective of this program, potentially through advances in distributed web3-based technologies. This would enable cancer centers to perform the methylation testing locally and obtain the class model predictions of the nation's neuropathology computational model.

HOW CAN WE HANDLE THESE CHALLENGES TODAY IN RESOURCE-POOR HEALTH CARE ECOSYSTEMS?

The state of Ohio represents in many respects a microcosm of the United States, with a mixed economy generating $695.4 billion, contributing about 3.2% of the GDP of the United States. Despite this economic output, Ohio has significant health care disparities. Note that 5-year survival is worst in the southeastern portion of Ohio, which correlates with predominantly rural and poor counties. Ohio State University Medical Center/James Comprehensive Cancer Center has significant penetrance in central Ohio and the rural portions of our state. The logistics of transporting these rural and poor patients from rural areas to Ohio State University for a clinical service that spans the boundaries of neurosurgery, neuro-oncology, radiation oncology, radiology, and pathology make efficient turnaround of diagnoses even more critical. This scenario of a tertiary care medical center serving an expanded catchment area of patients who are poor and rural and for whom logistics is challenging represents a common problem facing the brain cancer population in the United States and globally. However, cutting-edge molecular pathology workflows, including NGS and whole methylome, typically require extensive batching to reduce the per-patient cost, not to mention that the majority of patients worldwide suffering from CNS tumors do not have access to these workflows. Furthermore, treatment plans often require actionable decisions earlier. As a community, we therefore must accept that the current diagnostic framework with which we approach CNS tumors must adapt to this reality.

Our research group recently published a new framework from which to consider moving forward based on information theory.[19] Information theory rests on the notion that information can be objectively quantified into units called bits (or nats, where 1 nat = $\log_2(e)$*bit). In this cognitive framework, information is defined as the reduction of uncertainty. We specifically apply information theory in the neuro-oncology context as follows: if one patient-derived variable provides information about a patient diagnosis/prognosis, then knowing the state of that one variable, on average, would allow one to better predict the patient's diagnosis/prognosis. This permits the objective quantification of so-called mutual (or conditional) information by calculating the extent to which one variable reduces the uncertainty of the second variable. As a conceptual example to illustrate our application of information theory, consider a patient whose differential diagnosis includes an infiltrating low-grade glioma. The numerical unit implemented in information theory is entropy (H). Entropy measures the uncertainty contained within a variable (our differential diagnosis) and is provided by the following equation:

$$H(X) = \sum_{x \in X} p(x) \log_2 \left(\frac{1}{p(x)} \right)$$

where $x \in X$ refers to all possible states that x can take. In our example, X represents the patient's differential diagnosis and x is the patient's actual diagnosis, with $p(x)$ being the individual probability of each element of X. Therefore, the entropy (H) would describe the level of uncertainty regarding the patient's actual diagnosis. From this equation, one can also derive the conditional entropy, that is, the average uncertainty in a variable given the state of another variable.

$$H(X|Y) = \sum_{x \in X, y \in Y} p(x,y) \log_2 \left(\frac{1}{p(x|y)} \right)$$

Y may be the expression of a patient biomarker or clinical feature, with X referring to a patient's differential diagnosis or genomic alteration. Conceptually, the total entropy of X must be equal to the entropy that remains in X after Y is learned plus the information provided by Y about X, which may be determined as follows:

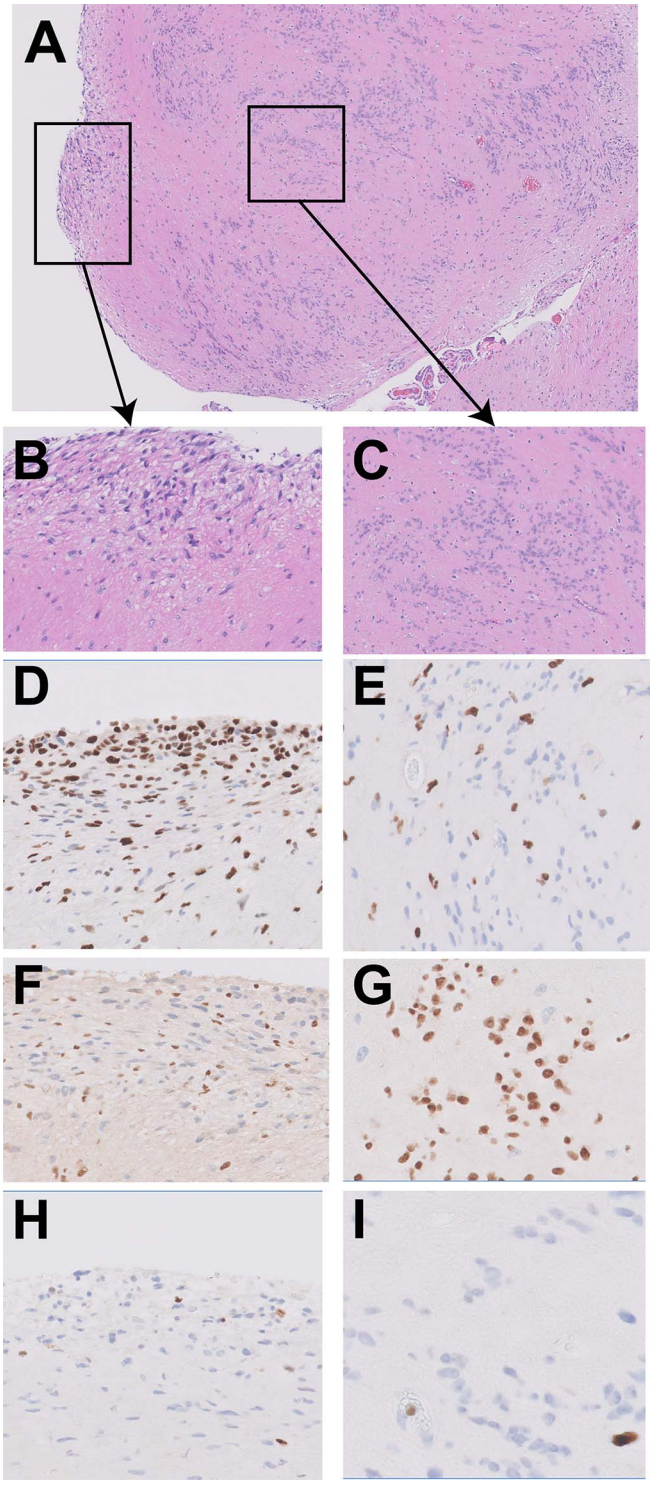

Figure 5. *Pertinent images of example 3. Hematoxylin-eosin (H&E)–stained sections are shown (A) with 2 regions highlighted on left and right smaller panels. Left panels (B, D, F, and H) represent high magnification of the rectangular box in A, and right panels (C, E, G, and I) represent high magnification of the square in A. B and C, Distinct morphology of the neoplasm on the H&E-stained section. D and E, Olig2 immunohistochemistry. F and G, ATRX immunohistochemistry. H and I, Ki-67. All images captured by Philips IMS region of interest (original magnifications ×10 [A] and ×40 [B through I]).*

$$I(X;Y) = H(X) - H(X|Y) = \sum_{x\in X, y\in Y} p(x,y)\log_2\left(\frac{p(x,y)}{p(x)p(y)}\right)$$

where $I(X;Y)$ is the information provided by Y (a biomarker) about X (the patient's differential diagnosis). The output of information theory is a continuous number that quantifies this relationship between the data. For instance, how much information on the diagnosis (actual diagnosis = x, with the possible diagnoses present in the differential diagnosis X) is gained if you know the value of the biomarker (biomarker = Y).

Continuing with the above conceptual example, given a patient with the aforementioned differential diagnosis, the mutual information formula above would calculate, on average, the information gain that a biomarker such as "*TP53* mutation–positive" status, a Boolean result, would inform on the actual patient diagnosis. In this case, diffuse expression of p53 by immunohistochemistry may reduce the probability that the tumor represents an oligodendroglioma, and therefore p53 contains a significant value of information. Using information theory, we would also be able to quantify the information gain of *TP53* status to other biomarkers such as proportion of tissue necrosis identified on H&E-stained sections, or Ki-67 proliferation indices, both of which are continuous numeric data types. Information theory is also capable of producing meaningful measurements when the data are continuous, such as patient age or tumor Ki-67 proliferation index; discrete, such as neuroanatomical locations; or Boolean, such as the presence or absence of contrast enhancement in the magnetic resonance image. Information theory is by nature multivariate and thus optimally suited as a measure of clinical decision-making. All data are also generated in units of bits or nats, and therefore straightforward comparisons between diagnostic biomarkers are possible, as is specific quantification of health care cost investment as it relates to diagnostic information. This capacity for across-the-board comparison between data is a central feature as to how we could compare distinct workflows to reduce costs.

I posit that approaches based on information theory calculations can be used to plan succinct workflows, can maximize biomarker information, and can accelerate TATs in neuropathology. In this context, focusing the workflow on rapid, single-assay biomarker analyses (be it immunohistochemistry, in situ hybridization, computer vision–based in silico biomarkers, or single-assay molecular tests) is sufficient to generate the majority of neuropathology diagnoses and usually is sufficient to commence treatment plan generation. In my own practice, I have used this combined approach of performing high-information biomarkers and single-assay molecular tests prior to ordering multiplexed sequencing assays.

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