A sizable shift in CNS tumor classification

Karen Titus

November 2021—Much has changed since the last WHO classification of central nervous system tumors was published five years ago.

Case in point: When the group of authors met in Utrecht, the Netherlands, in late 2019, everyone anticipated two more WHO meetings in Europe to work further on the 2021 classification.

Arie Perry, MD, a coauthor on both classifications, says the group photo was cheery. “Everybody was smiling.” The later trips to Europe were canceled because of the pandemic and the group met instead by Zoom. “Now everybody looked grumpy,” he says of a screen shot.

Fortunately, “We got everything done, even if it wasn’t quite as pleasant,” says Dr. Perry, professor of pathology and neurological surgery, Department of Pathology, Division of Neuropathology, University of California, San Francisco. The result is the latest WHO classification, which offers dramatic changes of its own.

“I’m really excited about the new WHO,” he says. “At first it takes a little getting used to”—like, say, a face mask—“but I think it’s another major advance, just like we had last time.”

“Last time” would be the 2016 classification, which itself was a decisive swerve, incorporating molecular methods into definitions in a substantive way. The impact on practices was substantive, too, says Dr. Perry. “It was major,” he says.

That set a high bar for change—one that the 2021 WHO classification managed to leap, a feat that surprised even Dr. Perry. “I didn’t think there would ever be another one that would shift things as much as the 2016 edition. But actually, I think this one, if anything, has shifted it even more.”

The authors have had to be nimble. Dr. Perry credits the role of cIMPACT-NOW, which was formed to keep up with changes in the field. Since new WHO classifications come out only every five to 10 years or so, the cIMPACT-NOW group published updates between more formal WHO updates, “so things wouldn’t be so overwhelming with one document all of a sudden.” Doing an interim review is unusual, he says. “But I hope other people start doing it, too.”

The group produced seven updates between WHO classifications, which enabled the 2021 classification to vault ahead. “I kind of joke that the ‘NOW’ stood for ‘not officially WHO,’” Dr. Perry says, though both groups comprised mostly the same members. “It was kind of a self-fulfilling prophecy that we would accept our own recommendations when the time came. And for the most part, of course, we did.” The interim recommendations have been incorporated into the newest WHO classification.

The evolution in classification, says another coauthor Daniel Brat, MD, PhD, “reflects the use of molecular approaches to establish a diagnosis and establish grading for brain tumors.” In 2007, molecular testing for brain tumors was more rumor than routine—the WHO did not incorporate it into diagnoses or classifications. “Basically all diagnoses were made under the microscope, based on morphology,” says Dr. Brat, the Magerstadt professor of pathology and chair, Department of Pathology, Northwestern University Feinberg School of Medicine.

That was the fourth edition; 2016 represented an updated version of the 2007 WHO, rather than a formal fifth edition. “The daily practice of neuro-oncology had changed so much,” Dr. Brat recalls. “We recognized that we really couldn’t wait until the fifth edition. There was so much data out there about the molecular classes of brain tumors that were critically relevant.”

The authors of the 2016 version added, for the first time, the integrated diagnoses that included both morphology and molecular features. The diffuse gliomas, as is their wont, led the way. Within that category, tumors could be

identified by IDH mutation status, which launched the additions of IDH-mutant astrocytomas and the IDH-mutant 1p/19q-codeleted oligodendrogliomas—two major classes of IDH-mutant tumors—as well as the IDH-wildtype astrocytomas grades 2, 3, and 4.

Those changes are more than interesting historical footnotes—they light the way for the latest edition. “In 2021 we have gone further and said these aren’t just subtypes of one another,” Dr. Brat says. “These are really two different diseases.”

Previously, diffuse astrocytomas were called either IDH-mutant or IDH-wildtype, as were anaplastic astrocytoma and glioblastoma. That terminology implied the same disease, one with a mutation and one without.

Dr. Dan Brat, coauthor of the WHO 2021 classification of central nervous system tumors. “For diseases that were lumped together in the past and now are recognized as different, based on molecular testing, we’re doing a disservice to patients unless we do appropriate testing,” he says. [Photo by Bruce Powell]

“Now we’ve separated that out completely,” says Dr. Brat. “We call the IDH-mutant astrocytomas what they are, and we grade them 2 through 4. There’s no longer such a thing as glioblastoma IDH-mutant—there’s a strong desire to get away from that terminology.” Previously, a grade 2 tumor was called diffuse astrocytoma; a grade 3 was an anaplastic astrocytoma; grade 4, a glioblastoma. With the shift in nomenclature, all are now called astrocytoma IDH-mutant, with the grade given within that overall entity.

The authors also made a new reckoning of tumors that used to be graded as 2 and 3 IDH-wildtype gliomas. At the molecular level, these were essentially wildtype glioblastomas, and they all behaved very aggressively as truly grade 4 tumors, says Dr. Brat.

Glioblastoma diagnoses have also taken a big leap forward, he continues. For decades, pathologists have used the

presence of necrosis and microvascular hyperplasia, seen under the microscope, to grade a diffuse IDH-wildtype glioma as a glioblastoma. Now they can make that same diagnosis absent those histologic features if the tumor has specific genetic alterations. *TERT* promoter mutations, *EGFR* gene amplification, and +7/-10 chromosome copy number changes are all criteria for a glioblastoma IDH-wildtype. "That's a *big* change," Dr. Brat says.

For IDH-mutant astrocytomas, the authors have also included a genetic alteration—*CDKN2A/B* homozygous deletion—that's sufficient to call a grade 4.

In short, molecular alterations, used as classifiers in the past, are now starting to be used in grading as well.

The pleasures of naming, of classifying, stretch back to earliest man strolling through the introductory pages of Genesis. Adam didn't last in Eden long enough to update his nomenclature, but the impulse persists whenever physicians ask: *What are we looking at? What does it mean? What should we call this?*

As with Adam's labeling system, the WHO classification by its nature has a global reach. The latest iteration in particular, with its rethinking of diffuse gliomas, should bring change to most labs, says Dr. Perry, given that these are the type of CNS tumors pathologists deal with most regularly. In particular, the pediatric classification has changed quite dramatically.

Pathologists will also notice that some of the embryonal and soft tissue tumors reflect a fair amount of advances since the 2016 classification. Ditto meningiomas, another extremely common entity.

As for pediatric tumors: The big change is in the pediatric diffuse gliomas, where "there's been a tremendous amount of improvement," Dr. Brat says. (The most common high-grade brain tumor of childhood is medulloblastoma, and the molecular classes for those, used in the 2016 update, haven't undergone any major changes in 2021.)

The adult IDH-wildtype diffuse gliomas are recognized as being very aggressive; in children, diffuse gliomas are rarely IDH-mutant. It might seem reasonable to call the pediatric diffuse gliomas IDH-wildtype, but they don't uniformly behave aggressively so this terminology would be misleading, Dr. Brat says. Advances now allow pathologists to identify specific molecular or methylation signatures in these gliomas and to base a more specific diagnosis on the presence or absence of defining alterations.

Histone H3-mutant gliomas (found in the brain stem, and clinically called diffuse intrinsic pontine gliomas, or DIPGs) are now known to have H3 K27 mutations, almost uniformly, and behave quite aggressively. Likewise, tumors that occur in the cerebral hemispheres and are H3 altered will have a G34 mutation, Dr. Brat explains, and are also high grade.

Both of these are WHO entities. Methylation profiling has further uncovered additional tumor types, called pediatric-type diffuse high-grade glioma, pediatric-type diffuse low-grade glioma, and infant-type hemispheric glioma. Each of these has distinctive clinical, histologic, and molecular features that have led to their recognition and are used to establish their diagnosis.

"We've learned a lot," says Dr. Brat.

Just as adult-type gliomas are now separated, so are the pediatric-type. "It's another big change," he says. Diffuse gliomas of adults that used to be recognized by the microscope and classified histologically are now identified as one of three types of entities: astrocytoma, IDH-mutant; oligodendroglioma, IDH-mutant, 1p/19q-codeleted; and glioblastoma, IDH-wildtype. "In addition to the histone H3-mutant diffuse glioma, we now have pediatric-type diffuse low-grade gliomas, with multiple subtypes depending on the type of associated molecular alterations. Infant-type hemispheric gliomas are uncommon but have defining genetic alterations that separate them from other childhood brain tumors. And then we have pediatric-type diffuse high-grade gliomas, and there are multiple types of those as well," Dr. Brat explains. "Within each of these broad clinical classes we can identify specific genetic or DNA methylation profiles that seem to correlate with clinical outcome."

Precision pays off. “For diseases that were lumped together in the past and now are recognized as different, based on molecular testing, we’re doing a disservice to patients unless we do appropriate testing,” he says. “Correct care requires the correct diagnosis.”

And not just for certain patients, he insists. “That’s a reality we’re going to have to deal with in the future in one manner or another,” whether that involves providing equipment and training to developing countries or to smaller community-based hospitals within this country. The response can’t be two-tiered, he says.

“I don’t buy the argument that we don’t have enough resources,” Dr. Brat continues. If there are resources to perform neurosurgery and provide quality in-hospital and outpatient postsurgical care that might include radiation therapy and chemotherapy, then molecular testing or its appropriate surrogates “are a drop in the bucket. In fact, that’s the reason the biopsy is being done in the first place—to get the right diagnosis.” The latest WHO classification should help flip the switch, he hopes, and reinforce that message.

This is not a sleepy field. “No,” says Dr. Brat. “Not at all.”

Nor is this a time to catch one’s breath, he says. “Let me tell you about the next paradigm shift.”

That would be DNA methylation profiling. Dr. Brat calls it a kind of bioinformatics separation of tumors based on their methylation fingerprint. The method, advanced by the Heidelberg group in Germany, is now considered a gold standard in brain tumor classification.

When faced with a challenging case that eludes even molecular profiling, Dr. Brat and colleagues will send it out for methylation profiling, which identifies tumor type based on its methylation pattern. “It gives you a degree of certainty on that call.” In a field already bursting with advances, he says, “It’s a huge breakthrough.”

The technique looks at thousands of genes throughout the genome for a level of methylation. As Dr. Perry explains, “The patterns you get overall seem to be related to a level of histogenesis.” Whatever the cell of origin—and in most cases it’s unknown—it tends to have a unique methylation profile, which is likely maintained in the tumors that arise from that cell, including some of the very high-grade or poorly differentiated tumors, he continues. Algorithms have learned to separate these tumors from one another.

It’s a powerful technique, Dr. Perry says, in neuropathology as well as in bone and soft tissue pathology. He predicts it will establish a role for itself in all other subspecialties eventually.

More breakthroughs should follow. Methylation profiling is specific for a given type of brain tumor. Once a methylation profile is identified, that tumor group will almost always have a defining genetic alteration and a fairly standard clinical course. This diagnostic path has given rise to many of the new entities recognized in the WHO 2021 classification. It represents a new wave in diagnostics. “And it’s going to be with us in the future,” says Dr. Brat. In some cases, it’s already here: The WHO classification has incorporated methylation profiling into some diagnoses, including it as a “desirable criteria.” (The other category is “essential criteria.”) In the next WHO classification, he predicts, methylation profiling will be one of the methods for establishing a primary diagnosis.

Dr. Brat estimates that only three or four centers in the country do methylation profiling. “So you can imagine that if there were serious considerations about inequality of technology for the 2016 edition, if we incorporate methylation profiling into classification, it’s going to be even more problematic.” Even many academic medical centers will find it out of reach, at least in the short term. At Dr. Perry’s lab, “We have it available for research, but we haven’t quite gotten to the point where we can do it for clinical testing.” When they need it clinically, they turn to the NIH. “They’re doing it right now free of charge. Free is always good news,” he says with a laugh.

Access is only one concern. “Once you add a new platform, it opens up all new types of applications, investigations, and practices,” Dr. Brat says.

He calls DNA methylation profiling a disrupter. Perhaps mindful that medicine has already had plenty of those recently, he hastens to add, “But I mean that in a positive way. Because if you think about patient care coming

first, methylation profiling is probably the most accurate predictor of a specific tumor type. And that goes along with prognosis in general."

Having said that, however, Dr. Brat notes that methylation profiling is not always adept at grading. The future, he says, will combine many steps: microscopy, NGS, other molecular profiling, and the gradual incorporation of methylation profiling into pathology practice. His own lab does perform the latter, and was about to go live with it diagnostically when he spoke to CAP TODAY in late September. And some tumor types are still primarily diagnosed purely on morphology, including the most common one, meningiomas, especially for grade 1.

The open-ended role for morphology will be a relief to some, given that practices change at different rates. In his own consult service, Dr. Perry sees the gamut—highly experienced neuropathologists, large private practice groups, and smaller private practice groups "that maybe take a little longer to implement changes."

Dr. Perry

For the most part, he says, adopting the molecular definitions set out in the 2016 document went smoothly. "People learn along the way," he says, adding that he tries to educate colleagues while explaining how he arrives at his diagnoses. "It takes time for everybody. We don't automatically, the next day, implement everything that's in the WHO."

Even with basic nomenclature changes that seem minor at first, says Dr. Perry, "It takes everyone a little time to get used to it. Whenever you change the name of something it causes confusion," as anyone who works for an oft-rebranded medical system knows.

The challenges can also be financial. As he works on consults, Dr. Perry says part of the education he does includes recommending adding, for example, NGS to a case, explaining why, and asking for permission.

That leads to the practical matter of billing. "I try not to assume anything," Dr. Perry says. Some practices want his lab to bill insurers (including Medicare); others prefer to have him bill the practice. It's not a scientific matter, "but it's a practical issue nonetheless. Certainly with the more expensive tests, I try to make sure they're OK with the billing before I go ahead and run them."

Worth noting is that third-party payers don't change overnight, either. They, too, need time to catch up to new classifications, and to understand the cost-benefits of even NGS, not to mention DNA methylation. It's a long game, although the new classification should bump things forward a bit. "I hope so," Dr. Perry says.

He takes up the perpetual choric: Payers too often eye the costs of specific tests for specific patients. "But you really have to factor in the global cost of giving the wrong therapy due to the wrong diagnosis or a less accurate diagnosis." While $3,000 to $4,000 for an NGS assay sounds like a lot of money upfront, it's not, he says, when compared with the costs of multiple neuroimaging studies, surgery, anesthesia, radiotherapy, and chemotherapies.

How will these freshly sharpened classifications shape the conversations between patients, their physicians, and those in the laboratory?

The new classification of pediatric diffuse gliomas aids clinical colleagues greatly, says Dr. Perry, even absent targeted therapies. "Pediatric oncologists really appreciate that, because they've known for many years even though we were calling many of these by criteria for adults, they're quite different for children."

Moreover, Dr. Brat says, additional mutations, deletions, or amplifications may provide additional prognostic

information. The aforementioned *CDKN2A/B* homozygous deletion indicates that an IDH-mutant astrocytoma that was thought to be grade 2 is actually going to behave in grade 4 fashion. Similarly, if it's IDH-wildtype and looks histologically grade 2 or 3 but has an *EGFR* amplification, it too will behave in a more aggressive fashion.

Dr. Perry calls his laboratory colleagues "lucky, because in our field, we're making discoveries fairly quickly. We're learning how a tumor forms and how it progresses." For oncologists, it's true that the conversation sputters a bit as researchers try to translate that knowledge into targeted therapies. Nevertheless, knowing about a unique genetic fusion that can be targeted or that drives a tumor is helpful. "Absolutely," he says, given that it can help determine prognosis as well as help physicians sort through available therapies.

Are therapeutics keeping pace with diagnostics? "I wish they were," says Dr. Brat. "It is a conundrum in this field that we probably have the most potential targets for therapy, but the actual targeting of them has been more challenging in the brain than in other organs." For example, *EGFR* amplifications are present in over half of IDH-wildtype glioblastomas, but targeted therapeutics have failed in clinical trials. Likewise, the finding of angiogenesis in glioblastoma raised the possibility of targeting the VEGF pathway, but those efforts came to an unsatisfying ending as well. And immunotherapy, a success in other organ systems, hasn't provided clear outcomes in brain tumors.

"So," says Dr. Brat, "we've got a lot of work to do. But if I can tell you one thing about the brain tumor community, it's that we work very well together as a team collaboratively across the world to uncover new targets and potential therapies, and do the clinical trials together." Since these tumors aren't as common as breast, lung, or prostate cancer, "We really need multi-institutional, sometimes multinational approaches to clinical trials."

People are still getting used to categories that were a prominent feature of the previous classification, Dr. Perry says, including NOS. "Some people use it a lot, and others don't." He suspects oncologists find it useful; it usually indicates that molecular testing was not done, and therefore the diagnosis was rendered purely on morphology.

Some, however, are reluctant to use the NOS designation. "I've noticed some groups—those that maybe don't have a lot of resources—feel a little resentful of having to put NOS on there," Dr. Perry says.

That ties into a common question Dr. Perry gets: "Can they get away with doing immunohistochemistry?" To give an example, several tumors are now defined as being a higher grade if there's homozygous *CDKN2A* deletion. Given that the protein product for that is p16, will IHC for p16 suffice? In most cases, Dr. Perry says, the answer is likely no—IHC alone provides some information, but it's not good enough to replace the more sophisticated molecular techniques. "There are still many, many diagnoses that are diagnosed by morphology alone. Fortunately, we do have a lot of good surrogate markers," Dr. Perry says. But p16 isn't one of them.

Dr. Brat suggests the NOS category will eventually fade as molecular testing lands in more hands.

While the 2016 classification discussed the problem of interobserver variations, that problem, too, is becoming diminished, for the same reason. "The molecular classes are actually very tight," Dr. Brat says, adding, "What I tell our neuro-oncologists and neurosurgeons is, 'Our diagnostic accuracy and our consistency among neuropathologists has gone up dramatically.'"

That's the good news. "The bad news: 'It's going to take two weeks to get your diagnosis.'"

For now, at least, that time frame is likely acceptable, Dr. Brat says, given that labs provide results in a stepwise fashion. IHC, with its next-day TATs, will generally allow pathologists to put tumors in the correct category of neoplasm, giving clinicians a diagnosis they can work with until the molecular profile is available.

Ultimately, he says, the goal is to identify tumors based on methylation profiling or NGS. "But then to identify more user-friendly and cost-efficient ways to establish the diagnosis, potentially by FISH or by immunohistochemistry, so that it, number one, isn't as costly, and, number two, doesn't take as much time to achieve a final diagnosis."

Dr. Perry is often asked which testing modalities are best. Not surprisingly, the answer is complicated.

Some guidance will come from a new CAP guideline that “dovetails nicely” with the WHO classification, says Dr. Brat. While the WHO classification makes a major change in what to call diffuse gliomas, the guideline shows how to test for them. It was done in collaboration with the American Association of Neuropathologists, Association for Molecular Pathology, and Society for Neuro-Oncology.

The focus is narrow but nevertheless “is a significant subset—diffuse gliomas are potentially the most important subset within the WHO,” says Dr. Brat, who is the guideline’s lead author. “The WHO provides classes and grades of neoplasm. It is agnostic on how you get there in terms of testing. So the guideline provides guidance to practitioners on how to do the biomarker testing, and what the test results imply, and the strength of evidence for that testing.” The guideline has been accepted for publication in *Archives of Pathology & Laboratory Medicine.*

Seeing how much the brain tumor classification systems have changed, says Dr. Brat, “The CAP recognized there would be a need for guidance around the diagnostic testing of diffuse gliomas as a family.” He and others on the expert panel (Dr. Perry is a coauthor) put together 10 guidelines and three practice statements as the basis for a diagnostic testing algorithm for community practice pathologists. When testing is as complex as it is for diffuse gliomas, it may seem straightforward only to those at academic medical centers who closely follow the evolution of classifications. “But for those who don’t have that historical grasp, it may seem like just a tremendous amount of unorganized diagnoses,” Dr. Brat concedes.

That would be where the flow chart comes in, he says. “It starts off with IDH testing, and then it branches into IDH-mutant gliomas and IDH-wildtype gliomas, and then within the IDH-wildtype gliomas it branches into age groups, locations, and it works through the diagnostic test results that end up in the WHO classification at the end.”

A certain coziness comes into play as experts develop guidance for their colleagues. With the added cIMPACT-NOW interludes, the latest WHO iteration brought few genuine shockers, apart, perhaps, from having to transfer the process to Zoom meetings.

Nevertheless, Dr. Perry says, “I’m always surprised that there’s always some controversial issue, or some questions for which there’s not quite enough data. And therefore people argue about which way they think we should go.” In most cases, he says, the answer is likely: Don’t go too far until we know more.

This isn’t unique to neuropathologists, he suspects. “Whenever you have experts around the world, all of us have strong opinions. And,” he laughs, “they’re not always the same opinions.”

A reasonable consensus does emerge eventually, he says. “And then, hopefully we get smarter again by the next WHO.”

Dr. Brat agrees. Putting together a brain tumor classification with a group of 12 internationally recognized neuropathologists is, he says, “often a discussion. It’s acknowledging we are on a journey and this isn’t the last classification that will be used. It’s really meant to be put in place as kind of a guidepost during our evolution to guide clinical care, to the best extent we can, at this point in time. I can guarantee you that the next classification of brain tumors will have evolved from the 2021.”

It always remains to be seen, for instance, how well a new classification of tumors will stand the test of time, especially if it’s based on morphology. Perhaps researchers will find a genetic subtype, or a methylation profile that provides additional information. “Until the 2016 classification, we didn’t incorporate genetic alterations at all into our classification,” says Dr. Brat, noting that just five years later, half of the brain tumor entities have essentially a requirement of molecular profiling to make the diagnosis.

Dr. Brat calls it an evolution, while noting, “We’ve come a long way in a short period of time.” They have a long way to go as well.

In the meantime, the only thing standing still might be the experts themselves. Dr. Perry says he used to travel 100,000-plus miles a year. “Now I’m a homebody. I don’t go anywhere.”

Karen Titus is CAP TODAY contributing editor and co-managing editor.

The 2021 fifth edition classification is due tentatively to be published online in November, with print to follow.