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NCI-CONNECT: Comprehensive Oncology Network Evaluating Rare CNS Tumors—Histone Mutated Midline Glioma Workshop Proceedings*

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*NCI-CONNECT Histone Mutated Glioma Workshop, please see full list of workshop participants at the end of the manuscript.

Abstract

Histone mutations occur in approximately 4% of different cancer types. In 2012, mutations were found in the gene encoding histone variant H3.3 (*H3F3A* gene) in pediatric diffuse intrinsic pontine gliomas and pediatric hemispheric gliomas. Tumors with mutations in the *H3F3A* gene are generally characterized as histone mutated gliomas (HMGs) or diffuse midline gliomas. HMGs are a rare subtype of glial tumor that is malignant and fast growing, carrying a poor prognosis. In 2017, the Beau Biden Cancer Moonshot Program appropriated $1.7 billion toward cancer care in 10 select areas. The National Cancer Institute (NCI) was granted support to focus specifically on rare central nervous system (CNS) tumors through NCI-CONNECT. Its mission is to address the challenges and unmet needs in CNS cancer research and treatment by connecting patients, providers, researchers, and advocacy organizations to work in partnership. On September 27, 2018, NCI-CONNECT convened a workshop on histone mutated midline glioma, one of the 12 CNS cancers included in its initial portfolio. Three leaders in the field provided an overview of advances in histone mutated midline glioma research. These experts shared observations and experiences related to common scientific and clinical challenges in studying these tumors. Although the clinical focus of this workshop was on adult patients, one important objective was to start a collaborative dialogue between pediatric and adult clinicians and researchers. Meeting participants identified needs for diagnostic and treatment standards, disease biology and biological targets for this cancer, disease-specific trial designs, and developed a list of action items and future direction.

Developing therapies for rare cancers of the central nervous system (CNS) is especially formidable for a number of reasons. First, obtaining tumor tissue, particularly from deep or midline regions of the brain and the spinal cord, poses strategic, pragmatic, and ethical challenges. Second, unlike other diseases and cancers, until recently there have been few, good preclinical models on which to evaluate new therapeutic approaches to ultimately build clinical trials. Third, there are inherent patient and research challenges in both providing care and conducting clinical research.[1] Patient challenges for some primary brain and spinal cord tumors, most notably the rare cancers, include the potential for delays in diagnosis, lack of well-defined standard of care treatments, limited social and advocacy support, and difficulty accruing and conducting trials because of the low incidence compounded by the limited experience of providers, even those in academic centers with specialized neuro-oncology programs. As these cancers are rare, some extremely so, it is often difficult to recruit a sufficient number of patients to adequately statistically power a study. To respond to these multifaceted challenges, the neuro-oncology community has to be particularly innovative, seeking partnerships and ensuring that each patient enrolled in a study is as informative as possible.

The Beau Biden Cancer Moonshot Program, part of the 21st Century Cures Act, appropriated $1.7 billion toward cancer care in 10 select areas, including patient engagement. The National Cancer Institute (NCI) was granted support for this program to focus specifically on rare CNS tumors through NCI-CONNECT, housed in the Neuro-Oncology Branch in NCI's Center for Cancer Research. Its mission is to address the challenges and unmet needs in CNS cancer research and treatment by connecting patients, providers, researchers, and advocacy organizations to work in partnership. NCI-CONNECT is first focusing on 12 types of tumors, each with less than 2,000 people diagnosed a year. NCI-CONNECT has 3 program goals: (a) develop an infrastructure across a network of national and international sites to study select adult rare CNS tumors; (b) collect, analyze, and share data to promote discovery and improve understanding of select adult rare CNS tumors; and (c) use the network to facilitate translation of discoveries into new therapies and methods to improve adult patient outcomes. To accomplish these goals, partnering with advocacy groups and patients is critical, providing insights about the patient community and outreach thereby increasing patient accrual to the program, care, and studies, which take place at the National Institutes of Health (NIH) Clinical Center or one of the more than 30 institutions in the Brain Tumor Trials Collaborative (BTTC). An important contribution of NCI-CONNECT is its convening power, that is, the ability to bring experts in rare CNS cancers together to share insights, ideas, and plans for collaboration. On September 27, 2018, NCI-CONNECT convened a workshop on histone mutated midline glioma, one of the 12 CNS cancers included in its initial portfolio. (See https://ccr.cancer.gov/neuro-oncology-branch/connect for the complete list.)

Histone mutated gliomas (HMGs) represent a rare CNS cancer. HMG is a diffusely infiltrative disease that develops within eloquent areas of the brain or spinal cord, cannot be readily surgically resected, and traditional chemotherapeutic agents have failed to change the median survival in pediatric clinical trials. There is a need to better define these diseases and address diagnostic challenges based on case definitions, molecular characterizations and profiling, and histological staining. A workshop of researchers, clinicians, and patients convened by NCI identified needs for diagnostic and treatment standards, the biology and identifying biological targets, and disease-specific trial designs. This workshop provides a roadmap to help advance the understanding of these cancers by developing standards for tumor sampling, processing, analysis, and storage. Participants endorsed novel ideas such as better molecular characterization of tumors, cerebrospinal fluid (CSF) analysis to avoid the need for brain biopsy, and infrastructure needs such as large patient registries and tumor repositories. The proposed strategies and action items represent a feasible roadmap that may be useful for other rare cancers and underscore the utility of convening focused workshops with dedicated experts.

Histone Mutated Gliomas

HMGs, also referred to as diffuse midline gliomas (DMGs), are a rare subtype of glial tumor that is malignant and fast growing, carrying a poor prognosis.[2] Until relatively recently, there were no known histone mutations in any human disease. In recent years, histone mutations have been described in approximately 4% of different cancer types.[3] Seminal studies in 2012 found mutations in the gene encoding histone variant H3.3 (*H3F3A* gene) in pediatric diffuse intrinsic pontine gliomas (DIPGs) and pediatric hemispheric gliomas.[4–6] As such, tumors with mutations in the *H3F3A* gene are generally characterized as HMGs. Histone-mutated DMGs occur more commonly in young children (average age 6–7) and are a leading cause of brain tumor-related death in children.[7,8] HMGs are seen less frequently in adults, with the largest retrospective series including 21 adult patients, and HMGs making up less than 10% of IDH-wildtype-infiltrating astrocytic tumors in adults.[9–11] Survival outcomes are poor across pediatric and adult cohorts of HMGs with median overall survivals ranging from 9 to 11 months for pediatric DIPGs and adult brainstem HMGs.[9,12–14] The median survival outcomes for thalamic-localized and spinal cord HMGs may be slightly better based on results from retrospective series, and the reason for this difference in survival outcomes is unknown.[15–17]

HMGs most often form in the pons, thalamus, spinal cord, cerebellum, and corpus callosum; hence, the reference to midline as the tumors are named, in part, based on the locations where they most often occur.[2,18] It is uncommon for these tumors to occur in other areas of the CNS, although hemispheric HMGs have been identified in pediatric patients and are associated with the H3G34R/V mutation, and of note, these tumors are not yet separately classified by the World Health Organization (WHO).[6,19] The prevalence of H3 G34R/V mutations in hemispheric, adult high-grade gliomas is not yet known.

It is rare for astrocytic tumors with H3 K27M mutations to occur in other areas of the CNS, outside of midline structures, although cases of non-midline H3 K27M-mutant gliomas have been reported.[16,20] Further complicating the classification of HMGs are recent reports of piloid and other low-grade glial neoplasms with H3 mutations leading to specific recommendations for classification of DMG with H3 K27M mutation by the Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy (cIMPACT) group.[18]

HMGs are a diffusely infiltrative disease, develop within eloquent areas of the brain or spinal cord, cannot be readily surgically resected, and traditional chemotherapeutic agents including temozolomide have failed to change the median survival in pediatric clinical trials. Historically in pediatric cases of DIPG, a diagnosis had been made based on radiological features alone. As a consequence, a dearth of tissue samples from patients has hindered progress in understanding this disease until recently, and especially in pediatric cases, when tumor sampling is now often considered and undertaken.[14]

Scientific Progress and Challenges in Midline Glioma Research

Three leaders in the field, Michelle Monje, Mario Suva, and Ali Shilatifard, provided an overview of advances in research on HMGs. In addition to describing research findings, they shared their observations and experiences related to common scientific and clinical challenges in studying these tumors.

Scientific challenges include the following:

- Gliomas are complex and heterogeneous ecosystems.
- Historically, lack of robust animal models, including both xenograft and patient-derived xenograft models, has slowed preclinical research.
- The tumor microenvironment is incompletely understood, particularly in HMGs.
- Despite recent efforts in pediatric studies, there remains a paucity of tumor specimens. Tumor samples from adult patients are even more scarce.
- There is a lack of uniformity of sample preparation and storage methods (e.g., fresh, frozen, fixed, cultured). This is particularly germane for histone mutated tumors, which are most commonly midline and biopsied rather than resected.

Clinical challenges include the following:

- Due to the diffuse nature and location of HMGs, there are limited surgical options for treatment and a reluctance from adult neurosurgical oncologists to perform biopsies in the adult population.
- The tumors are resistant to conventional therapy.
- A lack of established specific targets has stymied therapeutic options.
- In the absence of strong early-phase clinical trial results, trials require innovative study designs to enable early efficacy and failure readouts.

These issues were further discussed and developed by breakout groups (see below).

Cell-Intrinsic, Microenvironmental and Immunotherapeutic Targets in H3 K27M+ Diffuse Midline Gliomas

Michelle Monje, MD, PhD, Stanford University

Recent progress in the genomics of these tumors has shown that 80% of DIPGs, as well as the majority of thalamic and spinal cord high-grade gliomas in children, exhibit the H3 K27M mutation. Although the mutation is only present in 10% of the histone proteins in the cell, there is a dominant effect, inducing dysfunction of the Polycomb repressive 2 (PRC2) complex and dramatic alterations to gene expression.[21,22]

An effective therapeutic strategy will require a multipronged approach. In addition to targeting cell intrinsic vulnerabilities, it is becoming increasingly clear that we need to leverage effective immunotherapeutic strategies as well as target important microenvironmental dependencies. Monje and colleagues have begun to acquire patient tissue specimens, which are rapidly acquired postmortem. These tissues provide patient-derived DIPG, thalamic glioma, and spinal cord H3 K27M mutant tissue cultures available for preclinical studies and rapid drug screening. Subsequent work found that histone deacetylase (HDAC) inhibitors, such as panobinostat, were particularly effective at reducing tumor growth in vitro. In addition, this screen discovered potential beneficial effects of CDK inhibitors, proteasome inhibitors, and some specific kinase inhibitors.[23,24] In particular, Monje and colleagues found that HDAC inhibition helps to restore the trimethyl mark on histone 3 at lysine 27, which results in a broad normalization of gene expression in the histone-mutant cells. Furthermore, preclinical studies using in vivo rodent models have shown improved survival rates after treatment with panobinostat.[25] This agent has been further validated using high-throughput screening.[24]

Additional epigenetic strategies have been emerging from preclinical work. In addition to HDAC inhibitors, a number of other epigenetic modifying agents have proven to be potentially beneficial, including histone demethylase inhibitors, CDK7 inhibitors, and bromodomain inhibitors, all of which function to disrupt efficient transcription.[21–24]

Another emerging therapeutic category that is emerging is targeting dependencies of these diffusely infiltrating glioma cells on their microenvironment. Monje and colleagues have been studying the way that neuronal activity regulates both normal and malignant glial precursor cells and have found that neuronal activity robustly regulates the growth of high-grade gliomas through activity-regulated cleavage and secretion of a synaptic protein called neuroligin-3 (see Figure 1).[26] ADAM10 is the enzyme that mediates neuroligin-3 cleavage and release into the tumor microenvironment, suggesting that effective targeting may have clinical utility.[27,28]

Finally, Monje and colleagues are focusing on immunotherapeutic targets in DMGs. Screening neurosphere cultures against an existing panel of antibodies, high expression of an antigen called GD2, a disialoganglioside previously

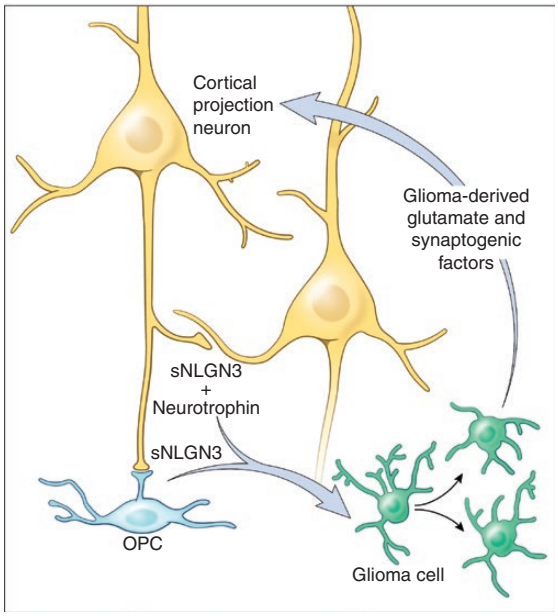

Fig. 1 Schematic illustrating the bidirectional relationship between normal neural cells and their malignant glioma counterparts. Activity-related factors, including a secreted form of neuroligin-3 (sNLGN3) from normal oligodendrocyte progenitor cells (OPCs, blue) and neurons (yellow) and neurotrophins such as BDNF, fuel the growth of glioma. Disentegrin and metalloproteinase domain-containing protein 10 (ADAM10, not shown in figure) cleaves neuroligin-3 before it is released into extracellular space. Adapted from ref. [25] with permission.

identified as an important immunotherapeutic target in neuroblastoma, has been seen across H3 K27M mutant tissue cultures. H3K27M+ DMGs exhibit higher expression of GD2 than even neuroblastoma, which provides an opportunity to use existing tools to target this antigen.[29] H3K27M is a potential novel immunotherapy target. Okada and colleagues have identified an H3.3K27M-derived peptide as an HLA-A*0201-restricted neoantigen epitope and cloned a high affinity T-cell receptor (TCR) cDNA that specifically recognizes the HLA-A*0201-H3.3K27M peptide complex.[30] Based on these data, a peptide vaccine trial (PNOC007; NCT02960230) is ongoing, and a phase I trial of TCR-transduced T-cell therapy is in development.

Dissecting Single-Cell Regulatory Programs in H3 K27M-Gliomas

Mario L. Suvà, MD, PhD, Massachusetts General Hospital

Suvà and colleagues are using a systems biology approach in trying to characterize both malignant cells and the tumor microenvironment by deploying single-cell analysis in clinical glioma samples, with a focus on single-cell RNA expression profiling (scRNA-seq).[31–33] Leveraging full-length scRNAseq they are able to infer both the genetics and expression program ("cellular states") of malignant cells in a tumor to better understand the type of lineage that malignant cells are recapitulating, infer lineages of differentiation, and create a putative phylogenetic tree in which genetic evolution is integrated into the cellular states of malignant cells.

In initial studies, Suvà and colleagues have successfully applied single cell expression profiling and genomic techniques to analyze the composition and function of DMGs (Figure 2).[34] They used 3 approaches to robustly separate malignant from nonmalignant cells: (a) expression clustering that characterized cells as being malignant or nonmalignant; (b) inferring copy number aberrations from scRNAseq data; (c) detection of point mutations in scRNAseq reads, in particular the H3 K27M mutation that they captured efficiently by adding locus-specific primers to their full-length scRNAseq protocol. Once the malignant cells are robustly identified, they can be further categorized into transcriptional subsets using unsupervised analyses methods such as non-negative matrix factorization. Suvà and colleagues used this information to infer the developmental trajectories of DMG. They found that these tumors are driven by oligodendrocyte precursor-like cells (OPC-like) that are in part sustained by *PDGFRA* signaling.

The authors also used their scRNAseq data to directly compare malignant cell programs in DMG to single-cell programs in other classes of human gliomas, for example, glioblastoma IDH wild type, astrocytoma IDH1 mutant, and oligodendroglioma IDH1 mutant, with the aim to highlight the signaling pathway particularly relevant to the histone mutation.[31,33] They found a signature of genes exclusively overexpressed in histone mutant cells, but not other types of gliomas, which is related to a dysfunctional epigenetic regulation and a differentiation blockade.[34] These findings suggested additional dependencies for DMG and have important implications for their origin.

Importantly, as a result of recent developments in single-cell sequencing technologies, these approaches can now be performed directly in frozen tissues by isolating nuclei from which RNA is sequenced. The information obtained is slightly different but overall comparable to what can be obtained from a single cell. Single-cell genomic profiling is now possible for RNA, DNA, DNA methylation, epigenome, and for multi-omics.

Principles of Epigenetics and Chromatin in Development and Human Disease

Ali Shilatifard, PhD, Northwestern University

Shilatifard and colleagues have focused on understanding the role of Polycomb and Trithorax group proteins as chromatin-modifying factors key in repressed or active gene expression states. An example of developmental chromatin regulation is the balanced activities of the Polycomb group proteins within the Polycomb repressive complexes 1 and 2 (PRC1 and PRC2), and the Trithorax group proteins within the COMPASS family, which are highly mutated in several human diseases.[21,22] Work by Shilatifard and colleagues has shown that these enzymes modify chromatin and that mutations within the

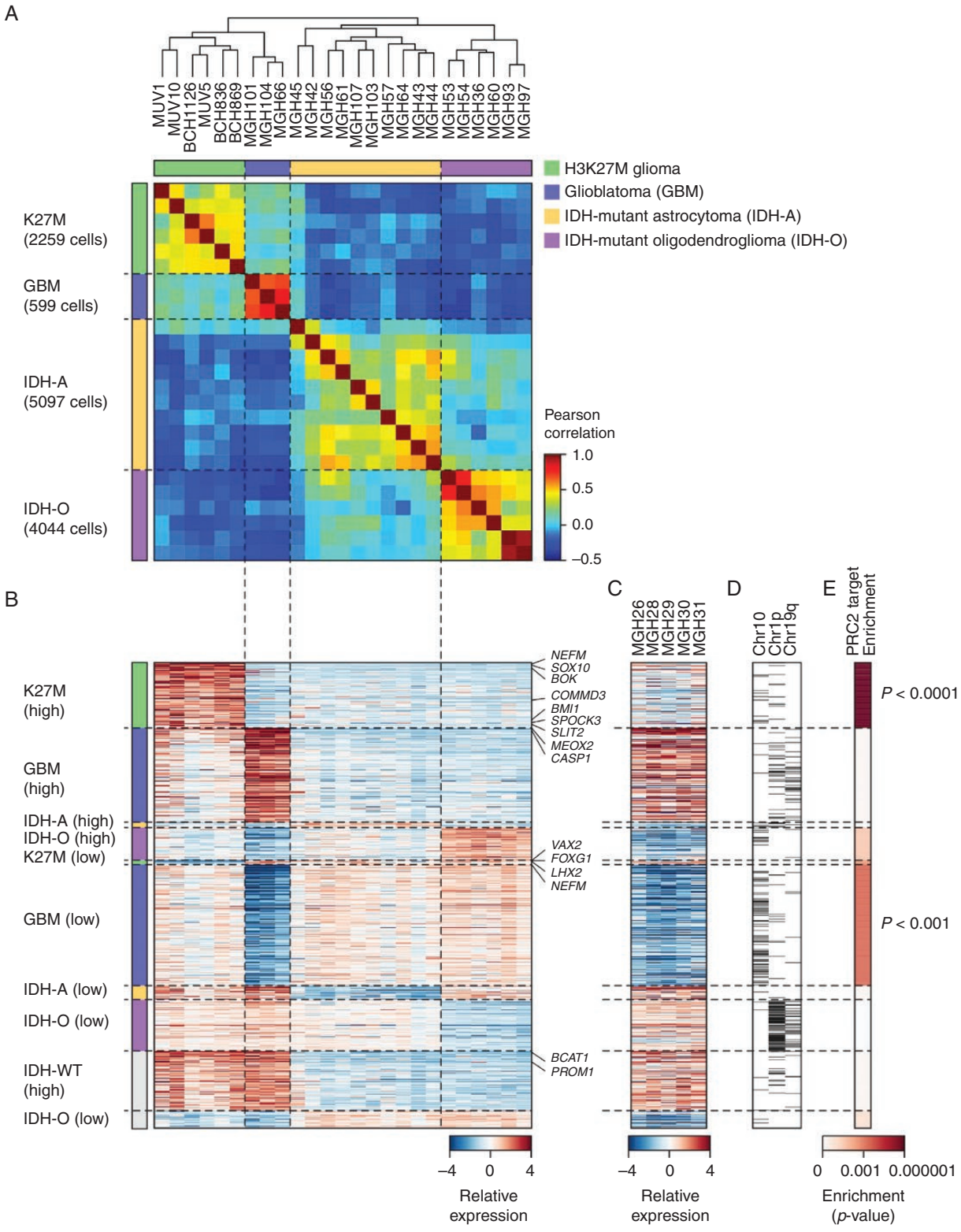

Fig. 2 (A) Pairwise correlation between the average expression profiles of malignant cells from 25 gliomas, ordered by hierarchical clustering (top) and marked by clinical classification (color bar). (B–E) Differentially expressed genes across gliomas, including H3K27M-mutant gliomas (as seen on the green bars in A and B). (E) Note the enrichment of PRC2 targets in each subset of differentially expressed genes (Fisher's exact test), with enrichment of PRC2 targets reaching the highest level of statistical significance in the K27M-mutant gliomas. Adapted from ref. [32] with permission.

components of the enzymatic machinery are associated with large numbers of malignancies.

Shilatifard has studied pediatric translocation of leukemia for the past 25 years, focusing on the gene for mixed lineage leukemia (MLL), which is a Trithorax homolog in humans. Seminal work by Janet Rowley defined the role of translocation of MLL and the pathogeneses of infantile acute myeloid and lymphoid leukemias.[35] Despite the seminal discovery by Dr. Rowley, the 5-year event-free survival rate for these patients has remained at less than 30%. Thus, understanding the molecular basis of translocations is critical to developing improved treatments for leukemia.

Polycomb is a negative regulator of gene expression in K27 methylation.[36] The discovery of H3 K27M led to the speculation that it can sequester with PRC2, and that this sequestration could be the cause of the disease, which was demonstrated in a *Drosophila melanogaster* model. This model for the pathogenic histone H3 K27M mutation revealed that its overexpression resembles PRC2 loss-of-function phenotypes, causing derepression of PRC2 target genes and developmental shifts.[21,22,36] The development of histone mutant models has become valuable in vivo tools for inhibiting methylation pathways that also function as biochemical reagents for capturing site-specific histone-modifying enzymes, which can provide molecular insight into chromatin-signaling pathways central to leukemia. Subsequent studies proposed that the inhibition of the transcription cycle in the elongation phase or a BET protein domain inhibitor could be curative for this disease.[21] Genome-wide analysis reveals that there is a mutual exclusivity between PRC2 localization, K27M methylation, and K27M acetylation. Furthermore, when an H3 K27M mutation is present, there is an activation of BET family members Brd2 and Brd4. It is speculated that perhaps inhibition of this cancer-specific binding could affect the disease biology.[21]

In 1996, Shilatifard and colleagues posed that transcription of elongation control must be central for leukemia pathogenesis. MLL translocation always gives way to the same diseases because they are components of the super elongation complex (SEC), which plays a role in transcriptional regulation.[37,38] This seems to be true for DIPGs as well. Therefore, disrupting the SEC through small molecules could disrupt the process of cancer pathogenesis, which has shown to be true for leukemia and other cancers.

Standards for Diagnosis and Treatment of Adult Patients with HMGs

A breakout group of meeting participants convened to discuss the need to establish standards for diagnosis and treatment of adult patients with HMGs, including case definition(s) for HMGs and histone-mutated glial neoplasms in adult patients; standard of care for newly diagnosed HMG in adult patients; and recommended treatment approaches to recurrent HMGs in adults.

Participants concluded that the current WHO classification of DMG H3 K27M-mutant is sufficient for the care of adult patients.[2] However, in the future, subgroups may be identified based on further mutational and molecular analysis. As stated previously, there have been cases with H3 K27M mutations with low grade and/or noninfiltrative astrocytic histology and non-midline tumor location, and these cases may not carry the same prognosis as DMG H3 K27M-mutant.[18] These unique cases must be identified and subjected to additional testing to include mutational profiling and methylation profiling as discussed subsequently. The classic DIPG radiographic appearance may not be as common in adult DMGs, and a more circumscribed radiographic appearance has been observed, thus far anecdotally, in some adult cases. Regardless, the case series reported to date suggest DMG H3 K27M-mutant have a uniformly poor prognosis in adults and prognostic estimates and treatment recommendations do not differ significantly from pediatric cases.[9,12] In pediatric DMGs, p53 mutations may be associated with a worse prognosis (compared with p53 wild-type tumors), a result that needs to be comparatively assessed in adult DMGs.[39] There may be subtypes within adult DMG H3 K27M-mutant tumors, for example, those with FGFR mutations or H3.1 K27M mutations, which may have unique clinical features, radiographic appearance, and prognosis.[14,40]

Minimum standards for diagnostic evaluation were discussed in detail, with careful attention and discussion to the widespread availability and cost of genomic testing. At a minimum, a stain for the H3 K27M mutation should be performed, which can easily be implemented in the community setting. The group recommended that H3 K27M staining should be done as a standard test if the IDH stain is negative, especially in midline-localized tumors. Additional immunohistochemistry (IHC) stains that might be informative include H3 K27M trimethylation, p53, and ATRX, although care must be taken not to exhaust limited tissue samples with additional IHC testing if genomic testing is planned. Loss of p53 function (by mutation or loss of chromosome 17p) carries prognostic significance in midline, pediatric high-grade gliomas[19] and ATRX mutations may be more common in certain subtypes of HMGs (e.g., H3 G34R/V mutated tumors),[6] but p53 and ATRX IHC testing are not necessary for a diagnosis of DMG H3K27M mutant. Per current WHO 2016 guidelines, an astrocytic neoplasm with H3 K27M mutation is classified as a WHO grade IV lesion and carries a homogeneously poor prognosis. To this end, neuropathology experts recommended performing a neurofilament stain to highlight the infiltrative tumor phenotype. Adult astrocytic tumors that are midline in location but IDH and H3 K27M wild type on immunostain may be challenging to classify based on histologic features alone and may require additional genomic testing. Patients might incur additional high costs if these tests are not covered by insurance plans, which is a frequently encountered challenge in oncology. The group recommended developing a tiered diagnostic algorithm for DMGs including histology, immunostaining, mutational profiling, and cases to be referred for methylome profiling.

In an adult patient with a suspected HMG, the group recommended maximum safe resection and avoidance of eloquent areas. Unfortunately, given the midline location, most of these lesions are not amenable to resection. Biopsy has been increasingly implemented in pediatric midline tumors as part of prospective studies, which utilize the molecular information obtained to guide therapy,

for example, selecting patients for clinical trials using molecularly targeted or immunotherapeutics. The importance of biopsy for purposes of diagnosis, therapeutic decision-making, prognosis, and discovery needs to be disseminated to adult neurosurgeons to assure that biopsy is pursued whenever possible to align with the pediatric approach.[41] The biopsy should be performed by an experienced neurosurgeon at a subspecialty institution. All participants in the clinical subgroup, and larger workshop, agreed that it is critical that every adult patient be biopsied, which might require referrals from community hospitals to academic medical centers with the appropriate neurosurgical expertise. Due to the potential for leptomeningeal spread of HMGs, the pediatric experts strongly recommended staging of the neuraxis with MRI of the cervical, thoracic, and lumbar spine, and considering cytologic analysis of the CSF if considered safe and necessary for additional confirmation of leptomeningeal dissemination. Staging of the entire neuraxis should be repeated at tumor recurrence and prior to clinical trial enrollment.

Standard adjuvant treatment after establishing a diagnosis of HMG with the H3 K27M-mutation should include involved-field radiation. Typical involved-field radiation for HMGs usually includes a total dose of 54–60 Gy delivered in 30–33 single-day fractions, the same treatment plans that are the standard of care for glioblastomas and anaplastic astrocytomas.[42–44] There is no known benefit of proton therapy over standard photon therapy, and the safety profile of proton versus photon radiotherapy, specifically rates of pseudoprogression and radiation necrosis, are unknown in adult midline tumors. Proton radiotherapy may be advantageous if craniospinal radiation is needed to treat disseminated disease.[45]

There is a lack of benefit of adjuvant temozolomide in the pediatric experience with DIPGs.[44] MGMT promoter methylation, which is associated with temozolomide sensitivity in high-grade gliomas in adults, is present in less than 5% of pediatric DMG H3K27M mutant[19,46]; 65% of H3G34R/V mutated pediatric high-grade gliomas have MGMT promoter methylation, but whether this is a specific group of pediatric high-grade gliomas that benefit from temozolomide is unknown.[19,47] Based on the observed lack of efficacy of temozolomide in pediatric patients and the lack of prospective or published experience in adult patients, concurrent and adjuvant temozolomide could not be recommended as the standard of care for a newly diagnosed, adult HMG by the clinical subgroup. Until further evidence is available, temozolomide could be considered on a case by case basis, for example, in an adult HMG with MGMT promoter methylation.

The dopamine receptor DRD2 has emerged as a therapeutic target in HMGs.[48] The oral imipridone ONC201 has been associated with durable, radiographic responses in H3K27M mutant gliomas in early-phase clinical trials.[49,50] Clinical trials of ONC201 are ongoing in adult and pediatric HMGs (NCT02525692, NCT03416530), and a first-in-human trial with the next-generation impipridone, ONC206 (https://oncoceutics.com/onc206/), is planned at the National Cancer Institute in recurrent, primary brain tumors (including HMGs).

Re-irradiation can sometimes be considered as a salvage therapy. At tumor recurrence, all patients should be considered for enrollment in a clinical trial if available, and given the lack of efficacy and experience with adjuvant chemotherapy, the group recommended developing clinical trials starting in the adjuvant setting following radiation therapy.

Although there was a strong consensus on the steps needed to define these diseases, there are diagnostic challenges based on case definitions, molecular characterizations and profiling, and histological staining. Additional testing must be considered against the cost to patients, safety, and the need for biopsy performed by an experienced neurosurgeon. Critically, there is a need for systematic evaluation of patients in the context of a clinical trial or natural history study to better define prognosis, treatment outcomes, and the patient experience.

Biology and Biological Targets

A second breakout group was tasked with discussing chromatin-related changes that contribute to HMGs with regard to understanding the mechanisms by which chromatin-pathways may be drivers of adult midline gliomas, and potential early detection methods and targets.

The key biological question is whether, and to what extent, the adult histone mutant DMGs are the same or different from the pediatric form. Determining whether the disease is the same across the age spectrum would enable investigations to focus on location-specific biology rather than age-specific biology. In turn, knowledge gained from pediatric studies would then become applicable to the adult disease. Despite ongoing efforts, fundamental questions regarding tumor biology and genesis remain, including definitely determining the cell of mutation and the cell of origin, and whether these events occur in the prenatal period, which in turn would imply a long latency in adults with HMG. Recent data suggest that DMGs may emerge from oligodendrocyte precursor-like cells (OPC)—a precursor cell population active in the postnatal and adult brain—providing an important clue to their possible origin.[8]

These precursor OPCs account for 8–10% of the cells in the brain and remain a proliferative cell type contributing not just to brain development in childhood but also to ongoing mechanisms of brain plasticity.[51] It remains unclear how the biology of these precursor cells makes them potentially permissive. Furthermore, it is unclear what makes the precursor cells or the microenvironment in the CNS midline region particularly susceptible to the transformation characterized by histone gene mutations. These questions underscore the need to study the unique biology of the precursor cells of the histone mutated midline gliomas. Another question is how H3 K27M mutations lead to a cancer with different biology than HMGs that occur in the cerebral hemispheres, where H3 G34R/V mutations are characteristic. For example, it remains to be determined whether H3 G34R/V-mutated high-grade gliomas have a similar cell of origin. The H3 G34R/V mutated tumors are far less studied than the H3 K27M mutated tumors; consequently, the resultant impact on chromatin biology is less

well characterized. This information may provide important insights about not only disease biology, but also potential therapeutic targets.

Although not yet established, there may be common tumor biology between the pediatric and adult forms of HMGs. Consequently, the separation of research efforts by age currently hinders progress. Glial development, that is, the establishment of the myelinated infrastructure of the brain, occurs over 35 years of human development, underscoring the overlap between the traditional age definitions of adult and pediatric tumors. If the biology of pediatric and adult HMGs turns out to be very similar, perhaps with some age specific differences, clinical trials including adult and pediatric patients would be important for accelerating progress and improving clinical trial accrual. Therefore, the current age-based infrastructure for the existing oncology groups imposes potentially arbitrary criteria for studies. There is an opportunity to leverage existing infrastructure for tissue banking and genomics, to make it available to researchers, and to raise awareness that investigations in these diseases should encompass the adult subtype as well. Although this will require coordination and standardization of terminology and protocols and more model systems, the potential synergy would outweigh the additional efforts to establish joint investigation.

Recognizing the challenges of obtaining large tumor samples and the inability to do serial tissue sampling, using CSF samples for longitudinal analysis was also discussed. This is an area of active investigations, particularly as it relates to cell free DNA. Early studies are demonstrating that while attempts at "liquid biopsies" using blood samples in patients with CNS cancers have been largely unsuccessful, there has been more success in finding tumor specific DNA fragments in the CSF. Preliminary studies suggest that increasing concentrations of tumor DNA in the CSF is an early indicator of tumor progression or recurrence. However, these results need to be validated and extended. In addition, other potential applications, such as monitoring cell-free circulating DNA from CSF for new mutations as potential therapeutic targets or as indication of malignant transformation have not yet been extensively studied. CSF sampling should only be done if deemed safe by the clinical team given the midline location of these tumors and the potential of associated increased intracranial pressure.

Clinical Trial Designs

A third breakout group was tasked to discuss clinical-translational paradigms for developing novel clinical trials for patients with HMGs. One of the greatest challenges to clinical trial design is recognizing that there is heterogeneity even within the group of CNS cancers defined by histone mutations. Depending on the molecular alterations, these cancers will have different biology, respond differently to treatment, and portend a different prognosis. For this reason, one of the key recommendations resulting from this discussion was strongly supporting the concept that sampling these midline tumors is a critical component of not only advancing our knowledge of these cancers, but also to guide the optimal treatment for the individual patient. Rapid advances in technology enable extensive molecular testing even from small samples and single cell analysis providing unprecedented insights into tumor biology and heterogeneity. The group concluded that tissue is needed not only for diagnostic specificity and targeted treatment but also to advance the field by informing clinical trials that require confirmation and direct molecular subtyping for either stratification or enrollment. The group recommended that biopsy become standard of care and that training be offered to neurosurgeons to increase their comfort level and skill.

Further recognizing that HMGs were only recently recognized in adults, a retrospective natural history series that looked exclusively at tumors in the midline, and stratified by various age groups, would enable an evaluation and determination of the biologic differences at various epochs in life and in different tumor locations. These results could potentially provide comparator groups for clinical trials, and if these results were suitably robust, provide an adequate comparator (historical control), enabling single arm clinical trials seeking an efficacy signal. As discussed earlier, the group emphasized that molecular subtyping is critical because it is likely that the underlying molecular changes drive the biology, not the histology.

As mentioned earlier, HMGs have only recently been recognized in adult patients and a standard of care has not been established. Therefore, clinical trial efforts that test specific targeted therapies will probably require further testing of a larger series of tumors with then appropriate preclinical testing before initiating a suitable clinical trial. In the interim and given that radiation is the standard of care for newly diagnosed HMGs, a trial that determines optimal radiation treatment can be launched. A prospective, randomized trial comparing hypofractionated versus hyperfractionated standard radiation including survival and patient reported outcome measures was proposed. The potential role of immunotherapy for HMGs was also discussed. The inherent challenges, including management of tumor enlargement secondary to an inflammatory infiltration also known as pseudoprogression and the potential neurologic consequences of this therapeutic response, were reviewed. The group also recommended identifying immune markers in CSF as both pretreatment predictors of response and serial evaluation to help distinguish pseudoprogression from true tumor progression.

Finally, recognizing the rarity of HMGs in adults, recruiting patients into disease-specific clinical trials will require creating patient registries, leveraging help from brain tumor advocacy organizations, and using social media outlets innovatively. The group recommended collaboration among the oncology cooperative groups, not only on study designs that cross the lifespan, but also on standardized collection and banking of data and tissue.

Action Items and Future Directions

Table 1 summarizes the major action items identified by the workshop participants. There was consensus that the accurate diagnosis of HMGs is critical for both treatment

Table 1. Action items for advancing HMG research and therapy

1. Limited available tissue remains a challenge for biological understanding of the disease. It is imperative to educate neurosurgeons about the need for tissue diagnosis, standardize the diagnostic evaluation to include molecular subtyping, and optimize tissue preparation, storage, and data sharing.
2. Develop protocols for collection of CSF and further develop cell-free DNA techniques using CSF for diagnosis and treatment monitoring.
3. Improve knowledge of natural history including the following: response to standard treatments including radiation and chemotherapy, outcome of midline versus non-midline tumors, H3 K27M-mutated tumors versus other histone mutations, rates of CNS dissemination, and patient-reported outcomes.
4. Limited data are available on adult HMGs and where overlap may occur with pediatric HMGs. Increased collaboration is needed between adult and pediatric research efforts.

and research, especially in adults where less is known. A strong recommendation agreed on by all participants was to make sampling of midline tumors the standard of care, especially when the results are used for therapeutic decisions. This will require training opportunities for neurosurgeons. At the current time, the only way to refine diagnoses and to better understand the biology of this rare cancer is to have more tissue available. Reliance on traditional histopathology alone is insufficient. As has been established in other CNS cancers such as medulloblastoma and ependymoma, molecular subtyping is essential, but this is not always possible because of cost constraints. In these cases, it may be possible to create a focused panel that initially implements IHC to start given its favorable cost and widespread availability and recommend further molecular subtyping based on the IHC results. For example, a case with an H3 K27M mutation on IHC may not require additional molecular subtyping, especially outside of a clinical trial or research setting.

The group recommended that tumor tissue preparation be standardized and, given the scarcity of this material, adherence to the established procedures should be highly encouraged. Standard procedures will need to be developed to encompass formalin-fixed, fresh, and frozen tissue protocols. These procedures would assist the community in ensuring collections serve both diagnostic and research purposes whenever possible. Regardless, diagnosis is the highest priority use of specimens, and institutions should establish systems where unused diagnostic material can also be used for further interrogation that might help provide an individual patient with prognostic information and as knowledge increases, for example, predictive markers that would determine optimal treatment. The group concurred that storage of frozen tissue should be standard of care and that a diagnostic algorithm that distinguishes hemispheric from midline tumors would be useful not only for treatment decisions but also to make the best use of scarce tissue.

As discussed earlier, there was extensive discussion and enthusiasm for exploring the use of CSF both to diagnose and to determine treatment-related changes in tumor biology. Much of this would be based on cell-free components such as DNA, recognizing that CSF cytology is often negative even when dissemination is evident by imaging studies. However, a recommendation was made to establish best practices and protocols for when to collect CSF for therapeutic or research purposes.

Clinical research initiatives are needed for HMGs, particularly in the adult patient population. Given the rarity of this disease and the only recent recognition of its occurrence in adults, creation of a patient and tumor registry was strongly supported. This will enable collection of clinical information from both retrospective and prospective patient populations. Retrospective tumor samples with comprehensive clinical annotation provide an outstanding opportunity to explore prognostic factors as there is typically mature survival and treatment data coupled with the ability to correlate outcomes with the molecular features of the tumor. Prospective collection of patient-reported outcomes, neurocognitive testing, and cancer-treatment-related toxicity measures are necessary to understand the impact of cancer and cancer treatment on HMG patients. To this end, a natural history study has been established as part of NCI CONNECT and adult patients with HMGs are currently being enrolled.

HMGs provide an excellent opportunity to generate collaborations between adult and pediatric clinical and laboratory research efforts. There was consensus that to determine whether adult and pediatric HMGs are distinct, the same, or a continuum of diseases, the community should leverage existing resources such as tissue banks, consortia, and expertise among investigators from adult and pediatric programs. These studies can help interrogate the genesis of these cancers to understand the role of the mutation in the initiation of cancer, the influence of development as a fetus and throughout life, and the general biology of the midline and why it might be predisposed to developing these cancers.

In conclusion, HMGs represent a rare CNS cancer that typically present clinically as midline tumors in pediatric patients. Pivotal studies from the initial series of biopsied DIPG samples in pediatric patients confirmed that this procedure can be done safely and revealed the signature histone mutations. Recently, with the readily available IHC stain, HMGs are being increasingly recognized in adults. This workshop provides a roadmap to help advance the understanding of these cancers by developing standards for tumor sampling, processing, analysis, and storage. Furthermore, novel ideas such as CSF analysis and creating large patient registries and tumor repositories to define the spectrum of the disease and outcomes for clinical trial planning were endorsed. Finally, collaborations between adult and pediatric investigators were encouraged. The proposed strategies and action items represent a feasible roadmap that may be useful for other rare cancers and underscore the utility of convening focused workshops with dedicated experts.

Keywords

clinical trials | histone-mutated glioma | NCI-CONNECT | rare brain tumors

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