Neuro-Oncology

Pediatric low-grade gliomas: implications of the biologic era

Roger J. Packer, Stephan Pfister, Eric Bouffet, Robert Avery, Pratiti Bandopadhayay, Miriam Bornhorst, Daniel C. Bowers, David Ellison, Jason Fangusaro, Nicholas Foreman, Maryam Fouladi, Amar Gajjar, Daphne Haas-Kogan, Cynthia Hawkins, Cheng-Ying Ho, Eugene Hwang, Nada Jabado, Lindsay B. Kilburn, Alvaro Lassaletta, Keith L. Ligon, Maura Massimino, Schouten-van Meeteren, Sabine Mueller, Theo Nicolaides, Giorgio Perilongo, Uri Tabori, Gilbert Vezina, Katherine Warren, Olaf Witt, Yuan Zhu, David T. Jones, Mark Kieran

Center for Neuroscience and Behavioral Medicine (R.J.P., R.A., E.H., Y.Z.); Gilbert Family Neurofibromatosis Institute (R.J.P., R.A., B.M., G.V., Y.Z.); Brain Tumor Institute (R.J.P., R.A., B.M., E.H., L.B.K., G.V.); Center for Cancer and Immunology Research (B.M., E.H., L.B.K., Y.Z.); Division of Neuroradiology (G.V.); Division of Pathology Children's National Health System (C.-Y.H.); Washington DC; Division of Pediatric Neuro-oncology, German Cancer Research Center (DKFZ) (S.P., T.D.); Department of Pediatric Hematology and Oncology, Heidelberg University Hospital (S.P., O.W.); Heidelberg, Germany; Paediatric Neuro-Oncology Program (E.B.); Division of Pathology (C.H.); Division of Haematology/Oncology (U.T.); Research Institute and The Arthur and Sonia Labatt Brain Tumour Research Centre, Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada; Department of Pediatrics (P.B.); Department of Radiation Oncology (D.H.-K.); Department of Medical Oncology (K.L.L., M.K.); Brigham and Women's Hospital, Harvard Medical School, and the Broad Institute, Dana-Farber/Boston Children's Cancer and Blood Disorders Centre, Boston, Massachusetts; Department of Pediatrics (D.B.); UT Southwestern Medical School, Dallas, Texas; Department of Pathology (D.E.); Department of Oncology (D.E., A.G.); St. Jude Children's Research Hospital, Memphis, Tennessee; Ann and Robert H. Lurie Children's Hospital of Chicago Department of Pediatric Hematology/Oncology and Stem Cell Transplantation (J.F.); Northwestern Feinberg School of Medicine, Chicago, Illinois; Children's Hospital Colorado (N.F.); University of Colorado, Aurora, Colorado; Brain Tumor Center, Brain Tumor Translational Research, UC Department of Pediatrics (M.F.); Cincinnati, Ohio; Department of Pediatrics (N.J.); McGill University, Montreal, Quebec, Canada; Pediatric Hematology-Oncology Department (A.L.); Hospital Infantil Universitario Niño Jesus, Madrid, Spain; Pediatric Unit (M.M.); Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Italy; Emma Children's Hospital AMC (S.-v.M.); Amsterdam-Zuidoost, Netherlands; Department of Neurology, Pediatrics and Neurosurgery (S.M., T.N.); University of California San Francisco, San Francisco, California; Department of Woman's and Child's Health (G.P.); University of Padua, Padua, Italy; National Cancer Institute (K.W.); Pediatric Oncology and Neuro-Oncology Branches, Bethesda, Maryland

Corresponding Author: Roger J. Packer, MD, Center for Neuroscience and Behavioral Medicine, Children's National Health System, 111 Michigan Avenue, Washington, D.C. 20010 (rpacker@cnmc.org).

For the past decade, it has been recognized that pediatric low-grade gliomas (LGGs) and glial-neuronal tumors carry distinct molecular alterations with resultant aberrant intracellular signaling in the Ras–mitogen-activated protein kinase pathway. The conclusions and recommendations of a consensus conference of how best to integrate the growing body of molecular genetic information into tumor classifications and, more importantly, for future treatment of pediatric LGGs are summarized here. There is uniform agreement that molecular characterization must be incorporated into classification and is increasingly critical for appropriate management. Molecular-targeted therapies should be integrated expeditiously, but also carefully into the management of these tumors and success measured not only by radiographic responses or stability, but also by functional outcomes. These trials need to be carried out with the caveat that the long-term impact of molecularly targeted therapy on the developing nervous system, especially with long duration treatment, is essentially unknown.

Keywords: low-grade glioma, neurofibromatosis type 1, pediatric brain tumor, pilocytic astrocytoma, RAS/MAPK pathway.

Low-grade glioma (LGG) is the single most common form of primary central nervous system tumor arising in childhood, accounting for over 30% of CNS tumors in this age group. According to the present World Health Organization (WHO) classification, various low-grade glial tumors are classified as grade I or grade II, and separation among variants can be difficult, subjective, and often arbitrary.[1,2] Classification becomes even more confounding when mixed glial-neuronal

tumors are considered. Pilocytic astrocytomas (PAs) represent the most common pediatric LGG, but even diagnosis of this entity and separation from more diffuse infiltrating gliomas can be difficult, especially when only small tissue samples are available for analysis.[2] Childhood tumors arising in the cerebellum are most common, but pediatric LGGs can occur in any region of the neuro-axis and have the proclivity to often arise in, and diffusely infiltrate, diencephalic and other midline structures.

Management of pediatric LGGs, although in part dependent on the location of the tumor, the age of the patient, and surgical resectability, remains strongly based on the histopathologic findings. The discovery, less than a decade ago, that the majority of pediatric LGGs harbor an alteration of the *BRAF* gene led to a seismic change in the conceptualization of the disease.[3,4] Subsequently, in a rapid fashion, molecular studies demonstrated that pediatric LGGs, including but not limited to PAs, as well as mixed glial-neuronal tumors such as ganglioglioma, harbor a variety of genetic abnormalities frequently causing aberrant intracellular signaling via the Ras–mitogen-activated protein kinase (MAPK) pathway.[5–10] These new understandings also have major management implications, in particular because agents are already available which can target diagnosable molecular abnormalities of an individual tumor. Thus, the relative roles of surgery and adjuvant treatments, which have been used with significant success to control and in many cases cure these tumors but may also result in associated toxicities, are being reassessed. These histologically and molecularly heterogeneous tumors are often indolent or extremely slow-growing, and decisions concerning management also must take into account that their clinical courses may be more akin to chronic diseases, highlighting the need to be increasingly diligent in assessing the long-term effects of any form of treatment, including molecularly targeted therapy.

In the fall of 2015, a group of clinical/translational and basic science researchers met to reach consensus on how to best utilize and integrate the novel biologic understandings of pediatric LGGs. The conference primarily focused on the implications of the novel molecular genetic understanding of LGGs on diagnosis, management, and prognosis. It did not attempt to draw conclusions about the relative roles of surgery, radiation or chemotherapy, other than how biologic findings could be best integrated with these conventional means of treatment to design more rational, biologic-informed therapies. Unifying concepts emanated from this consensus conference and their rationales are as summarized in this manuscript.

Molecular Characterization/Therapeutic Implications

LGGs in children comprise a heterogeneous group of tumors, but in a vast majority of cases they are seemingly driven by a single genetic hit, mostly in the MAPK pathway.[5,7,10] This is particularly true of PAs, with essentially 100% of cases showing an alteration somewhere in this axis.[6,8] First evidence for the role of this pathway in PA came from the association of

PAs (typically in the optic pathway) arising in the context of neurofibromatosis type 1 (NF1), with about 20% of NF1 patients developing a PA.[11] Additional alterations in other MAPK pathway members were then identified, culminating in the identification of *KIAA1549:BRAF* fusion genes in 70%–80% of cases.[12] Recent next-generation sequencing (NGS) studies have revealed an expanding repertoire of fusion gene partners for v-raf murine sarcoma viral oncogene homolog B1 (BRAF), with about 10 variants reported to date.[6,8,13] The common theme among these fusions is loss of the regulatory N′-terminal region of BRAF, although it remains to be seen whether there are additional modifying roles for the different 5′ partners (eg, inclusion of dimerization motifs that may enhance signaling). The *BRAF* gene is also the most common target of point mutations in LGG, with the vast majority being the typical V600E hotspot alteration, which is an extremely good target for small molecule inhibition.[14] The frequency of this change varies notably depending on histology, with ganglioglioma and pleomorphic xanthoastrocytoma showing a particularly high incidence, as well as by location (supratentorial PAs harboring this change more often than cerebellar cases).[5,15–18]

Besides *BRAF*, additional fusion genes involving upstream receptor tyrosine kinases have recently been identified in LGG. Both *NTRK2* (TrkB) and *NTRK3* (TrkC), for example, have been found to be rearranged in various LGG histological subtypes, with all fusions retaining the kinase domain.[6,8,9] Interestingly, related neurotrophic tyrosine kinase (NTRK)–family fusions were observed in pediatric high-grade glioma, particularly in infants,[19] suggesting potential overlap between high-grade glioma and LGG genetics in some instances.

A small number of *FGFR1:TACC1* and *FGFR3:TACC3* fusions, as reported in approximately 3% of adult glioblastoma,[20] have also been observed. These were not as common, however, as mutations of *FGFR1*, which are now known to be the second most common point mutations in LGGs after BRAF V600E.[6,8] These are hotspot alterations, usually affecting p.N546 or p.K656 according to NM_023110. An additional novel mechanism of fibroblast growth factor receptor 1 (FGFR1) activation was frequently observed in cerebral gliomas with dysembryoplastic neuroepithelial tumors (DNET) or oligodendroglial-like histology and in a handful of PAs,[6,8,9] whereby the whole kinase domain is duplicated (internal tandem duplication [ITD] or tyrosine kinase domain duplication [TKD]). Strikingly, germline mutation of *FGFR1* has also recently been described in DNET in addition to the common somatic *FGFR1* alterations seen in association with this histology.[21] These diverse *FGFR1* alterations highlight its growing importance as a previously unrecognized driver in LGG, and an important target for consideration in terms of novel treatment strategies.[22]

Interestingly, these various MAPK pathway alterations are not uniformly distributed across anatomic sites. *BRAF* fusions are extremely common in the cerebellum, but somewhat less so in supratentorial locations. Conversely, BRAF V600E and *NTRK*-family fusions are more common in hemispheric tumors, while *FGFR1* alterations are typically found in midline tumors.[6,8,9] Location-specific differences can also be observed in methylome and transcriptome profiles of these tumors,[23,24]

raising important questions about the interplay among cellular origins, tumor location, and susceptibility to particular oncogenic insults.

Outside of the canonical MAPK pathway, and enriched in non-PA low-grade neuroepithelial tumors (LGNTs), are alterations in the *MYB* and *MYBL1* oncogenes. Following an initial report a few years ago,[25] these alterations were confirmed both in a Pediatric Cancer Genome Project (PCGP) sequencing study[8] and in an independent analysis focusing on structural alterations in diffuse astrocytoma grade II (DA) and angiocentric glioma (AG).[26] This has also recently been confirmed in larger series of LGNTs, in which the vast majority of histological AGs as well as a proportion of diffuse astrocytoma grade II showed alterations in *MYB*.[9,27] In contrast to the typical kinase fusions, MYB and MYBL1 rearrangements result in loss of their C′-terminal portion (encoding a negative regulatory domain) but retention of the transactivating N′-terminus. Interestingly, the most commonly identified variant (*MYB:QKI*) seems to owe part of its transforming capacity to altered function of the partner gene (*QKI*; quaking homolog, KH domain RNA binding) in addition to aberrant MYB activity. *MYB* appears to "hijack" an enhancer element within *QKI* to drive expression of the fusion, while hemizygous loss of the tumor suppressive functions of QKI further facilitates oncogenesis.[27]

Isocitrate dehydrogenase (IDH) mutations may also be seen primarily in diffuse LGGs in adolescents and young adults and rarely, if ever, in PAs (8). In adulthood, the molecular spectrum of LGGs is markedly different from what is encountered in pediatrics, with IDH1/2-mutant astrocytic or oligodendroglial tumors being relatively common (1).

Substantial new insights into the molecular underpinnings of LGG have thus been gained in the "NGS-era," but greater concerted effort will be required to further delineate rare (but molecularly distinct) entities within the bulk of "LGG." It is clear that purely histological groupings fail to capture the substantial heterogeneity of an increasing number of molecular subclasses within LGG, many of which are only now starting to be identified. It does appear, however, that certain histologies are enriched for given molecular alterations (eg, *FGFR1* in DNET, *MYB* in AG, *BRAF* in PA and ganglioglioma). Further elucidating these relationships will be crucial for achieving a new standard of an integrated histopathologic-molecular diagnosis, as agreed upon in the Haarlem criteria of the WHO CNS tumors in 2014.[28] The revised WHO classification of childhood LGGs relies on histological criteria for classification and does not include molecular findings.[1] Recent work supports the inclusion of BRAF V600E mutation and *BRAF* fusion status in the classification of all non- NF1 LGGs. Sequential or concurrent evaluation for other molecular abnormalities, using next-generation diagnostic tools such as DNA methylation profiling and customized gene-panel and/or RNA sequencing, has been increasingly employed in *BRAF*-negative cases. Prospectively, evaluation for these molecular abnormalities will not only lead to increased diagnostic accuracy, but also identify patients potentially suitable for targeted therapies. Evaluation further allows for the discovery of novel molecular entities that are not identifiable by a distinct morphological pattern in the past. Detailed molecular analysis is rapidly becoming a crucial prerequisite for patients entering targeted therapy trials. Without such information it is impossible to retrospectively identify causes for successes or failures and to optimize treatment stratification. At present, however, there remains incomplete knowledge about the diagnostic, prognostic, or predictive value of most molecular genetic variables. There also remains variability on how characterization is performed across sites. As one example, determination of *BRAF* fusions by interphase fluorescence in situ hybridization is technically challenging, since the tumor cell content is variable and not all tumor cells harbor the fusion. On the other hand, some more advanced methods currently lack certification for use in clinical laboratories, and thus have to be developed further before they can become standard diagnostic tests.

Conclusions and Recommendations:

- Molecular analysis should be incorporated into histologically based tiered classification schema for all LGGs and glial-neuronal tumors: such analysis should at least include the determination of BRAF V600E mutation and *BRAF* fusion status.
- All presumed LGGs and other forms of low-grade glial-neuronal tumors, except those in children with NF1, should be resected or biopsied before adjuvant therapy is begun, biopsy for the area demonstrating growth should also be strongly considered at time of progression or relapse. All decisions concerning biopsy or resection at the time of diagnosis and progression must be carefully made weighing the potential risk of surgery versus the therapeutic benefit of elucidating the molecular subtype of the tumor.
- Standardization of determination of molecular genetic alterations are required to establish common standards, and strong considerations should be made for development of consortia of coordinated central reference laboratories, thereby improving quality and reliability of data and allowing pooling of available information on well-characterized patients.

Translational/Clinical Trials: Completed and Planned Trials

Over the past 2 decades, prospective therapeutic clinical trials primarily evaluating chemotherapy for children with newly diagnosed and recurrent/recalcitrant LGGs have been performed with variable results. The Children's Oncology Group (COG) trial A9952, a prospective, randomized clinical trial of chemotherapy for children less than 10 years old with newly diagnosed LGGs, was performed between 1997 and 2005.[29] Enrolled patients without NF1 were randomized to receive carboplatin and vincristine (CV, $n = 137$) or thioguanine, procarbazine, CCNU (lomustine), and vincristine (TPCV, $n = 137$). The 5-year event-free survival rates were 39% ± 4% for patients randomized to CV and 52% ± 5% for TPCV; this difference did not achieve statistical significance ($P = .10$). Factors which connoted poorer event-free survival and overall survival included younger age at diagnosis, tumor size of >3 cm^2, and primary thalamic involvement. Subsequently, the COG completed ACNS0223, examining the addition of temozolomide to carboplatin and vincristine in 65 eligible subjects less than 10 years of age with newly diagnosed LGGs.[30] The 5-year event-free

survival was 46% (95% CI: 33%–58%) and the 5-year survival was 87% (95% CI: 75%–93%).

The SIOP European Brain Tumor Committee coordinated the "Cooperative Multicenter Study for Children and Adolescents with Low Grade Glioma SIOP - LGG 2004." This study, a randomized first-line chemotherapy strategy for non-NF1 patients with progressive/symptomatic, unresectable tumors, investigated the role of induction intensification.[31] Standard vincristine-monthly carboplatin was compared with an intensified regimen which included etoposide in 497 patients. There was no statistically significant difference in radiologic response at 24 weeks.

Other agents have been studied as potential alternatives to carboplatin-based regimens. The hope associated with early results of single agent temozolomide in adult gliomas has not been confirmed in pediatric LGG studies—in a phase II study conducted by the COG, only one partial response was seen in 21 LGG patients.[32] Vinblastine at 6 mg/m^2 was used in 51 patients with recurrent tumors, demonstrating a response rate of 33% and with 75% of patients completing one year of treatment.[33] Therapy was well tolerated and the 5-year progression-free survival (PFS) was 42.3% ± 7.2%. A subsequent vinblastine trial, which enrolled 54 radiation and chemotherapy naive patients, demonstrated a 5-year PFS of 53.2% (95% CI: 41.3–68.5%) and a disease stabilization rate of 87%.[34]

The North American Pediatric Brain Tumor Consortium (PBTC) has conducted several trials focusing on LGG patients. The PBTC-018 trial, a phase I trial of CC-5013 (lenalidomide), demonstrated a 12-month PFS of 67±13%.[35] This trial provided the rationale for the ongoing COG phase II study evaluating and comparing the efficacy of lenalidomide in children with recurrent, refractory, or progressive LGGs at 2 different dose levels (clinicaltrials.gov: NCT01553149). A second trial (PBTC-022) evaluated the combination of bevacizumab and irinotecan in 35 evaluable children and showed a 6-month and 2-year PFS of 85.4% and 47.8%, respectively. There were 2 patients (5.7%) with sustained partial responses; however, over 80% of patients who had previously failed standard therapies had stable disease.[36] The toxicity of the combination was thought tolerable, and many clinicians have added this combination to the armamentarium of therapeutic options in children with multiply recurrent LGG.[37] There have also been some limited experiences suggesting that bevacizumab alone may slow or halt acute vision loss in children with progressive optic pathway glioma and visual deterioration.[37,38]

A glaring deficit is the lack of functional outcome data in the vast majority of these previous trials. As studies with bevacizumab have shown, clinical improvement can be seen after treatment with biologic agents, and such improvements must be prospectively captured to assess the true value of the agent being tested. Trials performed with chemotherapy in newly diagnosed children have, to date, been long in duration and have not incorporated biologic information or biomarker discovery. The reasons for success or failure on clinical trials are therefore often unclear.

The currently planned SIOP-E trial LOGGIC (Low Grade Glioma in Children) is a phase III randomized trial for non-NF1 patients, comparing different vinca alkaloids and different lengths of treatment. In addition, the trial will introduce targeted treatment in one arm, directed at MAPK activation, with the precise compound to be decided as soon as relevant phase I/II data are available. In this trial, the standard of care treatment arm will remain carboplatin plus vincristine, and a second treatment arm will contain vinblastine single agent chemotherapy. Collection of fresh frozen tumor tissue for molecular characterization will be mandatory. In addition to tumor size and PFS as measures, the trial utilizes visual function and adaptive behavior as primary endpoints.

Conclusions and Recommendations:

- Outcome of children with LGGs with chemotherapy remains far from ideal and its utilization should be compared with outcomes in children treated with molecularly targeted therapies, in addition to "standard" chemotherapy or in isolation, in prospective trials.
- Clinical trials for children with LGGs or glial-neuronal tumors should include functional endpoints.
- Clinical trials should be developed with not only strong radiographic and clinical end points, but with secondary aims including determination of predictive biomarkers.

Transition to Molecularly Targeted Trials in Children

Pediatric LGGs are excellent candidates for personalized approaches, although precision medicine has yet to definitively impact LGG therapy. Targeted therapeutics are available for the involved pathways; however, follow-up biological studies for each targetable alteration remain sparse and clinical trial designs addressing issues of resistance are lacking.

Studies have just been completed or are currently assessing inhibitors of the mammalian target of rapamycin (mTOR) and BRAF pathways as single agents in recurrent LGGs. Twenty-three patients with recurrent LGGs were treated with everolimus after failing a prior carboplatin-containing regimen, resulting in 4 with partial responses, 13 with stable disease, and 6 with progressive disease without significant toxicity.[39] Because this trial did not require tissue acquisition, a subsequent study is ongoing determining whether phospho-S6, a marker of phosphatidylinositol-3 kinase (PI3K) pathway activation, is a suitable biomarker for therapeutic response (NCT01734512).

AZD6244 (selumetinib) is a potent, selective, orally bioavailable and non-ATP competitive small molecule inhibitor of mitogen/extracellular signal-regulated kinase (MEK)1/2. The PBTC is presently completing the PBTC-029 trial, which included a phase I, a phase II, and a retreatment study of AZD6244 for recurrent or refractory pediatric LGGs.[40] In the completed phase I study, 12 of 19 evaluated tumors had *BRAF* abnormalities, but *BRAF* abnormalities were not prospectively required for enrollment onto the phase I portion of the trial. Patients received a median of 13 courses and 14/25 (56%) completed all protocol treatment with at least stable disease. Objective radiographic responses were seen. The most common toxicities observed were rash and mucositis.[40] The phase II trial is nearing completion and has enrolled more than 100 children in various strata. A unique portion of the phase II AZD6244 study is the allowance of retreatment of patients

with LGGs, previously treated on PBTC-029 phase I or II study, who experienced a response or prolonged stable disease of at least 12 months while on therapy and subsequently relapsed after completing therapy.

First-generation BRAF inhibitors, such as dabrafenib and vemurafenib, which have shown excellent results in melanoma patients with BRAF V600E mutations,[41] have also been studied in children with LGGs. Dabrafenib has shown an encouraging response rate in a multicenter phase I study, as 8 partial responses and 6 stable disease were seen in 15 children with BRAF V600E mutations and recurrent LGGs.[14] Conversely, one early generation BRAF inhibitor, sorafenib, demonstrated no efficacy, and in fact accelerated tumor growth, in the setting of the *KIAA1549:BRAF* fusion or *NF1* loss.[42]

For the less frequent IDH1/2 tumors, molecularly targeted therapy has been essentially exclusively studied in adults. IDH mutations are possible therapeutic targets, and selective inhibitors are being developed, as are mutation-specific peptide-based vaccines.[43,44]

Immunotherapeutic approaches for pediatric CNS tumors are rapidly evolving. LGGs have a stable genome and a low mutation rate, thus curtailing somewhat the enthusiasm for investigation with programmed cell death protein 1 (PD-1) and PD-ligand 1 agents. Nevertheless, the slow growth rate may render patients with LGGs particularly amenable to immunotherapy. A significant immune response against LGGs, including increased CD8+ T-cell responses, has been demonstrated,[45] and a vaccine trial for LGG is currently open (NCT02358187). Understanding of appropriate immunocorrelates in pediatric LGGs continues to evolve, and immunotherapeutic trials are expected to assume a more prominent role in clinical investigations going forward.

Conclusions and Recommendations:

- Molecularly based therapy is promising and should be considered for newly diagnosed patients with LGGs, where tumors have been molecularly characterized and have rigorous indications for treatment; these should only be undertaken as part of institutional review board approved trials.

Molecular Mechanisms, Resistance, and Transformation

The median number of somatic sequence alterations in whole-genome analysis of LGGs has consistently been one.[6,8] This suggests that the defining MAPK pathway alterations directly mediate critical cellular responses and subsequent abnormal growth and oncogenesis, although exactly how this is achieved in these tumors is currently unclear. While many of the patients on early MAPK pathway inhibitor trials, as well as single cases treated on an individual basis, are deriving clinical benefit,[46] some patients are nonresponsive or progress early on in therapy, consistent with innate resistance. Oncogene-induced senescence as a result of MAPK activation may be one reason for the slow growth and relative resistance to traditional chemotherapies in LGG,[47,48] but the role that this plays in determining response to targeted therapy is less clear. In addition,

some patients have exhibited recurrence after a period of response or stability consistent with acquired resistance. In responsive *BRAF* mutant melanoma, acquired resistance is a common phenomenon and emerges through diverse alternative routes for MAPK pathway reactivation including receptor tyrosine kinase amplification, *CRAF/RAS/NF1/MEK1* mutation, insulin-like growth factor receptor (IGFR)1/PI3K activity, and selection for a drug-resistant BRAF splice variant.[49] Second site mutations in *BRAF* have not been a common mechanism in melanoma. Innate resistance might be due to driver mutations outside of the MAPK pathway or secondary cooperating mutations, such as *PTEN* promoter methylation, in addition to a MAPK pathway alteration, thus rendering the tumors insensitive to monotherapy with MAPK pathway blockade.[50]

As the spectrum of alterations in LGG has expanded to include upstream MAPK alterations, such as in *FGFR1* and *NTRK2*, attention has focused also on other pathways which might be activated in parallel, such as the signal transducer and activator of transcription and PI3K pathways.[22] This suggests that inhibitors of receptor tyrosine kinases in themselves might show efficacy in this setting, and rational combination therapy may be more effective.[51] While there are only anecdotal reports of acquired resistance in pediatric LGGs, experience with other targeted agents suggests resistance will likely develop. Levy et al reported on a child with BRAF V600E mutant LGG who initially responded to a combination of vemurafenib plus vinblastine, and progressed 9 months later. At time of progression the child's tumor had developed resistance through upregulation of autophagy, a known cellular response to stress.[52] Through elegant preclinical studies, the authors showed that inhibiting autophagy with chloroquine resensitized the tumor to vemurafenib and the patient experienced a second clinical response.

Although preclinical models of LGGs are largely lacking, several models of BRAF V600E mutant high-grade glioma exist. Yao et al showed that while these tumors are initially sensitive to vemurafenib in vitro and in vivo, they developed resistance through activation of the epidermal growth factor receptor (EGFR).[53] EGFR is normally repressed through negative feedback loops downstream of MEK in LGG, but BRAF or MEK inhibition decreases this negative feedback, thus allowing the receptor tyrosine kinases to signal and activate parallel mitogenic pathways. Combination of an EGFR and BRAF inhibitor increased antitumor control in this model.

The *MYB* gene controls a large number of downstream genes, and the mechanism by which oncogenesis occurs in these subsets of LGGs is as yet uncertain, although the recent identification of a tripartite mechanism of action for *MYB:QKI* in angiocentric gliomas provides important insights.[27] Some MYB inhibitors have been evaluated, primarily in studies of hematological malignancies, but such inhibitors have not shown to be highly specific and are likely still far from clinical trials.[54] *MYB* oncogenes in LGGs have been suggested to activate the MAPK pathway, although this is not yet fully established. This may indicate, however, that the same combinations of MEK inhibitors used in *BRAF* mutant LGGs may later be promising, in combination with agents that more generally inhibit or alter transcription factor function (bromodomain and extraterminal domain family, histone deacetylase), for potential treatment of *MYB*-altered LGG.

Whether *BRAF* alterations and *FGFR1* mutations impart inherent resistance to standard chemotherapy and radiotherapy still needs to be determined.[55] Deletion or silencing of the *CDKN2A/B* locus may allow pediatric LGGs to bypass the oncogene-induced senescence seen in response to activation of the MAPK/extracellular signal-regulated kinase pathway,[45,46] and this may become an important marker in defining tumors with a greater propensity to become resistant to therapy. Stemlike cells have been identified in LGGs of childhood and may play a role in the remitting and relapsing course seen in many of these tumors.[56] Moreover, unlike in most tumors, relapse after a standard chemotherapy does not necessarily mean the tumor is resistant, as it may respond again to the same therapy.[57] While frequent recurrences are common in pediatric LGGs, the majority of patients are long-term survivors, and many patients appear to develop tumor quiescence once they reach their early twenties.[58,59]

Some studies have indicated that the abnormal fusion protein KIAA1549:BRAF regulates LGG cell growth in an mTOR-dependent manner.[60,61] Preclinical studies have demonstrated that combination therapies with the specific BRAF V600E inhibitor PLX4720, mTOR inhibitor everolimus, or MEK inhibitor AZD6244 are superior to single agent therapy for gliomas carrying the BRAF V600E mutation or wild-type *BRAF*, while the KIAA1549:BRAF fusion protein rendered cells highly sensitive to MEKi and thus combinations were only marginally more effective (Olow A, Mueller S, Haas-Kogan D, personal communication).

BRAF/MEK combinations are also likely to be effective in BRAF V600E mutant LGG such as ganglioglioma or pleomorphic xanthoastrocytoma based on preclinical data in *BRAF* engineered models. Strategies for overcoming *BRAF* mutant tumors with initial resistance to inhibitors have been proposed. The use of pan-RAF inhibitors in combination with MEK inhibitors (RAF/MEK) has shown promise particularly in BRAF V600E mutant colon cancer cell lines.[62] This may have greater synergy than that seen in standard BRAF/MEK combinations and may be an alternative choice to evaluate in unresponsive LGGs. Other combinations under evaluation have been BRAF plus IGFR1 inhibitors or BRAF plus immune checkpoint blockade, although the latter approach may be more effective in tumors with complex genomic alterations and may not be as useful for LGGs.[63] Combination therapy using conventional chemoradiation and targeted agents is yet another alternative approach to potentially overcoming resistance.

Still another issue which requires increased investigation is the incidence of malignant transformation of pediatric LGGs and mixed neuronal-glial tumors into higher-grade lesions. In a recent population-based study, 2.9% of pediatric LGGs transformed into higher-grade gliomas.[64] V600E-mutated diffuse LGGs, pleomorphic xanthoastrocytoma, and possibly glioneuronal tumors have a higher tendency for transformation, especially when they harbor concomitant cyclin-dependent kinase inhibitor 2A/B deletions.[16–19,64] In contradistinction, pediatric LGGs with KIAA1549:BRAF fusions rarely, if ever, mutate to higher-grade lesions. Significant issues remain whether all reported secondary pediatric LGGs are true transformations or are higher-grade lesions incorrectly diagnosed as LGGs because of sampling. However, common alterations found in adult LGGs which transformed, such as alpha thalassemia/mental retardation syndrome X-linked and IDH1, were not found in one pediatric series.[64] In the same series, pediatric LGGs with V600E mutations which transformed had longer latencies and occurred in older patients than those with secondary high-grade gliomas[64] without V600E mutations.[64]

One of the possible therapeutic implications of V600E mutated LGGs is whether aggressive therapy is indicated at the time of diagnosis, such as extensive resections because of the tendency of V600E LGGs to mutate to higher-grade lesions. Similarly, since it has been shown that V600E mutated tumors, including higher-grade lesions, can respond, at least transiently, to V600E inhibitors, the role of up-front molecularly targeted intervention requires investigation.[65]

Conclusions and Recommendations:

- Molecular mechanisms initiating and promoting growth of LGGs and low-grade glial-neuronal tumors need to be better understood, particularly mechanisms of tumor resistance to targeted therapy.
- Molecular targets other than BRAF should be explored in children with LGG or glial-neuronal tumors, especially in tumors which are resistant to molecularly targeted therapy or develop resistance after initial successful treatment.
- There is a need to obtain experience with molecularly targeted combination therapies which could include the use of the molecularly targeted agents with other molecularly targeted therapies, conventional chemotherapy or radiation therapy, as part of phase I and II studies.

Neurofibromatosis Type 1

NF1, caused by a germline heterozygous mutation of the *NF1* gene located on chromosome 17q,[66,67] is associated with the development of various forms of cancer.[68] Within the central nervous system, LGGs, primarily PAs, make up the vast majority of NF1-related intracranial neoplasms.[69] Due to the characteristic neuroradiographic findings of NF1-associated LGGs, individuals are most commonly diagnosed on the basis of MRI findings, and histological confirmation is infrequently obtained. Such neuroradiographic diagnoses are problematic on various levels, including: the increased use of screening techniques which identify "gliomas" in asymptomatic patients on the basis of MRI findings (many of these patients will never develop symptomatic lesions, suggesting they might not be true gliomas); the difficulty in separating gliomas from NF1 dysplastic or hamartomatous tissue; and the lack of tissue which can be used for biologic investigations.[70]

It has been recognized for decades that the majority of NF1-associated LGGs arise in the visual pathway.[69] Visual pathway NF1-associated LGGs will usually be diagnosed within the first three to four years of life, and almost never in patients older than 10 years. It is unclear whether these lesions are congenital tumors or arise later in development. The major morbidity associated with these LGGs is visual loss.[71] However, the mechanism of visual loss in children with NF1-associated gliomas is not well delineated and has not been clearly related to tumor size or extent of visual pathway involvement.

Visual pathway gliomas are not the only type of LGG that arise in children and adults with NF1. Other midline structures, including the brainstem, can harbor LGGs; brainstem lesions are usually diagnosed somewhat later in childhood.[69,72] Tumors of the corpus callosum and cerebral hemispheres also tend to be recognized later in life, often in the teenage years.

Many children with NF1-associated gliomas do not require treatment, because they are asymptomatic or have static deficits; even slowly growing lesions often do not need to be treated, because after a specific time window (commonly up to age 5 or 6) these tumors may spontaneously arrest and even regress.[73,74] For those tumors that require treatment, they may only need treatment for a finite period of time. Because of the location of these NF1 visual pathway gliomas, surgery is not a therapeutic option. Due to concerns of mutagenesis and vascular injury, radiation is also not utilized and the majority of patients are treated with chemotherapy. A variety of different chemotherapeutic agents have been employed, the most common of which has been the combination of carboplatin and vincristine.[75,76] Recent international collaborative trials have demonstrated the ability of carboplatin and vincristine to control tumor growth. Of 108 children with NF1 LGGs in the SIOP trial, 43 were initially observed and 55 treated with chemotherapy.[31] Many of the initially observed children went on to receive treatment. Those NF1 children who received treatment had a significantly better 10-year event-free survival than those without NF1 (50% vs 24%) and the 5-year PFS was 73%; overall survival was 96% at 12 years. The study demonstrated that location outside the visual pathway was associated with poorer survival. Similar results have been reported by the COG in 131 children with NF1 LGGs[75]; the 5-year event-free survival for children with NF1-related gliomas was nearly 70% compared with 39% of those without NF1 (the 10-year overall survival was 98%). In addition, patients with NF1 had a better objective rate of radiographic response than those without NF1. In the COG study, 3 children with NF1 developed second malignant neoplasms; all had relapsed and had received temozolomide as a salvage agent before the development of the second malignant neoplasm, highlighting the potential risk of the use of alkylator therapy in this patient population. However, the association between temozolomide and development of secondary tumors remains speculative because of the small numbers of patients affected and the possibility of spontaneous transformations into higher-grade gliomas. Although disease control as measured by radiographic response seems quite good in this patient population and only 25% of patients require other forms of therapy within 5 years, visual outcome was not carefully followed. In one retrospective review, over a quarter of children with NF1 lost vision despite stable radiographic studies.[71]

This experience with chemotherapy must be taken into account when new biologic agents are incorporated into therapy for children with NF1 and LGGs, and it may be difficult to show that the biologic agents are better in controlling newly diagnosed radiographically defined tumor growth. The primary benefit of novel therapies may be in improving functional outcome. The use of bevacizumab and irinotecan has demonstrated an excellent ability, in a small number of patients, to control disease in patients who have failed multiple different chemotherapeutic regimens.[36,37] Probably most important in the bevacizumab experience is the observation that some of these children have had restoration of prolonged neurologic and/or ophthalmologic dysfunction after treatment.[37,38]

The neurobiology of NF1-related gliomas is only partially understood and has been greatly elucidated by the use of mouse modeling, as human glioma tissue is usually unavailable for analysis.[77] It has been shown that bi-allelic inactivation of the NF1 gene occurs in NF1-associated LGGs, accompanied by increased Ras-MAPK signaling.[78,79] Accordingly, genetically engineered mouse models (GEM) using conditional bi-allelic inactivation of NF1 in the brain have been developed.[80,81] While hyperactive Ras signaling has been clearly shown in non-NF1 pilocytic LGGs, GEM-based research suggested that mTOR hyperactivation was also a major component of glioma growth, and mTOR inhibitors including rapamycin and RAD001 have been utilized in patients with NF1 and LGGs.[82,83] The results of the RAD001 studies are still pending, but rapamycin coupled with tarceva, an EGFR inhibitor, did show some degree of activity in patients with NF1 gliomas, as 6 of 9 patients had prolonged (greater than a year) disease control, with one patient's tumor demonstrating a partial response.[83] The use of MEK inhibitors is actively being explored in children with NF1 associated LGGs, and early results are more encouraging, as tumor shrinkage has been seen.[40]

Mirroring the human experience in LGGs, especially of the optic nerves and other regions of the visual pathway, GEM studies have demonstrated that tumors only arise in specific developmental windows.[84] Three recent studies showed that short-term preventative treatment with MEK inhibitors during neonatal stages improves glial defects in the corpus callosum and cerebellum, providing long-term benefits on motor functions.[85–87] Thus, MEK inhibitor treatment within these specific early developmental windows may be more successful in controlling and even preventing the development of such tumors, and abnormal brain development.

Clearly bi-allelic loss is needed in the astrocytic component; however, the tumor microenvironment is critical. In one mouse model, NF1 loss in the astroglial cell precursors alone was insufficient for optic glioma formation and NF1 heterozygosity in associated microglia and possibly other cell types in the tumor microenvironment were needed.[88] The loss of NF1 results in other biologic changes, including the production by microglia of various cytokines and chemokines.[89] Loss of retinal ganglion cells has been noted, and it has been postulated that this loss results from reduced levels of cyclic adenosine monophosphate (cAMP) and that pharmacological elevation of cAMP levels could reduce apoptosis.[90,91] Thus, understanding the interaction between NF1-null tumor cells and surrounding heterozygous cells would provide important insights into the mechanism underlying tumor growth, retinal ganglion cell loss, and visual impairment.

Conclusions and Recommendations:

- LGGs in children with NF1 are a distinct subset of LGGs and require different considerations concerning the need for surgery and other forms of treatment.

- Although PFS and overall survival are better for children with NF1-related gliomas, compared with those LGG patients without NF1, visual outcome is often suboptimal.
- NF1 patients are excellent candidates for treatment with molecularly targeted therapy, and functional outcome measures should be incorporated.

Evaluation of Toxicity and Response

Despite the excitement that surrounds targeted therapy, these agents have limited experience in children and no long-term safety profile—critical for children with LGGs. Their potential long-term impact on growth and development must be balanced against the need to make them available to patients expeditiously. Current primary endpoints in clinical trials that assess efficacy of agents for children with brain tumors are radiographic endpoints, based upon criteria defined more than 2 decades ago for adults with supratentorial malignant glioma.[92] While these criteria were applicable to solid enhancing lesions in adults receiving cytotoxic agents, where reduction in tumor size correlated with symptom improvement, there are a number of issues with the application of these criteria to children with non-enhancing, heterogeneous tumors, and those receiving cytostatic, anti-angiogenic or molecularly targeted therapies.[93] As noted in a recent report of the Response Assessment in Neuro-Oncology group in the assessment of outcome trials of diffuse LGGs, there are issues with even standard outcome measures, such as: overall survival due to the effects of salvage therapy at recurrence; event-free or PFS due to difficulties in radiographic interpretation, because of the infiltrating nature of the LGGs and treatment-related white matter changes occurring after radiotherapy; and response with varying criteria used and no uniform agreement on the significance of "minor response" or stable disease.[94] These issues also pertain to assessment of pediatric LGGs; however, there are differences as PAs, which predominate in pediatrics are more radiographically delineated and where there is less use of radiotherapy. On the other hand, pediatric LGGs have longer overall survival rates, allowing a greater impact for multiple therapies to prolong survival and may have more erratic natural histories, making stable disease a possibly less useful marker of efficacy.

Despite the noted limitations, radiographic response assessment remains important for children with LGGs and has been based on fluid attenuated inversion recovery (FLAIR), T2 or postcontrast T1-weighted images. There is consensus that response assessment must take into account FLAIR or T2 images and not be solely based on postcontrast T1 images, since: many tumors contain non-enhancing components (or, in some cases, the tumor may not enhance at all); enhancement characteristics of LGG can vary from one scan to the next even if there is no intervening therapy and without change in tumor size based on FLAIR/T2 extent; and enhancement can be biologically modified without matching change in tumor size—for example, a decrease in degree of tumor enhancement is commonly observed with anti-angiogenic therapies as a reflection of change in tumor permeability.[95]

A special consideration must be given for patients who have long-standing LGGs that contain areas that are not radiographically progressive (eg, their tumors have shown no change in size for many years), and who present with new progressive enlargement of a portion of the overall tumor or new extension beyond the original tumor distribution (while the remainder is radiographically stable). This often occurs in large/infiltrative lesions. If such a patient is given treatment for the newly growing aspect of the tumor, then response assessment should more rationally be based on the progressive aspect, not on the size of the entire tumor.

Advanced MRI techniques such as diffusion tensor imaging also hold promise to improve radiographic assessment, as fractional anisotropy changes in optic radiations has already been shown to correlate with visual acuity loss in children with visual pathway gliomas.[96] These and other potential biomarkers need to be incorporated into clinical trials and prospectively tested and validated.

Adult brain tumor trials have begun to incorporate clinical benefit as an endpoint, utilizing several components to determine a composite net clinical benefit; these include radiographic changes as well as changes in symptoms, cognition, and quality of life.[97] Translating these to the pediatric population represents methodological challenges, as validated, standardized measures of quality of life and symptom burden have yet to be developed or routinely utilized in this population. Self-report of symptoms is difficult in some children, and questions arise as to the correlation of proxy and patient reports.

Children with visual pathway involvement present unique challenges concerning reproducible measurements of functional outcome. Standardized visual assessments for children with visual pathway gliomas enrolled in clinical trials have been proposed.[98] Visual acuity testing methods designed for clinical trials report the results as a continuous variable which is preferable for longitudinal studies. Visual fields, defined as the extent of vision, have not been recommended as a formal outcome in visual pathway glioma clinical trials. Quantitative and reliable visual field assessments are infrequently obtained in children younger than 8 years.[98] Visual field loss is a frequent complication of LGGs, however, and more research is needed to determine algorithms that appropriately measure longitudinal change.

Other challenges remain for including functional visual outcomes (ie, visual acuity and visual fields) in clinical trials. Since visual pathway gliomas present at all ages, multiple different age-specific testing methods would need to be included in the clinical trial in order to capture this outcome in all patients. Results from different testing methods are not always comparable. To complicate matters further, the quality of the vision testing results rely heavily on patient effort, cognitive ability, and cooperation.

In children with visual pathway gliomas confined to the anterior visual pathway, optical coherence tomography (OCT) is under active study to detect early clinical progression or impending visual dysfunction, as well as confirm clinical stability.[99] OCT is a safe, noncontact ophthalmologic imaging device that uses a near infrared light source, similar to ultrasound, to produce a quantitative image of the retinal layers. OCT measures acquired over time demonstrate good reproducibility and are well suited for clinical trials.[100] Early results with OCT confirmed that a significant decline in retinal nerve fiber layer (RNFL) thickness occurs at the time of symptomatic vision loss,

and in some cases, it precedes vision loss. Also, visual pathway glioma patients who did not experience vision loss over time demonstrated stable RNFL thickness measures, even when MRI findings demonstrated tumor progression.

Conclusions and Recommendations:

- Both the short-term and long-term toxicities of molecularly targeted therapies for children with LGGs are incompletely understood and their assessment must be part of clinical trials.
- Radiographic evaluations remain critical components of evaluation, and standardization of techniques and assessment are needed.
- Functional outcome measure must be included in clinical trials and in certain circumstances should be primary outcome measures.
- For those with visual pathway involvement, present means of functional outcome are lacking and novel means of assessment should be explored and incorporated into trial design.

Summary

It is clear that great strides have been made in recent years in the understanding of childhood LGGs, and these new conceptualizations require careful integration into both classification and clinical management. Molecular characterization for the vast majority of pediatric LGGs, including low-grade glial-neuronal tumors, is a prerequisite for the appropriate use of molecular targeted therapy. As these tumors are increasingly studied, the complexity of aberrant molecular signaling becomes more evident. It is unlikely that these different genetic changes will respond to therapy in a similar fashion. Mechanisms of LGG development, resistance, and growth kinetics are just being explored. The early results of molecular targeted therapy are extremely encouraging but raise significant issues in how pediatric LGGs should be diagnosed and treated. Clinical trials utilizing molecular targeted therapy must become smarter, more focused on functional outcome and designed to not only assess radiographic and clinical improvement, but also carefully monitor the long-term toxicity of these new agents which may affect pathways critical in brain development.

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