Final 2/22/2017

Pediatric Low-Grade Gliomas: Next Biologically Driven Steps

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Acknowledgements: We would like to acknowledge the support of the A Kid's Brain Tumor Cure- PGLA Foundation and the Gilbert Family Neurofibromatosis Institute

Abstract

Despite the fact that they are not typically life-threatening, low-grade gliomas remain a significant clinical challenge in pediatric neurooncology due to co-morbidities associated with the tumor and/or treatment, and their propensity for multiple recurrences. This latter behavior means that this group of diseases, the most common brain tumors in childhood, can often become a chronic problem requiring decades of management. We present here the outcome of a 2nd international consensus conference on pediatric low-grade gliomas held in Padua, Italy in 2016, aimed at advancing the pace of progress with translating biological discovery into meaningful clinical benefit. Topics discussed included: the implications of our growing biological understanding of the genomics underlying these tumors; how to address the paucity of model systems available; what can be done to highlight the histopathologic differences with adult diffuse gliomas, and how best to move forward with bringing targeted therapy into late-stage clinical trials and newly diagnosed patients. Methods for the diagnostic assessment of alterations in the Ras/MAPK pathway, typical for these tumors, were also considered. While the overall tone was positive, with a consensus that progress is being and will continue to be made, the scale of the challenge presented by this complex group of tumors was also acknowledged. The conclusions and recommendations of the meeting panel are provided here as an outline of current thinking, and a basis for further discussion.

Keywords: low-grade glioma; pediatric brain tumor; neurooncology; molecular diagnostics; targeted therapy; MAPK pathway

Introduction

Pediatric low-grade gliomas and glioneuronal tumors (LGGs), defined as WHO grade I or II lesions of the central nervous system (CNS), are an extremely diverse group of tumors[1]. Together they comprise approximately one-third of all brain tumor diagnoses in children, making them the most common CNS neoplasia in the pediatric setting[2]. While a great deal has been learned over the past decade about some of the more common entities such as pilocytic astrocytoma, the complex spectrum of pediatric LGG is just beginning to be truly understood. There is no doubt that new knowledge is opening up significant opportunities.

Despite this clear potential, a number of challenges in how best to convert this growing biological understanding into improved clinical care exist. For example, how best to stratify tumors in terms of a combination of histological and molecular factors is still an open (and at times controversial) question. The lack of good model systems covering the range of genetic alterations observed in LGG in settings which are as close to the human disease as possible also limits at present the capacity for pre-clinical investigation. Further, the merging of (molecular) diagnostic stratification and information regarding molecular drug targets into optimal clinical trial design is also a pressing issue. This is all the more challenging for the fact that LGGs typically show a benign but often unpredictable growth pattern – they are curable by surgery alone in some instances, but in other cases (e.g. where total resection is not possible) the picture is often one of a chronic disease that can wax and wane over decades. These long-term effects on patient quality of life are becoming an increasing area of focus, and future large-

scale clinical trials must take functional endpoints into account in addition to just survival measures, since fortunately few children will die of their disease.

In the summer of 2016, a 2nd international consensus meeting was convened in Padua, Italy, to try to address these challenges and suggest a blueprint of how the scientific and clinical committees can come together to suggest a way forward. The group of clinicians and translational and basic researchers attending the meeting contributed to a lively discussion of these topics, resulting in a generally positive sense of progress despite recognition of the scale of the challenge. The conclusions of these discussions in the form of a summary of current thinking and outlook for future efforts, with recommendations, are presented.

Implications of tumor biology for diagnostics and therapy

Genetic alterations in pediatric LGG

A full description of all genetic alterations known to be present in pediatric LGG is not required here, and this topic was covered in some detail in a previous consensus report[3]. It is now clear, however, that the vast majority of tumors falling in the pediatric LGG spectrum are caused by one of a variety of alterations in the MAPK signaling pathway, including *BRAF* mutation or fusion, *FGFR1* mutation or structural rearrangement, *NF1* mutation, *NTRK*-family fusions and other rarer events (see e.g. [4-6]). Notable exceptions to this include subependymal giant cell astrocytoma (typically associated with germline *TSC1/2* mutations), and a histologically mixed group of tumors including a substantial fraction of angiocentric and diffuse gliomas that harbor activating alterations in *MYB* or *MYBL1*[6-8]. It is currently unclear whether the latter alterations, resulting in altered transcriptional activity, also function partly via the MAPK pathway, but the consequences are certainly more complex than that alone.

The discovery of these alterations over the last 5-10 years as a key driving alterations in pediatric LGG has led to excitement and optimism both from a biological standpoint, but also because of the parallel development of drugs specifically targeting several of these alterations (e.g. BRAF V600E, FGFR1, NTRK inhibitors) or the downstream mediators of the pathway (e.g. MEK inhibitors). Indeed, early phase trials with some of these compounds are currently in progress or nearing completion, as is summarized in a latter section of this consensus summary. In order for these new treatments to provide the greatest possible benefit, however, it will be crucial to properly stratify future trials to optimize the matching of

drugs to patients in a rational way. This is partly compounded by the fact that, while some degree of specificity for certain alterations in certain histologies has been observed (e.g. *KIAA1549:BRAF* fusion is much more common to pilocytic astrocytoma; BRAF V600E is enriched in ganglioglioma, pleomorphic xanthoastrocytomas etc.), there are no 100% concrete associations between LGG morphology and genetics. Teasing out the details of this interplay will be a major focus of ongoing efforts in the community, and a key aspect of translating the wealth of genomics data into patient benefit. Another major caveat is the fact that these genetic events are currently not routinely tested for in a majority of diagnostic laboratories.

Implications for diagnostic stratification and therapy

The recent 2016 update of the WHO classification of nervous system tumors has accepted in some tumor types the recognition of both morphological and molecular features as being important to tumor behavior, introducing the concept of a layered, integrated diagnosis[1,9]. As yet, however, it does not cover all of the pediatric-specific features of low-grade glial/glioneuronal neoplasms, especially diffuse gliomas. Not just molecularly (e.g. *IDH1* mutations are extremely rare in childhood gliomas, but partially defining in adult lower grade gliomas), but also clinically pediatric LGGs are distinct from their adult counterparts. Although fully comprehensive data is still lacking, it is likely that pediatric-type diffuse gliomas currently classed as WHO grade II do not have the same propensity for malignant progression, and thus have a better prognosis, than their adult counterparts. Gliomas in infants are yet another

special case, whereby apparently undifferentiated morphology and an elevated proliferation rate can represent the unique environment of the developing brain and do not always indicate malignancy. This is a further area where more work is needed to determine both the biological backgrounds and clinical outcomes of these rare tumors.

As noted, there are some clear enrichments of certain genetic alterations occurring more frequently in conjunction with specific histologies, and also in certain locations. For example, the classical *KIAA1549*:*BRAF* fusion is much more common in pilocytic astrocytoma than other LGGs, and is particularly common in the cerebellum[4,6]. *FGFR1* alterations, including point mutations and kinase domain duplications, are more frequent in dysembryoplastic neuroepithelial tumors (DNET)[6,10], while alterations of *MYB*/*MYBL1* seem to define a class of pediatric-type diffuse gliomas, often with features of angiocentric glioma[6,8]. DNA methylation profiles have been suggested to distinguish biological subgroups with enrichment for particular aberrations[5], in a similar way to what has been shown for e.g. medulloblastoma[11], ependymoma[12] and pediatric glioblastoma[13]. This is one of a number of molecular techniques worthy of further pursuit for its ability to more objectively subgroup these tumors.

Conversely, there are also some genetic alterations which seem to be distributed across different histologies, and which may have a varying prognostic impact. The BRAF V600E mutation is a good example of one such change, which is enriched in gangliogliomas and pleomorphic xanthoastrocytoma (PXA), but can also be found in DNET, pilocytic astrocytoma, and also high-grade gliomas, amongst others. It is clear that not all of these groups have a similar clinical course, with PXA having a worse

outcome than WHO grade I tumors. Notably, many PXAs additionally show a focal genetic loss of the *CDKN2A/B* locus at 9p21, and this combination (V600E + 9p21 loss) has recently been proposed to mark a subset of lower-grade gliomas that show a propensity towards malignant progression[14], as well as a group of histologically high-grade tumors that show a slightly more favorable prognosis than classical glioblastoma[15]. Further work is therefore required to precisely define the role of histology or other (epi-)genetic changes in determining the outcome of V600E-positive tumors – a task of particular urgency to ensure appropriate patient stratification in BRAFi clinical trials.

Even the role of the histone 3 K27M mutation, common in pediatric high-grade glioma and initially thought to perhaps be exclusive to them[16,17], is not yet fully clear. There are now multiple anecdotes of histologically low-grade tumors harboring this mutation (often in conjunction with a MAPK alteration such as BRAF V600E or *FGFR1* mutation) and showing longer-than-expected survival[4,6,18]. Thus, although true in the vast majority of cases, the presence of the K27M mutation is not automatically an indicator of aggressive behavior. In the authors' experience, however, K27-mutated LGGs show an increased propensity for later malignant progression, and should be followed up as such (a rationale for intensified up-front therapy is not clear as yet).

Further elucidating these complex relationships is important not just as an academic exercise, but also because of the expanding role that personalized therapy for these tumors is already playing and will continue to play in the future (see e.g. [19,20]). Since many targeted therapy trials are now recruiting

patients based on presence of a particular target across multiple histologies, rather than solely within one entity, one could imagine a framework for pediatric LGG that builds on the WHO 2016 concept of an integrated diagnosis to incorporate molecular aberrations. For example, low-grade neuroepithelial tumors (LGNET) may be categorized first by their particular genetic change, with a layer for histology in addition (see Figure 1).

The end goal of any such a framework should be to facilitate the routine assessment of these important characterizing features in a clinical diagnostic setting and the stratification of patients by prognosis (and possibly treatment response), but also to optimizing the way in which patients may be matched to targeted therapy trials. In order to achieve this, considerably more data is required on the natural course and prognosis of certain histologies in combination with certain molecular aberrations, and also on the response of these different groups to current therapies. This is something which can only be achieved in collaboration, and with the support of clinical trial groups for acquisition of sample material and outcome data. Once this data can be made available, however, it is hoped that this will rapidly translate into a robust classification scheme and will be one of the first priorities for the recently announced cIMPACT-NOW consortium for advancing nervous system tumor classification[21,22].

Molecular diagnostic methods

To answer the outstanding questions regarding this histology-biology interplay, and for the expansion of knowledge on diverse genetic alterations to be of utility in clinical practice, standardized methods of accurately and reliably detecting them are required. In terms of molecular subgrouping, most systems proposed to date make use of either gene expression or DNA methylation changes. As noted above, methylation analysis has been shown to be a valuable tool in other entities, and can identify molecular groups of LGG enriched for certain histologies and molecular alterations[5]. No clear consensus has yet been reached, however, as to how these groups should best be defined and diagnostically detected.

For genetic tests, there is currently no single gold-standard for assessing the multitude of changes that can potentially be found in pediatric LGG. Some laboratories are already implementing a comprehensive next-generation sequencing-based approach, applying whole exome/whole genome and transcriptome sequencing to newly diagnosed LGGs. While this clearly offers the best opportunity to cover the whole spectrum of possible changes, and is to be encouraged where possible, it is currently not feasible from a cost and logistic point of view to perform such an analysis in every pathology lab worldwide. A rationally planned series of more targeted tests, possible through a variety of methods (Sanger sequencing, FISH, RT-PCR, SNP array etc.) is also able to identify the majority of the more common changes. Based on the observed enrichment of certain alterations in particular locations and histologies, the order of these tests can potentially be optimized to minimize time and costs for the molecular diagnostic process. A suggested structure for this testing is outlined in Figure 2. There is uniform consensus that all future

clinical trials should make a provision to be able to collect sufficient tumor material to perform at least this panel of tests for every recruited patient (with the optimum being fresh frozen tumor material and a matched germline control). Other than for children with NF1, the optimal treatment of children with LGGs, in the molecular era, will mandate the obtainment of tissue and molecular characterization. This is also the only means to ensure that it is possible to learn from each case.

Conclusions & Recommendations:

- Expanding knowledge of tumor genetic alterations is already impacting on patient management in terms of diagnostics and targeted therapeutic options
- The current WHO classification, particularly for diffuse gliomas, does not satisfactorily address the spectrum of LGG seen in children
- More data is required, particularly on survival and functional outcomes, in order to further examine the complex interplay between genetics and histology in LGG
- The community should support and encourage efforts to redefine the classification of pediatric LGG on an integrated histo-molecular basis, as the backbone for future clinical trial stratification

Models of pediatric low-grade glioma

The other major topic of discussion from a tumor biology perspective was the pressing need for more and better model systems for pediatric low grade gliomas. Both cell-based and in vivo models are essential for learning more about the biology and mechanisms of transformation of this disease, as well as for preclinical screening and drug testing. The particular importance of the latter element is highlighted by the study of sorafenib, a drug which resulted in an unexpected acceleration of tumor growth when treating these typically BRAF-altered tumors with a RAF inhibitor[23]. This effect was subsequently explained as resulting from paradoxical MAPK pathway activation due to interactions between the drug and dimerization between mutant and wildtype B/CRAF[24]. The models used to investigate this effect, cells transduced with relevant oncogenic constructs, are a convenient tool for mechanistic and inhibitor studies. They have also been used in various other settings, from the initial demonstration of the transforming potential of *KIAA1549:BRAF*[25] and the abilities of the fusion to regulate neuroglial cell growth[26], to more recent functional investigations of *MYB* alterations[7,8]. While they certainly have a role to play for interrogation of signaling pathway dynamics and initial screening, and an expanded repertoire of such systems as being explored in various labs would be highly welcome, they do not accurately reflect the genetic/epigenetic background of the primary tumor. Many efforts to derive primary cultures of LGG that can be grown for more than a few passages have failed due to the intrinsic slow growth and benign behavior of these tumors, hampering the development of more accurate *in vitro* models. A novel approach was recently published using an inducible SV40 T antigen system in primary cells[27]. This method allowed the cells to overcome oncogene-induced senescence, the

main intrinsic growth barrier, for long enough to allow cell expansion and subsequent drug testing. Although still an artificial system, the advantages of being able to work with such patient-derived cells makes it a promising addition to the toolbox, especially if the same approach can now be used to generate a broader panel of lines with different MAPK alterations.

Finally, other more sophisticated *in vitro* approaches are also currently being investigated for their application in modeling LGG. For example, labs are exploring the potential for iPS cells derived from patients with Neurofibromatosis type I as a system for generating tractable MAPK-activated lines (Personal Communication, Weiss et al) Still in development at present, this represents a further promising method for expanding the repertoire available to the field.

A similar dearth of animal models is unfortunately also a feature of pediatric LGG research. One of the first models to be developed was an NF1 model, which elegantly demonstrated the need for an *Nf1* heterozygous microenvironment, as well as complete loss in astrocytes, in order to develop full blown optic glioma in mice [28]. Although this model, simulating hereditary optic pathway LGG, was first developed in 2003 (and subsequently used in several related follow-up studies), work to establish a model of the sporadic disease took substantially longer. The first such model was published in 2011, and used the RCAS somatic gene transfer system to introduce BRAF V600E into Nestin-expressing cells[29]. The resulting tumors histologically resemble human pilocytic astrocytoma, and show strong MAPK pathway activation as well as a very slow growth rate (to the extent that the animals typically die from old age

before they die of their tumor, despite an age of onset of ~4 weeks; (J Gronych, personal communication). Further investigations into the kinetics of these tumors and their response to targeted therapies are ongoing.

A second attempt to model the sporadic disease used the *KIAA1549*:*BRAF* fusion[30]. By introducing the fusion into different cell types, Kaul and colleagues were able to demonstrate that only neural stem cells (NSCs), and not mature astrocytes or NG2-positive progenitors, showed an increased proliferation in response to the oncogenic stimulus. The fusion-expressing NSCs also formed small glial lesions *in vivo*, but did not recapitulate a full tumor.

To our knowledge, there are no other representative genetic models available, although several are currently under development. An additional feature of these models which may be of substantial interest in the current era of excitement about immunotherapy is their fully immunocompetent background. This makes them well suited for investigation into, for example, immune checkpoint inhibitors or macrophage/microglia-modulating agents.

In addition to the genetic models, transplant models have been used as a way to rapidly investigate the tumorigenic potential of different oncogenes. For example, p53-null mouse astrocytes transduced with tyrosine kinase-duplicated FGFR1 generate tumors when implanted orthotopically into mouse brains[6]. Similarly, 3T3 cells transduced with activated MYB or MYBL1 were also able to generate tumors in the mouse flank[7,8]. These models are valuable tools for preclinical proof-of-concept studies, the artificial background (including cell cycle deregulation as a result of additional genetic alterations) makes their

broader utility as a faithful recapitulation of LGG somewhat limited. As long as these caveats are acknowledged, however, an expanded catalogue of similar models would certainly be of use.

Thus, there was a very clear consensus amongst meeting attendees that a lack of LGG models is currently a major hurdle and bottleneck for further advancing research into these tumors. Without such models, the advances that are possible in further interrogating the consequences of MAPK activation and how to inhibit it are limited. Both *in vitro* and *in vivo* models are also required for addressing the question of how drug resistance can emerge against targeted therapies including BRAFi and MEKi, and how we might overcome this in the clinic e.g. by combination strategies.

Conclusions & Recommendations:

- A lack of suitable in vitro and in vivo models of pediatric LGG is a bottleneck hampering functional and pre-clinical investigation
- Several efforts are ongoing with, importantly, a variety of different methods in an attempt to expand the catalogue of suitable model systems
- Such efforts should be strongly supported by the community, and should be pursued collaboratively where possible in order to reduce duplication and maximize efficiency

Low-Grade Gliomas: Clinical Trial Results and Design Implications

Critical issues for the development of future studies for children with low-grade gliomas (LGGs) remain, including whether there is enough information to justify the use of molecular-targeted agents in newly diagnosed patients and, if so, how clinical trials should be structured to best assess the efficacy and safety of such agents. As noted in Table 3 and 4, since 1998, there have been multiple prospective clinical trials performed by well-established consortia and working groups (31-39). Nearly 2000 patients have been treated on these studies and results have been relatively consistent between studies.

Prospective Chemotherapy Trials

Over 1400 children without Neurofibromatosis type 1 (NF1) have been treated (31-39). Although the majority of children treated had tumors of the optic nerves/chiasm/hypothalamus/optic tracks/optic radiations, many recent studies have included up to 50% of children with LGGs outside these regions, especially those of the brainstem. The most common regimen tested has been the combination of carboplatin and vincristine, utilized in nearly 1000 children both in single-arm studies and in randomized trials (32,33,35,36,37,38,39). Drug combinations directly compared to carboplatin and vincristine in randomized studies included the four drug combination of thioguanine, procarbazine, lomustine and vincristine and carboplatin and vincristine combined with either etoposide or temozolomide (32,33,38,39,40). Only one relatively small single-armed study utilized carboplatin alone (31). These prospective trials varied widely the number of patients entered on study, with the largest being the SIOP 2004 study, which is still in final analysis (39). Most studies entered children over a wide age range,

spanning as long as birth to 18 years of age. Response to the regimens tested was difficult to compare across studies, as some studies utilized only enhanced images, while others evaluated both enhanced and non –enhanced images (31-39). Overall response as assessed by either complete or partial responses (>50% reduction in tumor greatest bi-directional area) usually were in the 30-35% range; some investigations also utilized a minor response criterium connoting reduction between 25 and 49%. After adding minor responses, the carboplatin and vincristine regimens resulted in tumor shrinkage in up to 60% of patients. Making assessment across trials even more difficult was that there was no consistency in whether central neuroradiographic or local institution review was utilized or reported. Furthermore, the relationship between radiographic response and PFS was variable, with most studies showing no clear-cut association. The greatest consistency among studies was progression-free survival with 5-year rates in patients without neurofibromatosis being in the 35-45% range. Overall survival ranged between 85 and 100%.

For patients with LGG and NF1, studies designs were even more uniform, the majority being single-arm studies utilizing carboplatin and vincristine. The majority of the non-NF1 randomized trials had single strata for patients with NF1, as there was reluctance to expose patients with NF1 to alkylating agents (31-37,39). In over 550 patients with NF1 who have been treated on these prospective trials, the complete and partial response rates seemed somewhat higher than in patients without NF1; when reported, being closer to 50%. Similarly 5-year event-free or progression-free survival was better than that found in non-NF1 patients and was remarkably consistent in the 60-70% in almost all studies.

Overall survival also was consistent across studies and in most studies was between 90% and 100% at five years.

Smaller studies have tested other agents with overall similar results. The combination of cisplatin and etoposide, utilized at different dose intensities of cisplatin, has been prospectively evaluated in 50 children and had a high overall response rate (70%); the progression-free survival also seemed somewhat higher than seen in the carboplatin and vincristine prospective studies (41,42). Other drugs that have been utilized and abandoned in newly diagnosed patients because of lack of efficacy or excessive toxicity include high-dose cyclophosphamide alone, vinblastine in combination with carboplatin or temozolomide alone (40,43,44). Single-agent vinblastine has been utilized in 54 patients, including 13 with NF1 (45). The response rate to this regimen seems lower than what was reported after treatment with the carboplatin and vincristine regime, but the 5-year progression-free survival rate was quite similar.

What can be gleaned from these prospective studies includes that almost independent of the type of regimen utilized approximately 35-45% of children without NF1 and a higher proportion with NF1 will be event or progression-free 5 years following initiation of treatment (31-45). Disease control in those without NF1 progressively falls over the first 5-10 years. There is a seeming plateau in the trajectory of loss of disease control between years 3 and 5 in children with NF1, most likely due to the natural history of LGGs to grow primarily during the first few years of life. The vast majority of studies have utilized relatively lax entry criteria, allowing patients to be entered on the basis of either clinical or radiographic

progression, with the criteria being utilized for clinical progression being non-specific and at the discretion of the treating physician. In some studies, patients could be entered without progressive clinical or radiographic disease if the LGG was believed to have the potential to cause significant morbidity. In those trials evaluating children without NF1, the majority did not require tissue confirmation for tumors isolated to the optic nerve or the optic nerve and chiasm for entry. Even in those studies requiring tissue, molecular characterization was not done, which was not surprising given the era in which these prospective studies were initiated. The randomized trials took on average between 8 to 10 years to accrue and another 3 to 5 before results were reported. Response across studies is essentially impossible to compare and although some of these studies attempted to address functional outcomes, the vast majority did this only in an anecdotal fashion.

Molecularly-targeted Trials: Early Result

As regards the status of molecular-targeted approaches for patients with LGG, although there have been studies assessing receptor kinase inhibitors and m-TOR inhibitors, most efforts over the past five years have centered on evaluation of the efficacy of drugs aimed directly at interrupting RAS-MAPK hyperactivation. There are presently four MEK-inhibitors in active study including three evaluating tumor control and response in children with progressive LGG, with and without NF1 (46). The most mature results have been reported in a Pediatric Brain Tumor Consortium trial with the AstraZeneca drug, Selumetinib (46). Responses were seen in the phase I study in both patients with and without NF1. In the phase I study some non-NF patients did not have molecular testing performed for BRAF mutation.

The preliminary results of the phase II study in children with refractory or recurrent LGGs have been released in abstract form (47). Approximately one-third of patients with LGGs harboring either a v600E mutation or the BRAF KIAA 1549 fusion experienced a partial response to treatment and many others had some degree of tumor shrinkage, although less than the 50%. 2-year progression-free survival in these patients, who have failed at least one form of previous therapy, was 66 ± 11%. Responses seem to be somewhat higher in the children with the NF1-related LGGs, as 10 of 25 achieved a partial response (40%) and almost all patients have had some degree of tumor shrinkage. The 2-year progression-free survival for patients with NF1 was 96 ± 4%. The duration of response off treatment is being closely followed in the Pediatric Brain Tumor Consortium trial. In the Selumetinib trial, patients with responding or stable disease could elect to stop treatment between years 1 and 2 after initiation and if the tumor then progressed off treatment, they could be placed back on treatment. To date, only the minority of patients had to go back on treatment which is either evidence of the erratic nature of growth of LGGs, which can spontaneously cease growth as the child ages, or is evidence of a somewhat unexpected prolonged effect of the drug after cessation. Adding to the enthusiasm for the use of Selumetinib, or for that matter other MEK-inhibitors in patient with NF1, has been the recent published experience of the results of the phase I study performed in patients with NF1 and plexiform neurofibromas (48). In this single-armed study, partial responses were noted in 17 of 24 children with anecdotal evidence of decreases in tumor-related pain, disfigurement and functional impairment. The higher response rate should not be taken as clear evidence as plexiform neurofibromas are more responsive than LGGs; in the

plexiform trial a partial response was greater than or equal to a 20% reduction in volume, compared to the 50% criteria used for the LGG studies.

Results with the use of other MEK-inhibitors in children with LGGs should soon be forthcoming. The Novartis agent, Trametinib, has recently been studied in a completed phase II trial which included a strata for children with BRAF-mutated tumors (either fusion or point mutation). The Array drug (MEK 162) has been assessed in a nearly completed pediatric trial and is soon to be expanded into a phase II trial for children with LGGs with or without NF1. The Genentech, Cobimetinib, drug is presently in phase I trials.

The experience with the V600E mutation inhibitors is likewise growing. Ongoing pediatric phase II clinical trials include the two commercially available type I BRAF V600E inhibitors Vemurafinib (Roche/Genetech) and Dabrafenib (Novartis) (49). The phase I results for Dabrafenib in pediatric BRAF V600E positive tumors was reported at ASCO 2015 and the final phase II result for pediatric low grade gliomas treated with Dabrafenib has been recently reported in abstract and oral presentation at the European Society of Medical Oncology 2016 (ESMO). The clinical trial population included children 2-17 years of age with progressive pediatric low-grade gliomas that had failed at least one chemotherapy or radiation regimen. Overall, the drug was well tolerated with the most common side effects related to skin rash, similar to those observed in adults treated with this drug. Of the 32 patients enrolled on the trial with low-grade gliomas (15 in the phase I and 17 in the phase II), all but three had stoppage of tumor growth; 6 had minor responses, 11 had partial responses and 1 patient demonstrated a complete

response (49). A number of case reports of responses to BRAF inhibitors have also recently been published (50,51). While the response rate of BRAF V600E mutant low-grade gliomas is promising, the majority of pediatric low-grade tumors harbor the BRAF KIAA1549 truncated fusion and this variant is paradoxically activated by BRAF V600E inhibitors including Sorafenib (23). As such, it is critical that patients be properly profiled and only those with sequence confirmed BRAF V600E mutations be exposed to these type I inhibitors. To overcome the paradoxical activation of BRAF KIAA1549 truncated fusion variants of pediatric low-grade gliomas, newer types of BRAF inhibitors have recently been developed. Called type II inhibitors, these targeted agents shut down both BRAF and MEK signaling and thus can be considered for all BRAF pathway mediated tumors (52). A clinical trial of the first type II inhibitor in pediatric low-grade gliomas called TAK580, which has excellent CNS penetration, is just now opening and clinical results are therefore unavailable at the present time.

Experience from the use of BRAF V600E inhibitors in malignant melanoma has demonstrated rapid development of resistance to type I inhibitors. To address this, dual BRAF V600E and MEK inhibition is now approved and results in adults and results have demonstrated improved response rates, improved duration of response and in some cases, reduction in toxicity, especially related to the development of squamous cell carcinoma (personal communication, M. Kieran). Through an international consortium and funded by Novartis, the combination of Dabrafenib (targeting BRAF V600E) and Trametenib (targeting MEK) are now also being tested in pediatric patients including those with gliomas. The likelihood of resistance development in pediatric LGGs is likely different than melanomas and for that

matter, high-grade gliomas and this difference requires further study. The phase I component of both of these inhibitors has been completed and both single agent phase II Trametenib for BRAF KIAA1549 tumors, as well as the phase II combination of Dabrafenib and Trametenib for BRAF V600E tumors, are currently accruing patients. The safety and optimal duration of treatment remains unknown.

Conclusions and Recommendations

1. Over the past quarter century multiple prospective clinical trials, some randomized and some not, have been performed in children with progressive LGGs and they demonstrate very similar overall 5-year progression-free survival rates and better 5-year disease control in children with NF1 as compared to those without.
2. Studies have been consistently inconsistent in the inclusion criteria used and the prospective trials have, by and large, been very long in duration; because of the structure of the trials, results are not available for up to five years following completion of accrual.
3. Radiographic response has been used as a surrogate for efficacy, however the relationship between response and 5-year disease control has been variable among studies, as have the means utilized to assess response.
4. There is an increasing experience demonstrating that molecular-targeted therapies such as the BRAF inhibitors and the MEK inhibitors are effective in patients who have failed first line chemotherapy; these studies have incomplete, but somewhat reassuring short-term safety data.

5. The rationale for utilizing molecular-targeted therapy in clinical trials for children with newly-diagnosed LGGs is strong, however, it is unclear that this needs to be done as part of large, prospective randomized trials; there is a wealth of historical data available from chemotherapy-based trials and conventional randomized trials will likely require over 8 years to accrue and another 3-5 for evaluation.
6. A major flaw in all prospective studies that have been done to date is the lack of consistent evaluation of functional outcome and biology. Future studies utilizing biologic-targeted therapy should focus on functional outcome; functional outcome measures may supply a quicker read out of efficacy of the drug utilized and a more accurate test of its "true" benefit.

Evaluation of Therapies: Functional Outcome

Tumor measurements by MRI are increasingly being recognized as an insufficient assessment of the clinical response to therapy for children with low-grade gliomas (LGGs). Neurologic, cognitive, endocrine, behavioral, emotional and adaptive function are critical endpoints, if not the most critical, for pediatric LGGs.[53] Therapies may exacerbate patients' tumor-associated symptoms and thus may impair their overall function and quality of survival(QoS). The clinical impact of both the tumor and treatment effect may be measured by objective testing, clinician's rating of symptoms and functional status and patient-reported outcomes (PROs) scales. PROs are often used to evaluate the impact of the disease and treatment in terms of symptoms, impact on activities of daily living, and QoS. These instruments should be considered important outcome measurements for pediatric LGGs. Standardized guidelines on their

use and interpretation of these instruments are necessary to compare results across clinical trials and facilitate measurement of clinical benefit. The functional evaluations are summarized in table 5.

Neurological Function:

Tumor location, presence of hydrocephalus, and prior treatments may have substantial impact upon the neurologic examination. Assessments of neurologic function, including both neurologic examination and cognitive testing, are critical in assessing therapy response. There are currently no standardized neurological assessment scales for children with LGGs. The Lansky performance scale attempts to reflect global neurological function comparable to the Karnofsky Performance Status (KPS) scale, but has been shown to be subjective and lacking reproducibility.[54-57] Its use in response assessment is limited given that it is a global measure and may not address individual symptoms. The lack of a measurement scale of neurologic function specifically for brain tumor patients has been addressed by the Response Assessment in Neuro-Oncology (RANO) group with development of the neurologic assessment in neuro-oncology (NANO) scale.58 Assessment tools for specific functional motor skills, such as the the 6 minute walk test, are available for a variety of other neurological diseases but have yet to be evaluated for children with LGG.[59]

Visual Outcomes among Children with Optic Pathway Gliomas (OPGs):

Outcome measures for OPG clinical trials should include visual endpoints, and treatment success should be based on these endpoints. Several studies have identified limitations of imaging outcome measures for OPGs, including discordance with visual outcomes following treatment.[60-61] Consensus-based, evidence-driven recommendations for visual endpoints for OPG clinical trials, including Teller acuity cards, HOTV charts, optic disc pallor, and visual fields, have been defined by the Response Evaluation in Neurofibromatosis and Schwannomatosis (REiNS) Visual Outcomes Committee62 and are applicable to

sporadic OPG as well. Potential markers currently under evaluation for future testing include retinal nerve fiber layer measured by optical coherence tomography and MRI fractional anisotropy of the optic radiations. Both have been shown to correlate with vision in OPG patients 63-65 but require further validation prior to routine inclusion in OPG clinical trials.

Cognitive, Adaptive Functioning and Quality of Survival

Risk factors for poor cognitive outcomes include age at diagnosis, male gender, tumor size and location, hydrocephalus, surgical resection, and dose and treatment volume of cranial radiation therapy.[66-70] Although children with LGGs often demonstrate normal cognitive and adaptive functioning, they have increased risks for cognitive and adaptive functioning impairments. Due to costs and patients' access to testing, embedded studies of cognitive functioning within clinical trials have often been hindered by poor compliance with cognitive evaluations, thereby limiting progress in understanding the cognitive functioning of survivors.

The selection of evaluations for cognitive functioning for clinical trials for children with LGGs must meet several criteria: they must be clinically meaningful, validated in multiple languages, simple, brief and inexpensive to administer. The CogState is a relatively brief, validated, patient-completed, computer-based questionnaire of those neurocognitive processes which are known to be most affected among brain tumor survivors (i.e., attention, processing speed and memory) among children 5 years and older.[71,72] Adaptive behavior scales like the Vineland Adaptive Behavior Scales (VABS) address everyday performance in the following domains: Communication (Expressive and Receptive, Daily Living Skills (personal, domestic, and community), Socialization (Interpersonal relationships, play and leisure, coping skills), Motor Skills (Gross and Fine with a ceiling of abilities at 7 years), and Problem Behaviors.[73] The questionnaire is a widely available, multi-language assessment of adaptive functioning that has been

used in pediatric brain tumor populations including a LGG cohort.[74-80] It is applicable to all ages and can be completed from responses to telephone interview or to a parent- or caregiver-rating form.[81] The study design of the upcoming LOGGIC study in Europe will use the Vineland Adaptive Behavioral Scale as a primary endpoint. For this study, a trained research nurse will help to interview parent/guardians in an attempt to enhance participation and consistency of data. In addition, the 24-item PedsQL Brain Tumor Module, validated for children age 2 to 18 years and encompassing the following six scales: cognitive problems, pain, movement and balance, procedural anxiety, nausea and worry, has been incorporated into a recently published phase II study of weekly vinblastine for children with LGG.[82,83] Long-term assessments with the EORTC-QLQ-C30 and EORTC QLQ-BN20 questionnaires among adults surviving childhood LGG showed impaired QoL among those exposed to cranial radiotherapy.[84] Other studies using PedsQL, KINDL, and TACQOL-P revealed relatively high QoL concerning psychosocial, physical, emotional, social, and school-functioning scales among pediatric LGG patients.[85-88] Finally, the Patient-Reported Outcome Measurement Information System (PROMIS) QoL battery has been used for serial measurements of patient mobility, fatigue, pain interference, peer relationships, anxiety, and depressive symptoms among children with newly-diagnosed cancer and neurofibromatosis.[89,90]

Endocrine Outcome Measures:

Supratentorial midline LGG potentially disturbs the pituitary and / or the hypothalamus. Diencephalic syndrome (DS) is a rare syndrome of severe emaciation often associated with preservation or acceleration of linear growth, euphoria, hyperactivity, vomiting, irritability, nystagmus and increasing head circumference occurring especially in children < 2 years of age with diencephalic LGGs.[39-41] In response to therapy, severe emaciation may be conversely replaced by inappropriate and rapid weight gain and central precocious puberty.[94,95] Outcome measures for suprasellar tumours presenting with or without hormone dysfunction or failure require comprehensive auxological assessment (height, weight,

body mass index), pubertal assessment (Tanner staging) and full evaluation of hypothalamic pituitary axis (IGF-1, growth hormone dynamic testing, LH, FSH, testosterone/estradiol, 0900 cortisol, TSH, free T4, PRL, paired morning urine/ plasma osmolality measurements).

Conclusions and Recommendations

1. Outcome for children with LGGs cannot be assessed by neuro-imaging alone, and neurologic, cognitive, endocrine, behavioral, emotional and adaptive function, are critical endpoints.
2. Objective testing techniques are available to assess functional outcome, but have not been widely used in prospective studies to date.
3. Although visual outcomes, neurocognitive assessments, endocrinologic measures and to some extent, adaptive and quality-of-life measures have been employed in studies of children with brain tumors and are ready to utilize as endpoints in prospective clinical trials, other important assessments, such as that of neurologic function need to be better refined.
4. The potential cumulative toxicities of these approaches, especially given the chronic nature of LGGs and the need for repeated treatment, will require ongoing assessments of benefits and sequelae; distinguishing the toxicities of these different therapies and their potential synergistic affects may be difficult.

Table 1. Example of a possible framework for an integrated molecular-histological stratification of pediatric LGG.

LGNET – RAF fusion

- PA RAF-fusion positive
- GG RAF-fusion positive
- DLGNT RAF-fusion positive

LGNET-BRAF^{V600E}

- PA BRAFV600E positive
- GG BRAFV600E positive
- DG BRAFV600E positive
- DIA/DIG BRAFV600E positive

LGNET-RAF wt

- PA
- GG
- DIG/DIA

LGNET-NF1

- *Histopath. variants*

LGNET NOS

Table 2: Molecularly Targeted Testing Schema

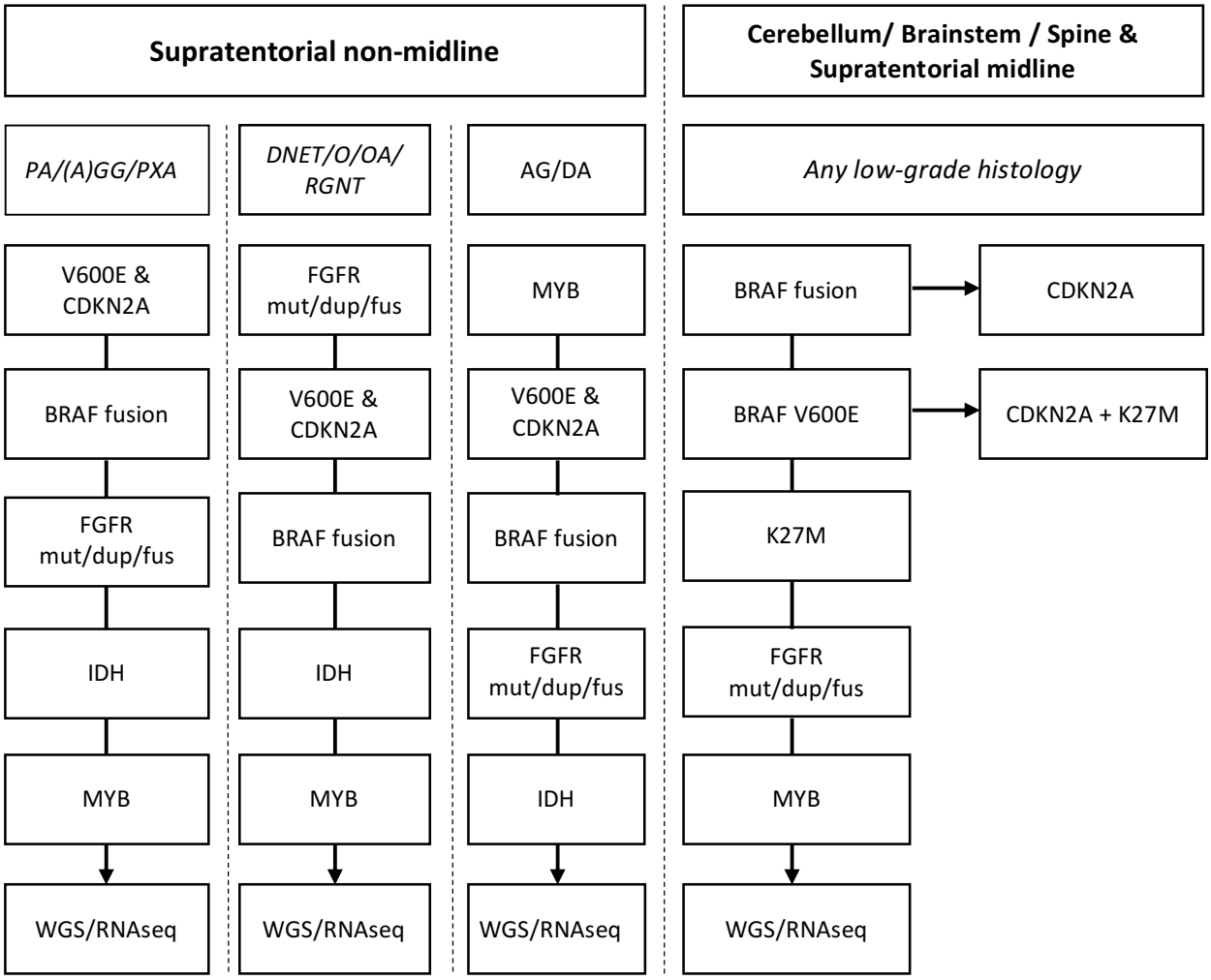

Table 2: Suggested order of investigation for rationally targeted molecular testing based on tumor location and histology. NB –up-front next-generation sequencing (DNA + RNA) can also be used to supersede this order and give the most comprehensive overview.

Table 3: Prospective Clinical Trials for Patients with Low-Grade Gliomas without NF1

STUDY TYPE	**Single-Arm[36] (multicentered)**	**Single-Arm[31] (POG)**	**Single-Arm[34] (SFOP)**	**Single-Arm[37] (HIT-LGG-1996)**	**Randomized[33] (COG)**	**Single-Arm[39] (SIOP)**	**Single-Arm[38] (COG)**	**Randomized[39] (SIOP)**
AGENT	CARBO/VCR	CARBO	PCV/CARBO; VP16/CPDD VCR/CYTOX	CARBO/VCR	CARBO/VCR VS. TPCV	CARBO/VCR	CARBO/VCR/ TEMO	CARBO/VCR VS. CARBO/VCR/VP16
NUMBER PATIENTS	63	29	62	161	274	166	66	497
YEARS UNDERTAKEN	1989-93	1989-94	1990-98	1996-2004	1997-2005	1993-2000	2004-2007	2004-2012
AGE RANGE	0-180 months	0-71 months	0-180 months	0-192 months	0-120 months	0-170 months	0-120 months	0-180 months
***NON-DIENCEPHALIC INVOLVEMENT**	*20%	0%	0%	*20%	*50%	*25%	*50%	*50%
EFS/PFS	2-year 79±11%	3-year 51±9%	3-year PFS 42±12%	5-year PFS 47%	5-year EFS 45±3.2%	5-year 40±11%	5-year 46±13%	5-year PFS 46%
OS 5-10 Years	97%	83%	89%	90%	86±2%	88±1%	87±12%	90±2%

*Includes both NF1 and non-NF1 patients when reporting sites of origin.
Legend:
Carbo-carboplatin; VCR-vincristine; PCV-procarbazine; VP16-etoposide; CPDD-cisplatin; cytox-cyclophosfamide; TPCV-thioguanine, procarbazine, CCNU and vincristine, temo-temozolamide; EFS-event-free survival: PFS-progression free survival; OS-overall survival.

Table 4: Prospective Clinical Trials for Patients with Low-Grade Gliomas with NF1

STUDY TYPE	Single-Arm[36] (multicentered)	Single-Arm[31] (POG)	Single-Arm[34] (SFOP)	Single-Arm[37] (HIT-LGG-1996)	Single-Arm[32] (COG)	Single-Arm[39] (SIOP1)	Single-Arm[39] (SIOP II)
AGENT	CARBO/VCR	CARBO	PCV/CARBO; VP16/CPDD VCR/CYTOX	CARBO/VCR	CARBO/VCR	CARBO/VCR	CARBO/VCR
NUMBER PATIENTS	15	21	23	55	127	44	284
YEARS UNDERTAKEN	1989-1993	1998-94	1990-1998	1996-2004	1997-2005	1993-2000	2004-2012
AGE RANGE	0-180 months	0-120 months	0-72 months	0-180 months	0-120 months	0-180 months	0-180 months
EFS/PFS	2-year 79±11%	5-year 61±12%	3-year 62±93%	5-year 68%	5-year 69±4%	5-year 60±6%	n/a
OS 5-10 Years	100%	100%		NG	98%	100%	n/a

Legend:
Carbo-carboplatin; VCR-vincristine; PCV-procarbazine; VP16-etoposide; CPDD-cisplatin; cytox-cyclophosfamide; TPCV-thioguanine, procarbazine, CCNU and vincristine, temo-temozolamide; EFS-event-free survival: PFS-progression free survival; OS-overall survival.

Table 5: Functional Evaluations of Response to Therapy:

Test	Endpoints of Test	Age range	Type of Measurement	Time Required	Caveats
Neurologic Function					
Lansky performance Scale[54]	Play performance; independence in daily living	1-16 years	Parent/Caregiver reported outcome; Scale: 10 – 100	1 minute	Subjective, inferior reproducibility
Neurological Assessment in Neuro-Oncology (NANO)[58]	Neurological function: 8 Domains by Likert scale of 1-4	>18 years	Functional performance status reported by a physician; 9 items on a 4 point scale	5 minutes	Validated only in adults with brain tumors; does not allow for inclusion of age and developmental measurements
6-minute-walking test[59]	Walking as fast as possible without running on flat surface, height, weight, BMI	5-17 years	Functional performance status, including both motor strength and endurance, reported by a physician	10 minutes	Ethnicity, Race, Socialization may cause bias, reproducible, valid and reliable in children with cardiorespiratory disease
Epilepsy[96] Engel/ILAE classification[97]	Frequency and severity of seizures	Any age	Functional performance status reported by physician	10 minutes	
Visual Function					
Visual acuity-Teller Acuity Cards[62]	Visual acuity – preferential looking	Any age	Functional performance status reported by physician	15 minutes	Availability of cards LogMAR mandatory of both eyes
Visual acuity – HOTV charts[62]	Visual acuity – recogntion acuity	>3 years	Functional performance status reported by physician	15 minutes	LogMAR mandatory of both eyes
Optic disc[62]	Presence or absence of optic disc pallor or edema.	Any age	Functional performance status reported by physician	15 minutes	Grading is unreliable. Should be reported as present or absent.
Visual fields	Age adapted – confrontation testing; perimetry when older	>5 years	Functional performance status reported by physician	15 minutes	Poor reliability in young children
Optic coherence tomography[63,64]	Retinal nerve fiber thickness	Any age if used with anesthesia and hand-held device in non-cooperative children	Functional performance status reported by physician	15 minutes	Young children require sedation / general anesthesia and hand-held device. Operator expertise required. Comparability of results with different machines.
Diffusion Tensor Imaging [65]	Fractional anisotropy of optic radiations (white matter integrity)	Any age	MRI technique	15 minutes	Norm values need to be generated for age. Comparability of results with different machines.
Cognition					
Cogstate-	Detection (DET) Psychomotor Function,	5-21 years	Semi-automated,	25 minutes	Screening tool, not designed to

Cognitive function[72]	Identification (IDN) Attention, One Back (ONB) Working Memory, Groton Maze Learning (GML) Test Executive Function, Continuous Paired Associate Learning (CPAL) Paired Associate Learning		computerized cognitive testing system that was developed as a rapid and accurate test of cognitive function		be a comprehensive measure of neurocognitive functioning, Requires computer and trained research assistant proctor
European short batteries of tests[53]	Cognitive operation, executive abilities, psychomotor abilities	5- 18 years			Vary within Europe
Patient Reported Outcomes					
Vineland Adaptive Behavioral Scale[29]	Communication (Expressive and Receptive, Daily Living Skills (personal, domestic, and community), Socialization (Interpersonal relationships, play and leisure, coping skills), Motor Skills (Gross and Fine with a ceiling of abilities at 7 years), and Problem Behaviors	0 - 90 years	Patient/Caregiver Reported Outcome, Telephone interview possible	60 minutes	Forms may be complicated. Should be administered by someone with training.
Behavior Rating Inventory of Executive Function (BRIEF)[98]	Parent-rated behavior rating composed of 86 items designed to assess various aspects of executive function, including ability to inhibit emotional and behavioral responses, cognitive flexibility, working memory, planning and organization skills, and initiative.	5 – 18 years	Parent-reported outcomes of problems related to attention, memory and executive function that occur in everyday life.	10 minutes	
PedsQoL-BT module[82]	1) Cognitive problems (seven items), (2) Pain and hurt (three items), (3) Movement and balance (three items), (4) Procedural anxiety (three items), (5) Nausea (five items), and (6) Worry (three items).	2-25 years	Patient Reported Outcome: Patient:8-18, 18-25 years Caregiver: 2-4, 5-18 Years	20 minutes	Not validated in children < 2 years of age
PROMIS[89,90]	Physical function, anxiety, depression, fatigue, sleep disturbance, and satisfaction with social role, as well as pain intensity and interference	> 5 years	Patient/Caregiver Reported Outcome	20 minutes	Not specifically evaluated in a childhood LGG population
Endocrine Function					
Auxological assessment	Height, weight, body mass index, Tanner stages	Any age	Functional performance status reported by physician	10 minutes	
hypothalamic pituitary axis[92,99]	IGF-1, provocative growth hormone testing, LH, FSH, testosterone/ oestradiol, 0900 cortisol, TSH, free T4, PRL, paired morning urine/ plasma osmolality measurements, Sexual maturation: inhibin, anti-Müller-hormone	Any age	Functional performance status reported by physician		Confirmatory testing >GH dynamic testing >TSH test

Summary

1. Louis, D. N. et al. *WHO Classification of Tumours of the Central Nervous System, Revised*. Fourth Edition. (IARC, 2016).

2. Ostrom, Q. T. et al. *Alex's Lemonade Stand Foundation Infant and Childhood Primary Brain and Central Nervous System Tumors Diagnosed in the United States in 2007-2011.* Neuro Oncol 16 Suppl 10, x1-x36, doi:10.1093/neuonc/nou327 (2015).

3. Packer, R. J. et al. *Pediatric low-grade gliomas: implications of the biologic era.* Neuro Oncol, doi:10.1093/neuonc/now209 (2016).

4. Jones, D. T. et al. *Recurrent somatic alterations of FGFR1 and NTRK2 in pilocytic astrocytoma*. Nat Genet 45, 927-932, doi:10.1038/ng.2682 (2013).

5. Qaddoumi, I. et al. *Genetic alterations in uncommon low-grade neuroepithelial tumors: BRAF, FGFR1, and MYB mutations occur at high frequency and align with morphology.* Acta Neuropathol 131, 833-845, doi:10.1007/s00401-016-1539-z (2016).

6. Zhang, J. et al. *Whole-genome sequencing identifies genetic alterations in pediatric low-grade gliomas.* Nat Genet 45, 602-612, doi:10.1038/ng.2611 (2013).

7. Bandopadhayay, P. et al. *MYB-QKI rearrangements in angiocentric glioma drive tumorigenicity through a tripartite mechanism*. Nat Genet 48, 273-282, doi:10.1038/ng.3500 (2016).

8. Ramkissoon, L. A. et al. *Genomic analysis of diffuse pediatric low-grade gliomas identifies recurrent oncogenic truncating rearrangements in the transcription factor MYBL1*. Proc Natl Acad Sci U S A 110, 8188-8193, doi:10.1073/pnas.1300252110 (2013).

9. Louis, D. N. et al. *The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary.* Acta Neuropathol 131, 803-820, doi:10.1007/s00401-016-1545-1 (2016).

10. Rivera, B. et al. *Germline and somatic FGFR1 abnormalities in dysembryoplastic neuroepithelial tumors.* Acta Neuropathol 131, 847-863, doi:10.1007/s00401-016-1549-x (2016).

11. Hovestadt, V. et al. *Robust molecular subgrouping and copy-number profiling of medulloblastoma from small amounts of archival tumour material using high-density DNA methylation arrays.* Acta Neuropathol 125, 913-916, doi:10.1007/s00401-013-1126-5 (2013).

12. Pajtler, K. W. et al. *Molecular Classification of Ependymal Tumors across All CNS Compartments, Histopathological Grades, and Age Groups*. Cancer Cell 27, 728-743, doi:10.1016/j.ccell.2015.04.002 (2015).

13. Sturm, D. et al. *Hotspot mutations in H3F3A and IDH1 define distinct epigenetic and biological subgroups of glioblastoma.* Cancer Cell 22, 425-437, doi:10.1016/j.ccr.2012.08.024 (2012).

14. Mistry, M. et al. *BRAF Mutation and CDKN2A Deletion Define a Clinically Distinct Subgroup of Childhood Secondary High-Grade Glioma*. J Clin Oncol, doi:10.1200/JCO.2014.58.3922 (2015).

15. Korshunov, A. et al. *Integrated analysis of pediatric glioblastoma reveals a subset of biologically favorable tumors with associated molecular prognostic markers.* Acta Neuropathol 129, 669-678, doi:10.1007/s00401-015-1405-4 (2015).

16. Schwartzentruber, J. et al. *Driver mutations in histone H3.3 and chromatin remodelling genes in paediatric glioblastoma.* Nature 482, 226-231, doi:10.1038/nature10833 (2012).

17. Wu, G. et al. *Somatic histone H3 alterations in pediatric diffuse intrinsic pontine gliomas and non-brainstem glioblastomas*. Nat Genet 44, 251-253, doi:10.1038/ng.1102 (2012).

18. Pages, M. et al. *Co-occurrence of Histone H3 K27M and BRAF V600E mutations in paediatric midline grade I ganglioglioma.* Brain Pathol [Epub] (2016).

19. Garcia, M. A., Solomon, D. A. & Haas-Kogan, D. A. *Exploiting molecular biology for diagnosis and targeted management of pediatric low-grade gliomas.* Future Oncol 12, 1493-1506 (2016).

20. Olow, A. et al. *BRAF Status in Personalizing Treatment Approaches for Pediatric Gliomas*. Clin Cancer Res 22, 5312-5321 (2016).

21. Louis, D. N. et al. *cIMPACT-NOW (the consortium to inform molecular and practical approaches to CNS tumor taxonomy): a new initiative in advancing nervous system tumor classification.* Brain Pathol [Epub] (2016).

22. Louis, D. N. et al. *Announcing cIMPACT-NOW: the Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy.* Acta Neuropathol 133, 1-3 (2017).

23. Karajannis, M. A. et al. *Phase II study of sorafenib in children with recurrent or progressive low-grade astrocytomas.* Neuro Oncol 16, 1408-1416, doi:10.1093/neuonc/nou059 (2014).

24. Sievert, A. J. et al. *Paradoxical activation and RAF inhibitor resistance of BRAF protein kinase fusions characterizing pediatric astrocytomas.* Proc Natl Acad Sci U S A 110, 5957-5962 (2013).

25. Jones, D. T. et al. *Tandem duplication producing a novel oncogenic BRAF fusion gene defines the majority of pilocytic astrocytomas.* Cancer Res 68, 8673-8677, doi:10.1158/0008-5472.CAN-08-2097 (2008).

26. Kaul, A., Chen, Y. H., Emnett, R. J., Dahiya, S. & Gutmann, D. H. *Pediatric glioma-associated KIAA1549:BRAF expression regulates neuroglial cell growth in a cell type-specific and mTOR-dependent manner*. Genes Dev 26, 2561-2566, doi:10.1101/gad.200907.112 (2012).

27. Selt, F. et al. *Establishment and application of a novel patient-derived KIAA1549:BRAF-driven pediatric pilocytic astrocytoma model for preclinical drug testing*. Oncotarget [Epub ahead of print] (2016).

28. Bajenaru, M. L. et al. *Optic nerve glioma in mice requires astrocyte Nf1 gene inactivation and Nf1 brain heterozygosity.* Cancer Res 63, 8573-8577 (2003).

29. Gronych, J. et al. *An activated mutant BRAF kinase domain is sufficient to induce pilocytic astrocytoma in mice.* J Clin Invest 121, 1344-1348, doi:10.1172/JCI44656 (2011).

30. Kaul, A., Chen, Y. H., Emnett, R. J., Gianino, S. M. & Gutmann, D. H. *Conditional KIAA1549:BRAF mice reveal brain region- and cell type-specific effects*. Genesis 51, 708-716 (2013).

31. Mahoney DH, Cohen ME, Friedman HS, et al. *Carboplatin is effective therapy for young children with progressive optic pathway tumors: a Pediatric Oncology Group phase II study*. Neuro-oncology. 2000 Oct 1;2(4):213-20.

32. Ater JL, Xia C, Mazewski CM, et al. *Nonrandomized comparison of neurofibromatosis type 1 and non–neurofibromatosis type 1 children who received carboplatin and vincristine for progressive low-grade glioma: A report from the Children's Oncology Group. Cancer*. 2016 Jun 15;122(12):1928-36.

33. Ater JL, Zhou T, Holmes E, et al. *Randomized study of two chemotherapy regimens for treatment of low-grade glioma in young children: a report from the Children's Oncology Group*. Journal of Clinical Oncology. 2012 Jun 4;30(21):2641-7.

34. Laithier V, Grill J, Le Deley MC, et al. *Progression-free survival in children with optic pathway tumors: dependence on age and the quality of the response to chemotherapy—results of the first French prospective study for the French Society of Pediatric Oncology*. Journal of clinical oncology. 2003 Dec 15;21(24):4572-8.

35. Gnekow AK, Kortmann RD, Pietsch T, Emser A. *Low grade chiasmatic-hypothalamic glioma-carboplatin and vincristin chemotherapy effectively defers radiotherapy within a comprehensive treatment strategy*. Klinische Pädiatrie. 2004 Nov;216(06):331-42.

36. Packer RJ, Ater J, Allen J, et al. *Carboplatin and vincristine chemotherapy for children with newly diagnosed progressive low-grade gliomas. Journal of Neurosurgery*. 1997 May;86(5):747-54.

37. Gnekow AK, Falkenstein F, von Hornstein S, et al. *Long-term follow-up of the multicenter, multidisciplinary treatment study HIT-LGG-1996 for low-grade glioma in children and adolescents of the German Speaking Society of Pediatric Oncology and Hematology*. Neuro-oncology. 2012 Oct 1;14(10):1265-84.

38. Chintagumpala, M, Eckel, S.P., Krailo, M, et al. A *Pilot Study Using Carboplatin, Vincristine and Temozolamide in Children with Progressive/ Symptomatic low-grade gliomas: a children's oncology group study*. Neuro-oncology 17(8) 1132-1138, 2015.

39. (Personal communication, E. Bouffet and D. Walker)

40. Jakacki RI, Bouffet E, Adamson PC, et al. *A phase 1 study of vinblastine in combination with carboplatin for children with low-grade gliomas: a Children's Oncology Group phase 1 consortium study*. Neuro-oncology. 2011 Aug 1;13(8):910-5.

41. Massimino M, Spreafico F, Riva D, et al. *A lower-dose, lower-toxicity cisplatin–etoposide regimen for childhood progressive low-grade glioma*. Journal of neuro-oncology. 2010 Oct 1;100(1):65-71.

42. Massimino M, Spreafico F, Cefalo G, et al. *High response rate to cisplatin/etoposide regimen in childhood low-grade glioma. Journal of clinical oncology*. 2002 Oct 15;20(20):4209-16.

43. Kadota RP, Kun LE, Langston JW, et al. *Cyclophosphamide for the treatment of progressive low-grade astrocytoma: a Pediatric Oncology Group phase II Study*. Journal of pediatric hematology/oncology. 1999 May 1;21(3):198-202.

44. Gururangan S, Fisher MJ, Allen JC, et al. *Temozolomide in children with progressive low-grade glioma*. Neuro-oncology. 2007 Apr 1;9(2):161-8.

45. Lassaletta A, Scheinemann K, Zelcer SM, et al. *Phase II weekly vinblastine for chemotherapy-naive children with progressive low-grade glioma: a Canadian Pediatric Brain Tumor Consortium Study*. Journal of Clinical Oncology. 2016 Aug 29;34(29):3537-43.

46. Banerjee, A, Jakack, R, Arzu Onar-Thomas, et al. A Phase 1 Trial of the MEK Inhibitor Salumetinib (AZD644) in Pediatric Patients with recurrent or Refractory Low-Grade Gliomas: A
Pediatric Brain Tumor Consortium (PBTC

47. To be added

48. Dombi E, Baldwin A, Marcus LJ, et al. *Activity of Selumetinib in Neurofibromatosis Type 1–Related Plexiform Neurofibromas*. New England Journal of Medicine. 2016 Dec 29;375(26):2550-60.

49. Mark Kieran, Eric Bouffet, Uri Tabori, et al. *The first study of dabrafenib in pediatric patients with BRAF V600–mutant relapsed or refractory low-grade gliomas.* ESMO Copenhagen Oct 2016 Abstract: LBA19_PR.

50. Skrypek M, Foreman N, Guillaume D, Moertel C. *Pilomyxoid astrocytoma treated successfully with vemurafenib*. Pediatr Blood Cancer. 2014 Nov;61(11):2099-100. doi: 10.1002/pbc.25084. PMID: 24821190

51. Lassaletta A, Guerreiro Stucklin A, Ramaswamy V, et al. Profound Clinical and radiological response to BRAF inhibition in a 2-month-old diencephalic child with hypothalamic/ chiasmatic glioma. Pedatr Blood Cancer. 2016 Nov;63(11):2038-41. Doi: 10.1002/pbc.26086. PMID: 27398937

52. Sun Y, Alberta JA, Pilarz C, et al. A brain-penetrant RAF dimer antagonist for the noncanonical BRAF oncoprotein of pediatric low-grade astrocytomas. Neuro Oncol. 2017 Jan 12. Pii: now261. Doi: 10.1093/neuroonc/now261. [Epub ahead of print] PMID: 28082416

53. Limond JA, Bull KS, Calaminus G, Kennedy CR, Spoudeas HA, Chevignard MP. Quality of survival assessment in European childhood brain tumour trials, for children aged 5 years and over. European Journal of Paediatric Neurology 2015;19:202-10.

54. Lansky SB, List MA, Lansky LL, Ritter-Sterr C, Miller DR. The measurement of performance in childhood cancer patients. Cancer 1987;60:1651-6.

55. Orr ST, Aisner J. Performance status assessment among oncology patients: a review. Cancer Treatment Reports 1986;70:1423-9.

56. Roila F, Lupattelli M, Sassi M, et al. Original article: Intra and interobserver variability in cancer patients' performance status assessed according to Karnofsky and ECOG scales. Annals of Oncology 1991;2:437-9.

57. Hutchinson TA, Boyd NF, Feinstein AR, Gonda A, Hollomby D, Rowat B. Scientific problems in clinical scales, as demonstrated in the Karnofsky index of performance status. Journal of Chronic Diseases 1979;32:661-6.

58. Nayak L, DeAngelis L, Wen P, et al. The Neurologic Assessment in Neuro-Oncology (NANO) Scale: A Tool to Assess Neurologic Function for Integration in the Radiologic Assessment in Neuro-Oncology (RANO) Criteria (S22.005). Neurology 2014;82.

59. Ulrich S, Hildenbrand FF, Treder U, et al. Reference values for the 6-minute walk test in healthy children and adolescents in Switzerland. BMC Pulmonary Medicine 2013;13:49.

60. Fisher MJ, Loguidice M, Gutmann DH, et al. Visual outcomes in children with neurofibromatosis type 1–associated optic pathway glioma following chemotherapy: a multicenter retrospective analysis. Neuro-Oncology 2012;14:790-7.

61. Shofty B, Ben-Sira L, Freedman S, et al. Visual outcome following chemotherapy for progressive optic pathway gliomas. Pediatric Blood & Cancer 2011;57:481-5.

62. Fisher MJ, Avery RA, Allen JC, et al. Functional outcome measures for NF1-associated optic pathway glioma clinical trials. Neurology 2013;81:S15-S24.

63. Avery RA, Liu GT, Fisher MJ, et al. Retinal Nerve Fiber Layer Thickness in Children With Optic Pathway Gliomas. American Journal of Ophthalmology 2011;151:542-9.e2.

64. Avery RA, Hwang EI, Ishikawa H, et al. Handheld optical coherence tomography during sedation in young children with optic pathway gliomas. JAMA Ophthalmology 2014;132:265-71.

65. de Blank PMK, Berman JI, Liu GT, Roberts TPL, Fisher MJ. Fractional anisotropy of the optic radiations is associated with visual acuity loss in optic pathway gliomas of neurofibromatosis type 1. Neuro-Oncology 2013;15:1088-95.

66. Ris MD, Beebe DW, Armstrong FD, et al. Cognitive and Adaptive Outcome in Extracerebellar Low-Grade Brain Tumors in Children: A Report From the Children's Oncology Group. Journal of Clinical Oncology 2008;26:4765-70.

67. Beebe DW, Ris MD, Armstrong FD, et al. Cognitive and Adaptive Outcome in Low-Grade Pediatric Cerebellar Astrocytomas: Evidence of Diminished Cognitive and Adaptive Functioning in National Collaborative Research Studies (CCG 9891/POG 9130). Journal of Clinical Oncology 2005;23:5198-204.

68. Tonning Olsson I, Perrin S, Lundgren J, Hjorth L, Johanson A. Long-Term Cognitive Sequelae After Pediatric Brain Tumor Related to Medical Risk Factors, Age, and Sex. Pediatric Neurology 2014;51:515-21.

69. Turner CD, Chordas CA, Liptak CC, et al. Medical, psychological, cognitive and educational late-effects in pediatric low-grade glioma survivors treated with surgery only. Pediatric Blood & Cancer 2009;53:417-23.

70. Ullrich NJ, Embry L. Neurocognitive Dysfunction in Survivors of Childhood Brain Tumors. Seminars in Pediatric Neurology 2012;19:35-42.

71. Roddy E, Sear K, Felton E, et al. Presence of cerebral microbleeds is associated with worse executive function in pediatric brain tumor survivors. Neuro-Oncology 2016;18:1548-58.

72. Maruff P, Thomas E, Cysique L, et al. Validity of the CogState Brief Battery: Relationship to Standardized Tests and Sensitivity to Cognitive Impairment in Mild Traumatic Brain Injury, Schizophrenia, and AIDS Dementia Complex. Archives of Clinical Neuropsychology 2009;24:165-78.

73. World Health Organization. International classification of functioning disability and health. Geneva: 2001.
74. Netson KL, Conklin HM, Wu S, Xiong X, Merchant TE. A 5-Year Investigation of Children's Adaptive Functioning Following Conformal Radiation Therapy for Localized Ependymoma. International Journal of Radiation Oncology*Biology*Physics 2012;84:217-23.e1.

75. Msall ME. Developing preschool surveillance tools for adaptive functioning: Lessons for neuro-oncology. European Journal of Paediatric Neurology 2010;14:368-79.

76. Ashford JM, Netson KL, Clark KN, et al. Adaptive functioning of childhood brain tumor survivors following conformal radiation therapy. Journal of Neuro-Oncology 2014;118:193-9.

77. Pastore V, Colombo K, Villa F, et al. Psychological and adjustment problems due to acquired brain lesions in pre-school-aged patients. Brain Injury 2013;27:677-84.
78. Vago C, Bulgheroni S, Usilla A, et al. Adaptive functioning in children in the first six months after surgery for brain tumours. Disability and Rehabilitation 2011;33:953-60.

79. Poggi G, Liscio M, Pastore V, et al. Psychological intervention in young brain tumor survivors: The efficacy of the cognitive behavioural approach. Disability & Rehabilitation 2009;31:1066-73.

80. Papazoglou A, King TZ, Morris RD, Morris MK, Krawiecki NS. Attention mediates radiation's impact on daily living skills in children treated for brain tumors. Pediatric Blood & Cancer 2008;50:1253-7.

81. Limperopoulos C, Majnemer A, Steinbach CL, Shevell MI. Equivalence Reliability of the Vineland Adaptive Behavior Scale Between In-Person and Telephone Administration. Physical & Occupational Therapy In Pediatrics 2006;26:115-27.

82. Palmer SN, Meeske KA, Katz ER, Burwinkle TM, Varni JW. The PedsQL™ brain tumor module: Initial reliability and validity. Pediatric Blood & Cancer 2007;49:287-93.

83. Lassaletta A, Scheinemann K, Zelcer SM, et al. Phase II Weekly Vinblastine for Chemotherapy-Naïve Children With Progressive Low-Grade Glioma: A Canadian Pediatric Brain Tumor Consortium Study. Journal of Clinical Oncology 2016;34:3537-43.

84. Nwachukwu CR, Youland RS, Chioreso C, et al. Health related quality of life (HRQOL) in long-term survivors of pediatric low grade gliomas (LGGs). Journal of Neuro-Oncology 2015;121:599-607.

85. Musial-Bright L, Panteli L, Hernáiz Driever P. Pediatric low-grade glioma survivors experience high quality of life. Child's Nervous System 2011;27:1895.

86. Aarsen FK, Paquier PF, Reddingius RE, et al. Functional outcome after low-grade astrocytoma treatment in childhood. Cancer 2006;106:396-402.

87. Bull KS, Liossi C, Peacock JL, Yuen HM, Kennedy CR. Screening for cognitive deficits in 8 to 14-year old children with cerebellar tumors using self-report measures of executive and behavioral functioning and health-related quality of life. Neuro-Oncology 2015;17:1628-36.

88. Bhat SR, Goodwin TL, Burwinkle TM, et al. Profile of Daily Life in Children With Brain Tumors: An Assessment of Health-Related Quality of Life. Journal of Clinical Oncology 2005;23:5493-500.

89. Dobrozsi S, Yan K, Hoffmann R, Panepinto J. Patient-reported health status during pediatric cancer treatment. Pediatric Blood & Cancer 2016:n/a-n/a.

90. Wolters PL, Martin S, Merker VL, et al. Patient-reported outcomes of pain and physical functioning in neurofibromatosis clinical trials. Neurology 2016;87:S4-S12.

91. Cappelli C, Grill J, Raquin M, et al. Long term follow up of 69 patients treated for optic pathway tumours before the chemotherapy era. Archives of disease in childhood 1998;79:334-8.

92. Gan H-W, Phipps K, Aquilina K, Gaze MN, Hayward R, Spoudeas HA. Neuroendocrine Morbidity After Pediatric Optic Gliomas: A Longitudinal Analysis of 166 Children Over 30 Years. The Journal of Clinical Endocrinology & Metabolism 2015;100:3787-99.

93. Gnekow AK, Falkenstein F, von Hornstein S, et al. Long-term follow-up of the multicenter, multidisciplinary treatment study HIT-LGG-1996 for low-grade glioma in children and adolescents of the German Speaking

Society of Pediatric Oncology and Hematology. Neuro-Oncology 2012;14:1265-84.

94. Brauner R, Trivin C, Zerah M, et al. Diencephalic Syndrome due to Hypothalamic Tumor: A Model of the Relationship between Weight and Puberty Onset. The Journal of Clinical Endocrinology & Metabolism 2006;91:2467-73.

95. Kilday J-P, Bartels U, Huang A, et al. Favorable survival and metabolic outcome for children with diencephalic syndrome using a radiation-sparing approach. Journal of Neuro-Oncology 2014;116:195-204.

96. Ullrich NJ, Pomeroy SL, Kapur K, Manley PE, Goumnerova LC, Loddenkemper T. Incidence, risk factors, and longitudinal outcome of seizures in long-term survivors of pediatric brain tumors. Epilepsia 2015;56:1599-604.

97. Durnford AJ, Rodgers W, Kirkham FJ, et al. Very good inter-rater reliability of Engel and ILAE epilepsy surgery outcome classifications in a series of 76 patients. Seizure - European Journal of Epilepsy;20:809-12.

98. Gioia GA, Isquith PK, Guy SC, L K. Behavior rating inventory of executive function. Child Neuropsychology 2000;6:235-8.

99. Clement SC, Meeteren AYNS-v, Boot AM, et al. Prevalence and Risk Factors of Early Endocrine Disorders in Childhood Brain Tumor Survivors: A Nationwide, Multicenter Study. Journal of Clinical Oncology 2016;34:4362-70.