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Optimizing preclinical pediatric low-grade glioma models for meaningful clinical translation

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Abstract

Pediatric low-grade gliomas (pLGGs) are the most common brain tumor in young children. While they are typically associated with good overall survival, children with these central nervous system tumors often experience chronic tumor- and therapy-related morbidities. Moreover, individuals with unresectable tumors frequently have multiple recurrences and persistent neurological symptoms. Deep molecular analyses of pLGGs reveal that they are caused by genetic alterations that converge on a single mitogenic pathway (MEK/ERK), but their growth is heavily influenced by nonneoplastic cells (neurons, T cells, microglia) in their local microenvironment. The interplay between neoplastic cell MEK/ERK pathway activation and stromal cell support necessitates the use of predictive preclinical models to identify the most promising drug candidates for clinical evaluation. As part of a series of white papers focused on pLGGs, we discuss the current status of preclinical pLGG modeling, with the goal of improving clinical translation for children with these common brain tumors.

Key Points

1. New and complementary in vitro and in vivo pLGG preclinical models are required, particularly those incorporating stromal interactions.
2. The advantages and limitations of each model must be considered.
3. Preclinical data should meet minimum criteria for successful clinical translation.

Primary tumors of the central nervous system (CNS) are the most common cause of cancer-related death in children.[1] In contrast to adults, the majority of these tumors are low-grade gliomas (LGGs), typically developing in children younger than 14 years of age. Specifically, gliomas account for 50% of all CNS tumors seen in children between the ages of 1 and 9 years, with pilocytic astrocytoma (PA; World Health Organization grade 1 astrocytomas) representing the most common circumscribed astrocytic glioma entity (15.3%) in children and adolescents. These tumors most frequently arise in the cerebellum, and harbor genomic aberrations involving the *BRAF* kinase gene.[2–4] *BRAF* alterations frequently include fusion of the *BRAF* kinase domain with the *KIAA1549* or *FAM131B* genes or oncogenic *BRAF* gene mutation ($BRAF^{V600E}$), resulting in increased BRAF kinase activation of the MAPK (MEK/ERK) signaling pathway.[5,6] Other locations include the optic pathway (optic nerve, chiasm, tracts, and radiations), specifically in children with the neurofibromatosis type 1 (NF1) cancer predisposition syndrome,[7] as well as the brainstem and other midline structures.[8] In NF1-associated LGGs, bi-allelic loss of *NF1* gene/protein expression is observed, which leads to increased MEK/ERK signaling as a result of impaired RAS GTPase activating protein function.[9–11] While less common, some children with midline LGGs harbor mutations or fusions involving the fibroblast growth factor receptor-1 (*FGFR1*), Raf-1 proto-oncogene (*RAF1*) or TrkB (*NTRK2*) receptor, as well as other less frequently altered genes,[12] also result in elevated MEK/ERK growth pathway signaling.[13] The common effect of all the described molecular alterations is MAPK (ERK) pathway hyperactivation, thus representing one of the hallmarks of pediatric LGGs (pLGG). Finally, in contrast to adult gliomas, additional mutations are not commonly detected, and *IDH1/2* gene

mutations are extremely rare and more typically found in other WHO histopathologic entities (diffuse astrocytoma, oligodendroglioma).[12,14]

At the histologic level, PAs are characterized by a predominant solid pattern of growth, with variable compact areas composed of elongated cells with thick eosinophilic processes and frequent Rosenthal fibers ("Piloid" morphology), alternating with myxoid rich, microcystic areas with variable oligodendrocyte-like cells and eosinophilic granular bodies. Detailed morphologic and immunophenotypic analysis of the individual cell components of pLGGs reveals that the tumor cells are embedded in a microenvironment composed of nonneoplastic cells, including neurons, T cells, and monocytes.[10,15–17] These noncancerous stromal cells are key contributors to pLGG pathobiology, where they provide paracrine factors that support tumor growth and survival.[18–24] The dependence on stromal cells is additionally illustrated by the challenges encountered when attempting to maintain human pLGG cells in vitro or as patient-derived xenografts in vivo, where the required supportive microenvironment does not exist.[25–31]

While pLGGs are not usually fatal in children (98% 5-year overall survival rates), they are associated with significant neurological and neuroendocrine deficits, such as vision loss, motor deficits, seizure disorders, and precocious puberty.[32–35] In this respect, pLGGs should be considered a nonfatal chronic disease of neurodevelopment, often requiring multiple lines of treatment and a thoughtful consideration of quality of life issues, where preservation of intact neurological function is paramount. As such, there is a need for chemopreventative strategies that focus on delaying the need for definitive treatment by interrupting the supportive tumor microenvironment or targeting glioma cell senescence.

The current standard of care encompasses maximal safe neurosurgical resection, chemotherapy (e.g., carboplatin, vincristine; vinblastine), and, in some rare situations, radiotherapy. Since these tumors are largely diseases of MAPK hyperactivation, MEK inhibitors (eg, selumetinib, trametinib) have entered the clinical workplace through successfully closed[36–38] and ongoing clinical trials for progressive pLGG. In addition, novel drugs targeting the MAPK pathway, such as the combination of a BRAF inhibitor (dabrafenib) and a MEK inhibitor (trametinib), have recently been approved by the FDA for first-line therapy in *BRAF*^{V600E}-mutant pLGG (NCT02684058). Similarly, the BRAF inhibitor, tovorafenib, is currently in phase I/II trials for *BRAF*-altered pLGG (eg, FIREFLY-1; PNOC026, NCT04775485), as well as in a global randomized phase III registration trial for newly diagnosed *BRAF*-altered pLGG (LOGGIC-Firefly 2, NCT05566795).

Prior to clinical translation into phase I/II trials, preclinical data must fulfill a set of requirements that center on the histologic type, composition, and specificity of the preclinical models used, such as (but not limited to) target dependency, blood-brain-barrier (BBB) penetrance, pharmacokinetic (PK) and -dynamic (PD) properties. Unfortunately, there is currently a paucity of authenticated pLGG preclinical models and a lack of guidelines to optimize effective clinical translation. In this white paper, a team of clinicians and scientists was coalesced to review the current preclinical platforms, provide recommendations for their authentication and use, and suggest approaches to ensure successful translation to clinical practice.

Preclinical Tissue Culture Models of Pediatric Low-Grade Glioma

A wide range of mouse,[39,40] as well as patient- and human iPSC-derived LGG cell lines[26,27,29,31,41–47] have been developed (Table 1). Over the past 2 decades, these platforms have been successfully used for drug-target development and testing. Although lacking a supportive tumor microenvironment and significant cellular heterogeneity, these cell line models have the advantage of scalability, long-term usage, short-term availability, and comparatively low cost.

One of the major barriers to establishing these cell line culture systems has been the induction of oncogene-induced senescence (OIS)[25,30] and the senescence-associated secretory phenotype (SASP).[28] The use of specialized media has enabled these lines to be maintained in vitro, while still preserving pLGG growth properties and genetic driver expression.[29,31]

As detailed in Table 1, current human pLGG cell lines are derived from children with PA (*NF1* loss, *BRAF*^{V600E} mutation, or *KIAA1549: BRAF* expression) or pleomorphic xanthoastrocytoma (PXA) (BT40 line[42]), and have been used for in vitro modeling studies.[29,45,46] As such, the Res186 PA tumor has a homozygous *PTEN* deletion and loss of heterozygosity at the *TP53* locus,[46] alterations not typical of pLGG, whereas the BT40 PXA cell line[42] harbors both a *BRAF*^{V600E} mutation and *CDKN2A* deletion, a combination common in PXA. Additionally, the DKFZ-BT66, -BT308, -BT314, and -BT317 cell lines with *BRAF* fusions or *BRAF*^{V600E} mutation were derived from primary pediatric PA tumors, where long-term expansion was achieved by blocking OIS induction through inducible SV40-TAg expression. The use of inducible SV40-TAg expression allows these models to switch off SV40-TAg expression and enter OIS with activation of the SASP, making it possible to study senescence in a cell line model system.[26,44] To bypass oncogene-induced senescence in the NF1-PA cell line, JHH-NF1-PA1, an alternative strategy leading to conditional cellular reprogramming was applied through exposure to a Rho kinase (ROCK) inhibitor in the presence of irradiated fibroblast cells or medium. This technique reversibly blocks senescence in epithelial cells,[56] and subsequently maintains molecular features and phenotypes in a variety of normal and tumor cell cultures.[57]

In addition to human pLGG cell lines, human iPSC engineering methods have been employed to generate preneoplastic cell types with specific pLGG-associated mutations. As such, hiPSC lines harboring homozygous *NF1* loss or ectopic *KIAA1549: BRAF* expression allow for differentiation into multiple progenitors, including neural stem cells, glial restricted progenitors, and oligodendroglial progenitor cells to study the consequences of these alterations on progenitor cell biology in vitro and gliomagenesis in vivo.[31] Moreover, the deployment of specific immunodeficient mouse strains has allowed for in vivo modeling using the human iPSC-derived pLGG cell lines.[31]

Table 1. Existing Preclinical Pediatric LGG Models

Model Type	Name of Model	Usage	Origin Species	Reference	pLGG Type	Tumor Latency	Detailed Genetics of Model	Additional Alterations
Mouse models								
GEMM	*Nf1*-OPG	In vivo	Mouse	[48]	OPG/LGG	3 months	$Nf1^{neo/flox}$; GFAP-Cre	None
GEMM	*Nf1*-OPG	In vivo	Mouse	[49]	OPG/LGG	6 weeks	$Nf1^{flox/flox}$; GFAP-Cre	None
GEMM	*Nf1*-OPG	In vivo	Mouse	[50]	OPG/LGG	6 months	$Nf1^{neo/flox}$; Olig2-Cre	None
GEMM	*Nf1*-OPG	In vivo	Mouse	[50]	OPG/LGG	3 months	$Nf1^{neo/flox}$; CD133-Cre	None
GEMM	*Nf1*-OPG	In vivo	Mouse	[51]	OPG/LGG	3 months	$Nf1^{neo/flox}$; GFAP-Cre-ER	None
GEMM	*Nf1*-OPG	In vivo	Mouse	[52]	OPG/LGG	3 months	$Nf1^{neo/flox}$; BLBP-Cre	None
GEMM	R681X-*Nf1*-OPG	In vivo	Mouse	[53]	OPG/LGG	3 months	$Nf1^{R681X}$/flox; GFAP-Cre	None
GEMM	R618X-*Nf1*-OPG	In vivo	Mouse	unpublished	OPG/LGG	6 months	$Nf1^{R816X}$/flox; GFAP-Cre	None
GEMM	Y2083X-*Nf1*-OPG	In vivo	Mouse	unpublished	OPG/LGG	3 months	$Nf1^{Y2083X/flox}$; GFAP-Cre	None
GEMM	*Nf1*-OPG; *Pten*-mutant	In vivo	Mouse	[54]	OPG/LGG	3 months	$Nf1^{neo/flox}$; $Pten^{wt/flox}$; GFAP-Cre	None
GEMM	*Nf1*-OPG; *KIAA1549:BRAF*	In vivo	Mouse	[54]	OPG/LGG	3 months	$Nf1^{neo/flox}$; *KIAA1549:BRAF*^{flox}; GFAP-Cre	None
RCAS-TVA system	*BRAF*V600E KD-RCAS-Ntv-a	In vivo	Mouse	[55]	PA	2 months	*BRAF*^{V600E} kinase domain	None
Human models (patient-derived)								
Patient-derived cell line	BT40	In vivo and in vitro	Human	[41,42]	PXA	n/a	$BRAF^{V600E}$	*CDKN2A* del
Patient-derived cell line	BT35 PDX	In vivo (zebrafish) and in vitro	Human	[43]	AA	n/a	$BRAF^{wt}$	Unknown
Patient-Derived cell line	DKFZ-BT66	In vivo (zebrafish) and in vitro	Human	[27, 44]	PA	n/a	*KIAA1549:BRAF*	None
Patient-derived cell line	DKFZ-BT308	In vivo (zebrafish) and in vitro	Human	[26]	PA	n/a	*KIAA1549:BRAF*	None
Patient-derived cell line	DKFZ-BT314	In vivo (zebrafish) and in vitro	Human	[26]	PA	n/a	BRAF V600E	None
Patient-derived cell line	DKFZ-BT317	In vivo (zebrafish) and in vitro	Human	[26]	PA	n/a	*KIAA1549:BRAF*	None
Patient-derived cell line	JHH-NF1-PA1	In vivo and in vitro	Human	[29]	PA	n/a	*NF1* loss	None
Patient-derived cell line	JHH-PXA-1 PDX	In vivo and in vitro	Human	[29]	PXA	n/a	*TSC2*-mutant	Unknown
Patient-derived cell line	Res186	In vivo and in vitro	Human	[45-47]	PA	n/a	none detected	Homozygous *PTEN* del, *TP53* LOH
Patient-derived cell line	WUPA1	In vivo and in vitro	Human	unpublished	PA	n/a	*KIAA1549:BRAF*	None
Patient-derived cell line	WUPA2	In vivo and in vitro	Human	unpublished	PA	n/a	*NF1* loss	None
Patient-derived cell line	WUPA3	In vivo and in vitro	Human	unpublished	PA	n/a	*NF1* loss	None
Patient-derived cell line	WUPA4	In vivo and in vitro	Human	unpublished	PA	n/a	*NF1* loss, *FGFR1*-mutant	None

Table 1. Continued

Model Type	Name of Model	Usage	Origin Species	Reference	pLGG Type	Tumor Latency	Detailed Genetics of Model	Additional Alterations
Humanized models (iPSC derived)								
iPSC-derived cell line	*NF1*-null iNPCs, iGRPs, iOPCs	In vivo and in vitro	Human	[31]	LGG	1 month	*NF1*-2041C>T	None
iPSC-derived cell line	*NF1*-null iNPCs, iGRPs, iOPCs	In vivo and in vitro	Human	[31]	LGG	1 month	*NF1*-6513T>A	None
iPSC-derived cell line	*KIAA1549:BRAF*-expressing iNPCs, iGRPs, iOPCs	In vivo and in vitro	Human	[31]	LGG	1 month	*KIAA1549:BRAF*	None

Abbreviations: del, deletion; iPSC, induced pluripotent stem cell; KD, kinase domain; LGG, low-grade glioma; OPG, optic pathway glioma; LOH, loss of heterozygosity; n/a, not available or not analyzed; PA, pilocytic astrocytoma; PDX, patient-derived xenograft; PXA, pleomorphic xanthoastrocytoma; RCAS, Replication-Competent ASLV long terminal repeat (LTR) with a Splice acceptor; wt, wild type.

Critical Features of pLGGs for In Vivo Modeling

The rarity of low-grade tumors of glial origin in adulthood, as well as the clear biological and molecular differences between pediatric MAPK-activated compact low-grade gliomas and adult, typically *IDH1/2* mutated, diffuse low-grade gliomas, suggests the existence of one or more neural stem or progenitor cell population(s) in the developing brain. These progenitor cell populations are susceptible to hyperactivation of the MEK/ERK/MAPK signaling pathway and are vulnerable to neoplastic transformation. Pediatric LGGs typically arise in specific brain locations (optic pathway, brainstem, cerebellum), variably cause clinical signs or symptoms, and rarely, if ever, evolve into malignant gliomas. These unique disease characteristics argue that optimal in vivo model(s) should recapitulate: (1) an early developmental origin, (2) a dependency of the susceptible cell(s)-of-origin on MEK/ERK signaling (MEK/ERK dependency), and (3) histopathologic features seen in human pLGGs.

The early development of these tumors in childhood suggests that the cell of origin must undergo genetic alteration either during embryonic or early postnatal life. Support for this derives from the use of an inducible LoxP/Cre transgenesis system in which *Nf1* loss can be induced in GFAP- or CD133-expressing neural progenitor cells of *Nf1*-mutant mice by the administration of tamoxifen. Using this platform, *Nf1* mouse optic gliomagenesis required that bi-allelic *Nf1* inactivation occurs prior to birth,[50,51] consistent with an early cellular origin for pLGGs. Indeed, this principle of expression of genetic drivers in neural progenitor cells is not restricted to NF1-LGGs, but can be applied to other drivers, such as mutant $BRAF^{V600E}$. Introduction of the $BRAF^{V600E}$ kinase domain into nestin-expressing mouse neural progenitor cells successfully generated PAs in mice.[55]

While sporadic and NF1-associated pLGGs arise in different brain regions, these tumors share a common feature, MEK/ERK dependency during development. Prior research revealed an essential role for Mek/Erk signaling in glial differentiation from neural stem cells in different regions of the developing brain. Loss of both *Mek1* and *Mek2*, which eliminates Erk signaling, does not overtly affect neuronal differentiation but blocks the transition from the neurogenic to gliogenic phase of radial glial stem cells during cerebral cortical development.[58] This developmental arrest completely blocks the generation of astrocytes in the cerebral cortex. Similarly, loss of Erk signaling due to the inactivation of a positive pathway regulator, Shp2, blocks the generation of an astrocyte lineage, which normally differentiates into Bergmann glia during cerebellar development.[59] As such, the dependency on Erk signaling in the genesis of astrocytic lineages in the developing cerebral cortex and cerebellum can potentially cause developmental vulnerability of a particular neural stem cell or astrocyte precursor cell population(s) to abnormal Erk pathway activation, and consequently, make it susceptible to pLGG formation.[60] In this manner, pediatric low-grade gliomagenesis usurps some of the same cellular

and signaling dependencies observed during normal brain development.

Importantly, pLGG formation in mice and other species should be evaluated using similar criteria used to classify human brain tumors.[61] These include the presence of (1) a mass-occupying lesion with architectural distortion by standard H&E staining (and by MRI when possible), with (2) increased proliferation (Ki67 labeling index > 1%), and (3) immunopositivity for glial immunohistochemical markers (eg, GFAP and OLIG2). Attention should also be paid to the brain location and age when these tumors arise, as well as the presence of neurologic signs seen in children with the same tumors (eg, vision loss).

Preclinical Mouse Models of Pediatric Low-Grade Glioma

Current preclinical models largely reflect the two most common genetic alterations seen in children, those with sporadic pLGGs (*KIAA1549: BRAF* genomic rearrangement) and those arising in the setting of the NF1 (biallelic *NF1* loss). These experimental platforms include genetically engineered animals (pigs and mice), human-induced pluripotent stem cells, and patient-derived low-grade glioma cell lines (Figure 1).

While genetically engineered mouse models of sporadic pLGG have not been successfully established to date,[62] mice and swine have been created that develop the most common brain tumor in children with NF1, the optic pathway glioma (OPG). These models differ with respect to their genetic engineering: miniature pigs created with germline *NF1* gene mutations develop OPGs with an estimated penetrance of 15% following stochastic loss of the remaining wild-type *NF1* allele.[63] In contrast, mice with germline *Nf1* gene mutations have conditional loss of the wild-type allele in specific neuroglial progenitor cells at particular times during embryonic development, leading to OPG development in nearly all mice.[48,49,64] Using conditional and inducible transgenesis methods, somatic *Nf1* loss can be limited to specific cell types (eg, neural stem cells, astrocytes, glial progenitors, oligodendroglial progenitors) at particular times of embryonic and postnatal life or in the setting of particular germline *Nf1* gene mutations, resulting in different degrees of tumor penetrance and growth.[50,51,65]

To complement models in which tumors spontaneously arise in animals with intact immune systems, explant (in vivo) models have been generated (Table 1). These fall into two general categories: engineered human stem cell and tumor-derived implantation model systems. Tumor cells from low-grade gliomas can also be derived from genetically engineered mouse tumors, maintained in culture as primary cell lines or allografted into naïve immunocompetent animals.[29,50] Similarly, patient-derived tumor cell lines can be generated under conditions that limit senescence to study their growth control pathways or xenografted into immune-competent zebrafish (survive for <7 days) or immune-defective mice (for at least 6 months).[29,31,44,66] Alternatively, human induced pluripotent or embryonic stem cells can be engineered with biallelic *NF1* mutations or *KIAA1549: BRAF* expression, differentiated into appropriate neuroglial progenitors or organoids and studied

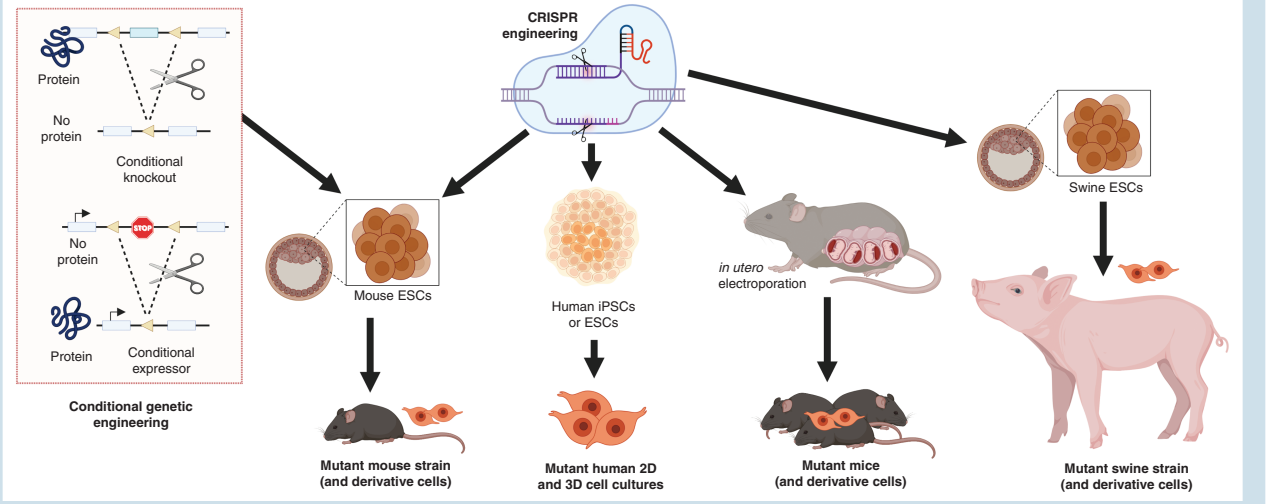

Figure 1. Genetic engineering of pLGG models. Genetically engineered mice can be generated to conditionally lose expression of a tumor suppressor gene (eg, *NF1* gene; conditional knockout) or gain expression of an oncogenic gene (eg, *KIAA1549: BRAF*, *BRAF*^{V600E}; conditional expression) using Cre-mediated excision. In these models, LoxP sites (denoted by the triangles) are inserted into the genomic DNA either flanking an exon of a tumor suppressor gene or a stop codon, such that when the Cre recombinase enzyme expressed in a cell type- or tissue-specific manner, it results in inactivation of the tumor suppressor gene (loss of protein expression) or removal of the stop codon (gain of protein expression). In addition, CRISPR/Cas9 engineering can be used to introduce specific genetic alterations in mouse embryonic stem cells (ESCs) to generate mutant mouse strains (and derivative tumors), human induced pluripotent stem cells (iPSCs) or ESCs to establish progenitor cell 2D cultures or 3D organoid cultures, or individual embryos by electroporation to generate litters of mutant mice (and derivative tumors) in a more rapid through-put fashion. This figure was produced using BioRender.

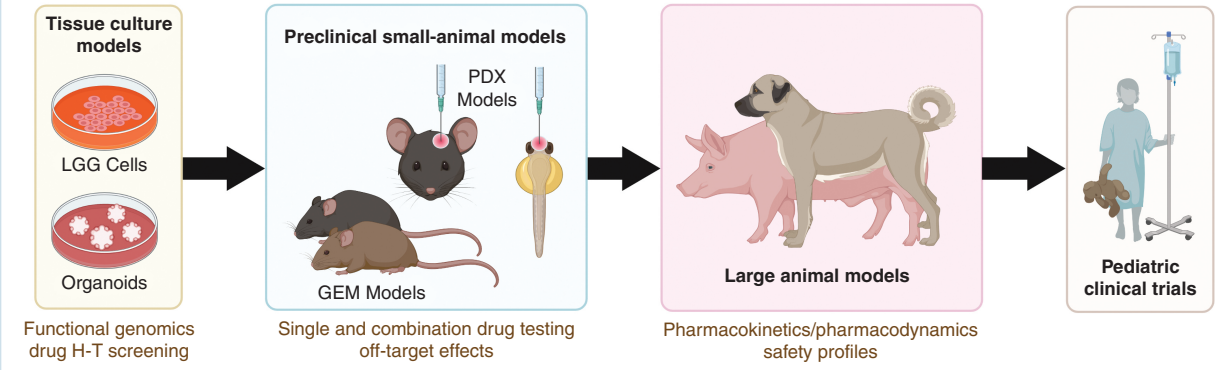

Figure 2. An optimized pipeline for pLGG could encompass tissue culture models for rapid drug screening and functional genomics, yielding a limited number of high priority candidates for in vivo testing for their impact on tumor growth and progression (both as single agents and combinatorial therapies), as well as off-target effects, in authenticated preclinical PDX (mice, zebrafish) and genetically engineered mouse models. Prior to clinical translation, candidates could be evaluated in large animal models, where drug pharmacokinetic/pharmacodynamic (PK/PD) properties and safety profiles are more similar to humans. Typically, in pediatric phase 1 clinical trials, there is adult recommended phase 2 dose (RP2D) and safety data available, which is used to inform drug dosing in children. This figure was produced using BioRender.

for their growth properties in vitro or following xenotransplantation into immune-defective mice to generate pLGGs.[31] Lastly, mouse progenitor cells can be engineered to express *KIAA1549: BRAF* (or *BRAF*^{V600E}) and injected into immune competent mice for in vivo tumor analysis.[18,55,67] Using this approach, introduction of the *BRAF*^{V600E} kinase domain into nestin-expressing cells within the hemisphere or brainstem of neonatal mice resulted in LGG formation by 8 weeks of age.

Advantages and Disadvantages of Model Platforms

Each model has its own unique advantages and disadvantages that impact upon its use as a preclinical platform (Table 2). For studies aimed at targeting basic properties of the low-grade glioma cells, tissue culture models afford rapid, high-throughput screening of compounds, and have led to the discovery of actionable therapeutic targets.[70] However, it is clear that pLGG cells are heavily dependent on mitogenic cues from non-neoplastic cells, which are not present in primary culture systems. Refinements to this platform could include the addition of non-neoplastic cells, like microglia or T cells, or the maintenance of tumor cells in cerebral organoids containing these stromal cellular elements. For basic science, discoveries focused on understanding stromal dependencies,[22,71] brain tumor risk factors (eg, sex, comorbid diseases, germline *NF1* mutation, systemic conditions)[53,72] and the developmental origins of tumors,[50] intact genetically engineered mouse models are superior. These platforms have been quite instructive for defining the cell of origin,[73] the requirement for immune system cells and neurons,[19–21,74] and the impact of systemic disease (eg, asthma) on tumorigenesis and progression.[75]

While preclinical models involving xenotransplantation provide opportunities to assess tumor responses to tumor-directed therapies (eg, MEK inhibitors), they lack an intact immune system, thus limiting their utility for immunomodulatory or stroma-directed therapeutic studies. For this reason, many preclinical studies employ authenticated genetically engineered mouse models with intact immune systems and preserved stromal dependencies.[19,68,71,76]

Other limitations to these models include differences in drug metabolism and bioavailability between mice and humans, and the inability of the mouse models to capture the clinical diversity of these tumors in children with respect to tumor location within the brain, age at development, clinical symptoms, and response to therapy. Some of these disadvantages could be mitigated by using multiple strains with different tumor locations, ages of development, cells of origin, and genetic changes, thus more closely resembling the clinical diversity of children with pLGGs. With future characterization of their natural history and biological properties, the use of swine models may likewise circumvent some of these issues. Moreover, there is a continued need to develop new pLGG models with different genetic mutations (*NTRK2*, *FGFR1* mutations[13]), brain locations (cerebellum, deep midline structures), and histopathology (HGAP[77]) with additional genetic alterations, as well as those that more accurately capture stromal interactions (mice with humanized immune systems).

Validation and Optimal Selection of Preclinical Models

As many pLGGs in children are slow-growing lesions with the tendency to spontaneously growth arrest or, rarely, even regress, it is challenging to recapitulate this level of heterogeneity in a single model. As such, preclinical models need to be well-validated and carefully selected to reflect their intended use. Moreover, it is unlikely that any one model will capture all of the features of their human counterparts at the neuropathological, molecular and biological

Table 2. Advantages and disadvantages of existing preclinical pediatric LGG models

Model	References	Advantages												Disadvantages
		High penetrance	Non-fatal	Rapid course of disease	Intact immune system	Useful for studying stromal cell dependencies	More representative of human tumor biology	More representative of human molecular biology	Ability to engineer any genetic alteration or any neuroglial cell type	Assessment of drug PK possible	Useful for evaluating tumor-directed therapies in vivo	Useful for high-throughput evaluation of tumor-directed therapies in vitro	Cost effective	
Nf1-OPG mice	[68]	✓	✓		✓	✓				✓				Lacks some histological features of NF1-PA, gliomas limited to optic nerve and chiasm, species differences
Nf1-OPG mice	[60]	✓		✓	✓					✓				Lacks some histological features of NF1-PA, gliomas limited to optic nerve and chiasm, early adult lethality, not useful for studying Nf1+/– stromal cell dependencies, species differences
Nf1-OPG swine	[63]		✓		✓	✓	✓			✓				Incompletely characterized model, 15% penetrance, expensive
Human LGG cell lines	[26,27,69]							✓				✓	✓	Does not capture stromal dependencies, no assessment of drug PK possible, no BBB
Human LGG cell lines injected into mice	[31,42]	✓					✓	✓		✓	✓			Does not capture stromal dependencies, lack of an intact immune system, species differences
Human LGG cell lines injected into zebrafish	[29,44]	✓						✓			✓			Does not capture natural stromal dependencies, limited propagation time, species differences, lacks tumor heterogeneity, no assessment of drug PK possible, no BBB
Mouse LGG cell lines injected into mice	[39,67]	✓								✓	✓			Does not reflect natural tumor evolution, species differences, lacks tumor heterogeneity
Engineered human iPSC lines injected into mice	[31]	✓							✓	✓	✓			Does not reflect natural tumor evolution, lack of an intact immune system, species differences, lacks tumor heterogeneity

Abbreviations: BBB, blood-brain-barrier; iPSC, induced pluripotent stem cell; LGG, low-grade glioma; OPG, optic pathway glioma; PA, pilocytic astrocytoma; PK, pharmacokinetics.

Table 3. Desired Preclinical Model Characteristics for Clinical Translation

Characteristics	Measures
Histologic and molecular features	• Similar cellular composition • Similar histologic appearance • Similar brain location(s) • Similar molecular profile (DNA sequence and methylation profile; transcriptome) • Similar prognostic and/or predictive markers
Growth dependencies	• Similar tumor cell signaling pathway dependency • Similar stromal cell-induced tumor cell dependency

levels, necessitating the deployment of multiple complementary models. In this respect, pLGGs encompass a wide range of clearly delineated molecular-neuropathological entities.[14,61] Nonetheless, validation and authentication should include a demonstration that the model faithfully represents key aspects of the tumor at numerous levels. This would include a full neuropathological (histology, cytology, immunohistochemical marker expression), molecular (DNA methylation profile, DNA sequencing, RNA sequencing), and biological (tumor-associated neurologic or endocrinologic problems) analyses (Table 3).

Evaluating Preclinical Data for Clinical Trial Translation

Given the excellent overall prognosis for pLGG, careful thought and evaluation of the available preclinical data must be performed prior to testing an experimental treatment in clinical trials for children, to ensure that the risks to participants are reasonable compared with the potential therapeutic benefits (Figure 2). Important factors to consider prior to clinical testing include the biologic rationale for the treatment, safety profile (both preclinical and clinical, if available), pharmacokinetics, and preclinical efficacy.

Since most pLGGs are driven by a single pathway (mitogen-activated protein kinase, MAPK), targeting a protein in this pathway (eg, RAF, MEK, ERK) often provides a strong biologic rationale. This makes pLGGs unique relative to their high-grade counterparts, in which the number and complexity of genetic and genomic changes make it more challenging to predict the likelihood that targeting a single protein and/or signaling pathway will lead to a tumor response. Nonetheless, preclinical evaluation of agents (including rational combinations) to assess efficacy, adaptive signaling and resistance are critical. One prominent example in pLGG was the phase II study of sorafenib for recurrent pLGG, in which 82% (9/11) of participants were removed from the trial after 2–3 treatment cycles for an accelerated progression, including all 3 with NF1 and 5 of 6 with fusion *BRAF* pLGG.[78] Subsequent evaluation in cell lines expressing *KIAA1549-BRAF* demonstrated paradoxical activation of MEK/ERK to this agent.[78] Similarly, an increase in cell proliferation was observed following treatment of *KIAA1549-BRAF* models with first-generation RAF inhibitors,[79] which were designed to target oncogenic BRAF, rather than non-mutant RAF proteins, including accelerated tumor growth in xenograft mouse models.[79] Had this data been available prior to clinical testing, these paradoxical progressions might have been predicted. In contrast, selumetinib was shown to induce partial responses in a PDX mouse model of *BRAF*^{V600E}-mutant LGG,[80] which successfully resulted in drug efficacy in a phase 2 clinical trial.[36]

Unlike aggressive cancers, the risk-to-benefit ratio of moving a new pLGG therapy into clinical trial requires a higher bar of safety. Despite excellent overall survival rates, a large percentage of pLGG survivors have impairments in neurological, endocrine, and neurocognitive function,[81] with many reporting impairments in measures of adaptive functioning.[82] Therefore, in most cases, moving a novel therapy forward to a clinical trial in pLGG requires prior human safety data with a low risk of toxicity. Therapies with a higher toxicity profile are best evaluated in clinical trials focused on recurrent disease, given the generally good outcomes of standard-of-care frontline therapy in pLGG. In addition, except in cases for which the therapy cannot be evaluated in an adult tumor (eg, fusion *BRAF* LGG, which is likely a pediatric tumor), first in human studies should be avoided in children for ethical reasons.

One crucial factor in drug efficacy is adequate exposure of the tumor to the drug. One of the unique considerations in achieving this for brain tumors is the requirement that the therapy crosses the blood-brain barrier (BBB), which restricts the entry of drugs to the central nervous system (CNS). To evaluate this, it is important that preclinical studies assess CNS pharmacokinetics. In general, when selecting drugs in the same class, those with better BBB penetration are preferable. That said, the BBB is often disrupted in brain tumors, potentially allowing agents with poor CSF penetration into the tumors. An example of "blood-tumor" barrier disruption is the enhancement seen on MRI after gadolinium administration, which is common in pLGG. Thus, an effective agent in preclinical testing that does not cross the BBB well would not necessarily be excluded from clinical testing, otherwise an agent such as selumetinib, with excellent response rates for recurrent pLGG,[36,37] but poor preclinical CSF penetration,[83] would not have been pursued.

Prior to moving to a clinical trial, critical evaluation of the preclinical efficacy of any given experimental therapy is required. Factors to consider include whether the model adequately reflects the subtype of pLGG of interest (eg, *BRAF*^{V600E}, *BRAF* fusion, *NF1*-mutant, *NTRK*-mutant, or *FGFR1*-mutant) and the specific clinical scenario. For example, efficacy from *Nf1* mouse models is insufficient evidence to move an agent to a clinical trial for *BRAF*-altered pLGG. In addition, the genetic background needs to be

considered. For example, evaluating the efficacy of an immunotherapy approach requires an immunocompetent model. Similarly, pLGG tumor-derived models carrying inducible SV40 large T antigen are excellent to screen MAPK drugs and senolytic agents, but they have limitations when it comes to evaluating apoptosis-inducing drugs.[26,27,44] The design of the preclinical experiments, the magnitude of the effect, whether the response was durable, and whether the results were replicated in multiple models are also important. Preclinical study design should be rigorous with adequate sample size, and appropriate controls, including comparisons to standard of care treatment (eg, carboplatin-based regimens in NF1-associated pLGG, RAF inhibitor plus MEK inhibitor for *BRAF*^{V600E} pLGG) in addition to vehicle controls, assessment of the dose–response relationship, and evaluation of not just tumor outcomes, but also pharmacodynamic endpoints, such as target inhibition, should be performed. In particular, for in vitro studies, clinically achievable therapeutic concentrations need to be considered. Although in vitro evidence is important, ideally this is supported by in vivo studies, which are more likely to resemble the human disease and should consider human exposure pharmacokinetic parameters when choosing the dosing regimen. Tumor outcomes are ideally assessed by applying clinical trial endpoint parameters. It is preferable to allow the tumor to grow to a size that enables measurement of response (ie, both growth and shrinkage of tumor size compared with baseline), rather than initiating treatment prior to this point (ie, secondary prophylactic treatment approach) and comparing tumor size between arms at the end of treatment, as agents that result in tumor shrinkage (and those with a larger effect size) are likely to be more promising than those that simply prolong time to progression. Efficacy in more than one model (and more than one laboratory), provides greater confidence.

While the ideal preclinical evidence for clinical translation may include strong biologic rationale, excellent safety profile, good BBB penetration, and efficacy in multiple models, in reality, this may not always be feasible given the currently available models. In such cases, a drug with strong biologic rationale, excellent safety data in prior studies, and evidence of efficacy in other agents within the same class, may be enough to move to clinical trial in relapsed/refractory pLGG. In contrast, treatments with higher toxicity, invasive delivery (eg, intratumoral), or unknown toxicity (first in humans) require a higher bar of evidence of preclinical efficacy to move forward into children.

Discussion and Recommendations

In this consensus statement, we review the state of the field of preclinical pLGG modeling, providing a comprehensive overview of the models currently available. We advocate that stringent neuropathological analyses and molecular diagnostics be applied to validate each model and to define the faithfulness with which it represents the studied pLGG (sub-) entity, as defined using current neuropathology criteria. Ideally, pLGG models need to recapitulate the defining molecular alteration (eg, *NF1* loss, *BRAF*-fusion, *FGFR1* mutation) without additional technical alterations for modeling purposes, which may change the phenotype to higher-grade tumors (ie, *TP53* mutation) or interfere with biological functions necessary for drug development (ie, ectopic SV40-TAg expression to block p53-mediated cell death). Depending on the desired experimental design, certain models have advantages and limitations, which need to be considered prior to study interpretation and translation. Consideration of the advantages and limitations of each model used is needed for realistic and clinically meaningful interpretation of preclinical data.

To meet the needs of future pLGG research, these preclinical models will need to incorporate novel tractable and accurate in vitro and in vivo platforms. Experimental 3-dimensional in vitro model systems that combine pLGG neoplastic cells with non-neoplastic stromal cells, including, but not limited to, immune cells, will help to elucidate the bidirectional interactions between these cells relevant to tumor pathobiology and response to treatment. In addition, these in vitro platforms are amenable to single-cell discovery studies, as well as medium to high-throughput drug screens. Similarly, genetic modeling using conditional and CRISPR-based engineering methodologies can be employed to create pLGG variants not currently modeled, including *FGFR1*-, *NTRK*-, fusion *BRAF*- and *BRAF*^{V600E}-driven PAs, arising within an intact brain environment. The availability of such complementary and diverse authenticated platforms provide systems to validate next-generation molecularly-targeted treatments.

Moreover, the use of novel technologies, such as single-cell sequencing, large genetic/genomic datasets, and artificial intelligence approaches encompassing clinical, imaging, genomic, transcriptomic and proteomic data, may supplement, inform, and even improve the results obtained with classical in vitro and in vivo testing. Lastly, we desperately need more preclinical models that more fully represent the spectrum, as well as the cellular heterogeneity, of pLGGs. With the rapid advances in cellular and genetic engineering and the development of methods to utilize big data, it is likely that a highly operational pipeline will be constructed for effective preclinical translation to improve the therapeutic options for children with these common brain tumors.

Keywords

genetically engineered mice | MAPK | pediatric low-grade glioma | preclinical models | translation

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Conflict of interest

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Authorship statement

T.M., D.H.G., J.F., M.J.F., C.H., F.J.R., U.T., O.W., and Y.Z. wrote specific sections of this manuscript, while T.M. and D.H.G. edited, compiled, and organized this submission.

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