



## mTOR: a new therapeutic target for pediatric low-grade glioma?



Fausto J Rodriguez<sup>\*,1,2</sup> & Eric H Raabe<sup>1,2,3</sup>

“Recent clinical trials with small numbers of patients support the feasibility of mTOR pathway targeting for pediatric low-grade glioma in general in the clinic.”

Pediatric low-grade gliomas (PLGG) represent the most frequent primary brain tumors in children. Despite their low growth rates, a subset may progress or cause substantial morbidity when located in critical brain regions where aggressive surgical resections are not possible. Conventional treatments for young children with unresectable PLGG have avoided radiation therapy due to the significant effects of radiation on the developing brain [1]. Chemotherapy using either carboplatin/vincristine or a lomustine-based regimen is the treatment of choice in the first-line setting [2]. The majority of patients will achieve stable disease or a reduction in tumor size with these low-intensity chemotherapy regimens. A subset of these patients will not have tumor regrowth, probably due to a process of oncogene and chemotherapy-induced senescence [3]. However, approximately 50% of patients will have tumors that start to grow again or are refractory to primary therapy [2], underscoring the need to develop new therapeutic options for patients with PLGG.

The development of high-throughput molecular analysis and next-generation

sequencing platforms have led to an unparalleled dissection and discovery of key somatic genetic alterations underlying tumorigenesis. These studies have proven valuable for the study of cancer and have highlighted novel possibilities for rational therapeutic targeting of PLGG (reviewed in [4]). One critical pathway that has been highlighted in PLGG specifically using high-resolution techniques is the MAPK pathway. In fact, one recent study demonstrated activating mutations in components of this pathway in essentially 100% of pilocytic astrocytomas [5], the most frequent PLGG subtype. The predominant molecular alteration leading to MAPK pathway activation is duplication of the *BRAF* gene segment encoding for the kinase domain, usually resulting in a *BRAF-KIAA1549* fusion [6–8]. Therapeutic targeting of this pathway has met with some success in cancer. However, responses in well-studied tumor types (e.g., melanoma) are transient and resistance usually develops. Furthermore, it is critical to think about the specific molecular abnormality present when planning treatments, since a paradoxical increase in cell growth and MAPK activation may be triggered when

### KEYWORDS

- mTOR • neurofibromatosis
- optic nerve • pediatric glioma
- pilocytic astrocytoma

<sup>1</sup>Division of Neuropathology, Johns Hopkins University, Baltimore, MD, USA

<sup>2</sup>Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University, Baltimore, MD, USA

<sup>3</sup>Pediatric Oncology, Johns Hopkins University, Baltimore, MD, USA

\*Author for correspondence: Tel.: +1 443 287 6646; Fax: +1 410 614 9310; [frrodig4@jhmi.edu](mailto:frrodig4@jhmi.edu)

cells containing wild-type BRAF or BRAF fusions are treated with inhibitors of other BRAF mutant proteins (e.g., BRAF V600E) [9].

The PI3K–mTOR pathway is a key signaling pathway that has generated significant excitement in oncology because inhibitors of the pathway (i.e., rapamycin and its analogs) are currently available for therapeutic purposes. Novel strategies for targeting mTOR are now in preclinical and clinical studies [10]. At the cellular level, mTOR can exist as part of two multiprotein complexes that are distinct in function and response to rapamycin: mTORC1 and mTORC2. Activation of mTORC1 (rapamycin sensitive) stimulates protein translation and cell growth. mTORC2 (rapamycin resistant) may participate in cell metabolism and cytoskeletal organization, although the extent of its role has not been completely characterized.

mTOR activation has been explored as a feature of brain tumors in general and pediatric gliomas specifically [11]. Key experimental observations have increasingly highlighted a role for this pathway in PLGG. For example, prior work by David Gutmann’s laboratory in Washington University, St Louis (USA) has demonstrated that the mTOR pathway is active in neurofibromin-deficient cells and NF1-associated gliomas, and that it mediates growth in astrocytes [12]. More recent work from the same laboratory also showed that mTOR mediates proliferation in murine stem cells containing *BRAF* fusions [13].

Although mTOR activity appears to be important for PLGG biology, there may be different mechanisms for activation, as well as variable activation in tumor subsets and individual tumors. Our prior work has highlighted an increase in immunohistochemical markers of PI3K–mTOR activation, as well as *PTEN* gene deletions, in the rare subset of pilocytic astrocytomas that develop anaplasia and an aggressive phenotype [14]. More recently, we studied the frequency of mTOR pathway activation in pilocytic astrocytoma and various PLGG subgroups using phospho-S6 immunohistochemistry [15]. Interestingly, moderate-to-strong immunoreactivity for this marker was present in approximately 60% of PLGG. Further testing for markers of mTORC1 and mTORC2 complexes, and activation, demonstrated that mTORC2 was predominantly activated in tumors of the optic pathways, as well as those NF1 associated. These findings suggest that although mTOR pathway activation is frequent

in PLGG, its context and extent varies by biologic subtype. PLGG is a relatively heterogeneous category, including pilocytic astrocytoma, diffuse astrocytoma, pleomorphic xanthoastrocytomas and subependymal giant cell astrocytoma, among others, as pathologic subtypes. In our recent study, we also tested the sensitivity of mTOR inhibition in two PLGG-derived cell lines and found different sensitivities for each line to mTORC1 inhibition. The less sensitive line (i.e., Res259) showed a pronounced increase in pAKT (s473) levels, consistent with compensatory mTORC2 activation, a known phenomenon that limits therapeutic efficacy with single mTORC1 inhibitors. This suggests that combination therapies and dual mTORC1–mTORC2 inhibition may be required in individual cases.

Many of these studies suggest mTOR pathway inhibition as an attractive therapeutic strategy for PLGG and is timely given the exciting success of mTOR inhibition in patients with subependymal giant cell astrocytoma [16], a PLGG subtype almost defined at the molecular level by mTOR pathway activation. Recent clinical trials with small numbers of patients support the feasibility of mTOR pathway targeting for PLGG in general in the clinic. In a Phase I/II study using the mTOR inhibitor rapamycin and the tyrosine kinase inhibitor erlotinib focusing on recurrent PLGG, two NF-1 patients stabilized or had responses to the drug [17]. In preliminary results of a more recent trial that has just been completed of 23 PLGG patients treated at progression with the mTOR inhibitor everolimus [18], a subset had partial responses or stable disease [19]. A study of everolimus in NF1-associated PLGG is ongoing [20]. A follow-up Phase II trial of everolimus in refractory PLGG is actively recruiting patients [21]. This study requires tumor tissue for correlative testing and hypothesizes that tumor activation of mTORC1, as evidenced by increased phosphoribosomal protein S6, will be associated with drug response. These studies are encouraging and suggest that mTOR inhibition may become an important component of PLGG treatment.

However, much remains to be learned about the optimal approaches to mTOR inhibition in the laboratory. What underlies the variability of mTOR activation in PLGG subsets? What combination of therapies should be attempted? What are the best biomarkers to identify susceptible and resistant tumors, and make sure that the patient receives biologically driven therapy? Is mTOR activation seen in non-BRAF-driven

“...much remains to be learned about the optimal approaches to mTOR inhibition in the laboratory.”

PLGG? Further work will require testing dual mTORC1/mTORC2 inhibitors or targeting of multiple pathways simultaneously to identify the optimal therapeutic approaches for PLGG. Now that we know more about the underlying biology of PLGG, better *in vitro* and *in vivo* models need to be developed and clever ways to bypass the practical problems associated with oncogene-induced senescence must be entertained in developing these models [3,22]. The development of genetically accurate models of aggressive PLGG will be essential for screening and deployment of novel therapies.

## References

- Merchant TE, Conklin HM, Wu S, Lustig RH, Xiong X. Late effects of conformal radiation therapy for pediatric patients with low-grade glioma: prospective evaluation of cognitive, endocrine, and hearing deficits. *J. Clin. Oncol.* 27(22), 3691–3697 (2009).
- 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. *J. Clin. Oncol.* 30(21), 2641–2647 (2012).
- Raabe EH, Lim KS, Kim JM *et al.* BRAF activation induces transformation and then senescence in human neural stem cells: a pilocytic astrocytoma model. *Clin Cancer Res.* 17(11), 3590–3599 (2011).
- Raabe E, Kieran MW, Cohen KJ. New strategies in pediatric gliomas: molecular advances in pediatric low-grade gliomas as a model. *Clin. Cancer Res.* 19(17), 4553–4558 (2013).
- Jones DT, Hutter B, Jäger N *et al.* Recurrent somatic alterations of *FGFR1* and *NTRK2* in pilocytic astrocytoma. *Nat. Genet.* 45(8), 927–932 (2013).
- Bar EE, Lin A, Tihan T, Burger PC, Eberhart CG. Frequent gains at chromosome 7q34 involving *BRAF* in pilocytic astrocytoma. *J. Neuropathol. Exp. Neurol.* 67(9), 878–887 (2008).
- Jones DT, Kocialkowski S, Liu L *et al.* Tandem duplication producing a novel oncogenic *BRAF* fusion gene defines the majority of pilocytic astrocytomas. *Cancer Res.* 68(21), 8673–8677 (2008).
- Pfister S, Janzarik WG, Remke M *et al.* *BRAF* gene duplication constitutes a mechanism of MAPK pathway activation in low-grade astrocytomas. *J. Clin. Invest.* 118(5), 1739–1749 (2008).
- Sievert AJ, Lang SS, Boucher KL *et al.* Paradoxical activation and RAF inhibitor resistance of BRAF protein kinase fusions characterizing pediatric astrocytomas. *Proc. Natl Acad. Sci. USA.* 110(15), 5957–5962 (2013).
- Guertin DA, Sabatini DM. The pharmacology of mTOR inhibition. *Sci. Signal.* 2(67), pe24 (2009).
- Mueller S, Phillips J, Onar-Thomas A *et al.* *PTEN* promoter methylation and activation of the PI3K/Akt/mTOR pathway in pediatric gliomas and influence on clinical outcome. *Neuro Oncol.* 14(9), 1146–1152 (2012).
- Banerjee S, Crouse NR, Emnett RJ, Gianino SM, Gutmann DH. Neurofibromatosis-1 regulates mTOR-mediated astrocyte growth and glioma formation in a TSC/Rheb-independent manner. *Proc. Natl Acad. Sci. USA* 108(38), 15996–6001 (2011).
- Kaul A, Chen YH, Emnett RJ, Dahiya S, Gutmann DH. Pediatric glioma-associated *KIAA1549:BRAF* expression regulates neuroglial cell growth in a cell type-specific and mTOR-dependent manner. *Genes Dev.* 26(23), 2561–2566 (2012).
- Rodriguez EF, Scheithauer BW, Giannini C *et al.* PI3K/AKT pathway alterations are associated with clinically aggressive and histologically anaplastic subsets of pilocytic astrocytoma. *Acta Neuropathol.* 121(3), 407–420 (2011).
- Hütt-Cabezas M, Karajannis MA, Zagzag D *et al.* Activation of mTORC1/mTORC2 signaling in pediatric low-grade glioma and pilocytic astrocytoma reveals mTOR as a therapeutic target. *Neuro Oncol.* 15(12), 1604–1614 (2013).
- Franz DN, Belousova E, Sparagana S *et al.* Efficacy and safety of everolimus for subependymal giant cell astrocytomas associated with tuberous sclerosis complex (EXIST-1): a multicentre, randomised, placebo-controlled Phase 3 trial. *Lancet* 381(9861), 125–132 (2013).
- Yalon M, Rood B, MacDonald TJ *et al.* A feasibility and efficacy study of rapamycin and erlotinib for recurrent pediatric low-grade glioma (LGG). *Pediatr. Blood Cancer* 60(1), 71–76 (2013).
- Everolimus (RAD001) for Children With Chemotherapy-Refractory Progressive or Recurrent Low-Grade Gliomas. [www.clinicaltrials.gov/show/NCT00782626](http://www.clinicaltrials.gov/show/NCT00782626)
- Kieran M, X Yao, Macy M. A prospective multi-institutional Phase II study of everolimus (Rad001), an mTOR inhibitor, in pediatric patients with recurrent or progressive low-grade glioma. *Pediatr. Blood Cancer* 60(S3), Abstract O-310 (2013).
- Study of RAD001 (Everolimus) for Children With NF1 and Chemotherapy-Refractory Radiographic Progressive Low Grade Gliomas (NFC-RAD001). [www.clinicaltrials.gov/show/NCT01158651](http://www.clinicaltrials.gov/show/NCT01158651)
- PNOC 001: Phase II Study of Everolimus for Recurrent or Progressive Low-grade Gliomas in Children. [www.clinicaltrials.gov/show/NCT01734512](http://www.clinicaltrials.gov/show/NCT01734512)
- Jacob K, Quang-Khuong DA, Jones DT *et al.* Genetic aberrations leading to MAPK pathway activation mediate oncogene-induced senescence in sporadic pilocytic astrocytomas. *Clin. Cancer Res.* 17(14), 4650–4660 (2011).

## Financial & competing interests disclosure

Funding sources include the Childhood Brain Tumor Foundation (to FJ Rodriguez), the Pilocytic/Pilomyxoid Fund (to FJ Rodriguez) and the Knights Templar Eye Foundation (to EH Raabe and FJ Rodriguez). EH Raabe is a St Baldrick's Scholar. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.