

ACVR1 Mutations in DIPG: Lessons Learned from FOP

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Abstract

Whole-genome sequencing studies have recently identified a quarter of cases of the rare childhood brainstem tumor diffuse intrinsic pontine glioma to harbor somatic mutations in *ACVR1*. This gene encodes the type I bone morphogenic protein receptor ALK2, with the residues affected identical to those that, when mutated in the germline, give rise to the congenital malformation syndrome fibrodysplasia ossificans progressiva (FOP), resulting in the transformation of soft tissue into bone. This unexpected link points toward the importance of developmental biology processes in tumorigenesis and provides an extensive experience in mechanistic understanding and drug development hard-won by FOP researchers to pediatric neurooncology. Here, we review the literature in both fields and identify potential areas for collaboration and rapid advancement for patients of both diseases. *Cancer Res*; 74(17); 4565–70. ©2014 AACR.

Introduction

Recent sequencing of the whole genome or coding regions (exome) of cancer cells has provided an unprecedented level of insight into the biologic processes underlying the development of numerous tumor types (1, 2). Such approaches have shown a remarkable ability to spring surprises, few more so than in the field of pediatric neurooncology. Numerous childhood brain tumors have been found to be driven by a diverse series of unexpected genetic and epigenetic processes, which differ substantively from adult cancers, with medulloblastoma (3–5), ependymoma (6, 7), and glioma (8, 9) now known to comprise a varied series of subentities defined by age, anatomic location, and biology. These insights likely reflect unique origins of these tumors and highlight the important interface of developmental biology and cancer. Here, we discuss a novel link between these processes suggested by the remarkable discovery of mutations present somatically in a subset of lethal childhood brainstem tumors, which when found in the germline give rise to a rare congenital malformation syndrome of soft tissue. What can cancer researchers studying diffuse intrinsic pontine glioma (DIPG) learn from the experience of the fibrodysplasia ossificans progressiva field?

DIPG

Although considerably less frequent than histologically similar lesions occurring in adults, high-grade gliomas in children represent a major unmet need in clinical neurooncology (10). The identification of specific molecular subgroups of these

tumors linked to anatomic location and age of incidence (11), and marked by specific gene mutations (8), has strengthened the contention from earlier molecular profiling studies (12) that they harbor unique biology and disease origin (13). An unusual high-grade glioma variant restricted to the pediatric setting is DIPG, a brainstem lesion arising in the ventral pons at a peak age of incidence of 6 to 7 years (Fig. 1A). These tumors are universally fatal, with a median overall survival of 9 to 12 months (10). DIPGs are diffusely infiltrating, and although may harbor regions of lower-grade histology, are largely indistinguishable from World Health Organization grade IV glioblastoma multiforme (GBM) of the cerebral cortex. Efforts to improve survival in these children have thus far failed—surgical resection of these tumors is not possible due to their anatomic location and clinical trials based upon promising targets from the adult GBM literature have shown no benefit (14).

To improve this dismal situation, efforts have focused on collecting tumor material for detailed molecular analysis. In Europe, the reintroduction of stereotactic biopsy procedures in typical DIPG cases has been pioneered with low morbidity and mortality (15). Elsewhere, rapid autopsy protocols have been opened to obtain tumor material postmortem (16), as review boards have been reluctant to allow biopsies in all but atypical cases due to the requirement only for imaging and a short clinical history for the diagnosis of DIPG (17). Use of such material has provided evidence for distinct DNA copy number and gene expression profiles of DIPG compared with non-brainstem pediatric and adult GBM (18, 19), and more recently, the identification of highly recurrent and selective mutation in genes encoding the histone variants H3.3 (*H3F3A*) and H3.1 (*HIST1H3B*; ref. 9). These mutations were initially found in 60% and 18% of cases, respectively, and resulted in an amino acid substitution conferring a change of lysine to methionine at position 27 on the histone tail (K27M). Remarkably, such mutations have not been identified in any other cancer type, but are also found in approximately 50% of thalamic GBM (8, 11), an anatomic location generally restricted to children,

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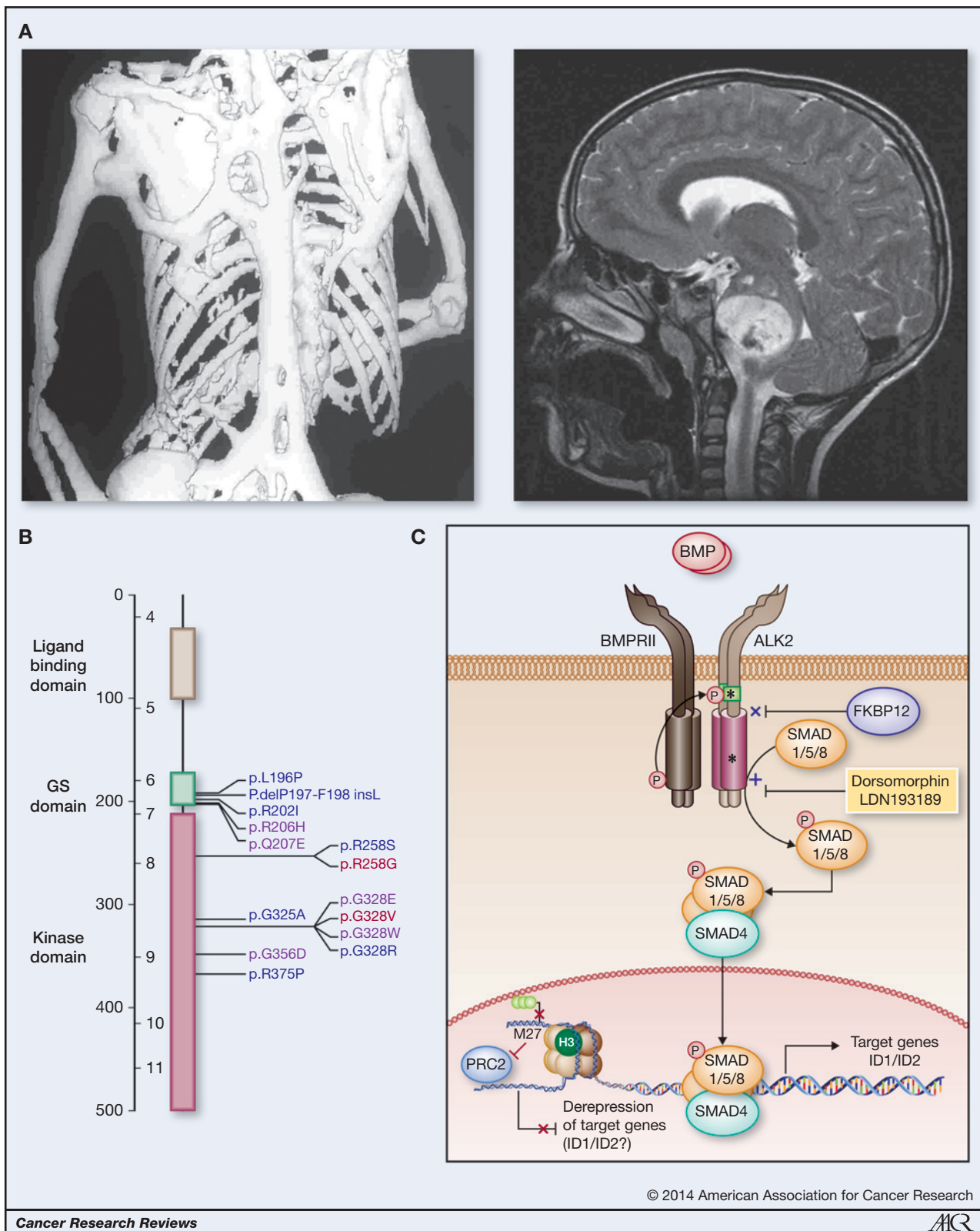


Figure 1. ACVR1 mutations link FOP and DIPG. **A**, left, dorsoventral CT scan image showing extensive heterotopic bone formation in the skeletal muscles of an aged individual with FOP. (Courtesy of James T. Triffitt, University of Oxford, United Kingdom; Martyn Cooke, Humanitarian Museum, London, United Kingdom, and Margot Rintoul, The Princess Grace Hospital, London, United Kingdom.) Right, T2 weighted sagittal MRI scan of an individual with DIPG at time of diagnosis (Courtesy of Darren Hargrave, Great Ormond Street Hospital, London, United Kingdom.) **B**, schematic representation of ACVR1 mutations in FOP and DIPG. (Continued on the following page.)

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hinting at a common origin of these tumors and DIPG. Although targeting only one of the many genes encoding histone H3 proteins, the mutation exerts a powerful transdominant negative on cellular H3K27 trimethylation (20), a posttranslational modification that usually binds the polycomb repressive complex 2 (PRC2) to repress gene transcription. K27M-mutant tumors consequently have distinct gene expression patterns (21) and global hypomethylation (22), in addition to a restricted age of onset and poor clinical outcome (23). Although this mutation clearly represents a fundamental genetic driver in DIPG, it is currently unclear how to directly target K27M tumors therapeutically.

Recently, four independent studies have been published that apply whole-genome or -exome sequencing to a collectively large series of DIPG biopsy and autopsy specimens, and that have begun to shed light on the wider genetic background against which these H3 K27M mutations are found, providing novel targets for desperately needed treatments. These data, from groups in Paris/London (24), Toronto/Duke (25), St. Jude (26), and Montreal/Boston (27), comprise data from a total of 195 DIPGs, representing a remarkable series of collaborative efforts worldwide in this rare disease. Common themes to emerge from these studies include a relatively low mutation rate for such an aggressive tumor (0.8–0.9 mutations per megabase; refs. 24, 26), recurrent alterations in the PI3K (40%–68% cases) and p53 pathways (57–76% cases), and a prevalence of mutations in genes encoding chromatin modifiers (26%–30% cases; refs. 24–27).

Most strikingly, however, was the unexpected identification of the most recurrently mutated gene in DIPG after the histone variants, *ACVR1*. This gene, encoding the receptor serine/threonine kinase ALK2, was found to harbor nonsynonymous heterozygous somatic mutations in 46 of 195 (24%) cases at five specific residues (24–27; Fig. 1B). Patients harboring *ACVR1* mutations were predominantly female (approximately 2:1) and had a younger age of onset (approximately 5 years) and longer overall survival time (approximately 15 months) compared with wild-type tumors (24, 26, 27). *ACVR1* mutations also strongly cosegregated with K27M mutations in the gene encoding histone H3.1 (*HIST1H3B*), which themselves are now reported to represent an accumulated 22% of DIPG (24–27). These tumors were also largely *TP53* wild-type (90%) and harbored additional alterations in the PI3K pathway (56%; refs. 24–27). The specific base changes in *ACVR1* conferred seven different amino acid substitutions, namely R206H (9/46, 20%), Q207E (1/46, 2%), R258G (6/46, 13%), G328E (11/46, 24%), G328V (13/46, 28%), G328W (2/46, 4%), and G356D (4/46, 9%; refs. 24–27). These mutations are located in the glycine-serine-rich (GS; R206H, Q207E) or protein kinase (R258G, G328E/V/W,

G356D) domains, with more than half (26/46, 57%) occurring at the glycine at position 328. These mutations seem to be remarkably specific for DIPG—the Catalogue of Somatic Mutations in Cancer database (28) version 68 lists only 18 confirmed somatic *ACVR1* mutations in 9,170 tumors (0.2%), with only a single amino acid substitution in common with DIPG (a case of hepatocellular carcinoma with G328V). It is worth noting, however, a further series of three endometrial carcinomas with R206H mutations for whom no matched normal DNA sequence was available. These specific alterations are important, as most remarkably of all, the somatic mutations observed in DIPG are the same as those found in the germline of patients with the congenital malformation syndrome fibrodysplasia ossificans progressiva (FOP).

FOP

FOP is an autosomal dominant disorder of skeletal malformation and disabling heterotopic ossification (Fig. 1A) that arises in 1 in 1,500,000 live births due to sporadic germline mutations in *ACVR1* (29). All five sites of mutation newly described in DIPG are also found in cases of FOP, as well as a further five sites across the GS and kinase domains of the encoded ALK2 protein (Fig. 1B; refs. 30, 31). Some 95% of FOP cases harbor the recurrent GS domain mutation R206H (c.617G>A), in contrast to the high proportion of ALK2 kinase domain mutations in DIPG (31). Perhaps as a result of this bias, two specific amino acid substitutions found in DIPG samples, R258G and G328V, have yet to be observed in patients with FOP (24–27).

Classical cases of FOP harboring the R206H mutation may be diagnosed at birth by a signature malformation of the great toes (31). Ectopic bone formation in muscle, tendons, and ligament is typically observed by 5 years and progresses to restrict joint movement, such that most individuals are confined to a wheelchair by their third decade of life (31). Episodic flare-ups are additionally precipitated by soft tissue injury, viral infection, and inflammation, which induce painful localized swellings that may resolve or harden into bone (31). Tissue metamorphosis first involves the catabolism of soft tissue before an anabolic phase involving the differentiation of osteogenic progenitor cells. Unfortunately, flare-ups in children are too often misdiagnosed as malignancy, resulting in harmful surgery and devastating postoperative ossification. FOP may ultimately become life threatening in middle age due to thoracic insufficiency (31).

All FOP-associated mutations activate the canonical bone morphogenic protein (BMP) pathway to varying degrees to promote osteogenic differentiation and endochondral bone formation (30). BMP ligands belonging to the TGF β

(Continued.) Protein structure is given, highlighting ligand binding (gray), GS (green), and kinase (purple) domains. Amino acid substitutions identified to date in FOP (blue), DIPG (red), or both diseases (purple) are labeled. C, cartoon representing a simplified BMP/ALK2 signaling pathway. ALK2 is a type I BMP receptor that dimerizes, and upon ligand binding, forms a heteromeric complex with two type II receptors (e.g., BMPRII), which themselves phosphorylate the ALK2 GS domain. Mutations (yellow stars) in either the GS or kinase domains inhibit interactions with the negative regulator FKBP12 and enhance recruitment and phosphorylation of SMAD1/5/8. Both types of mutation therefore confer constitutive pathway activation as evidenced by increased expression of transcriptional targets of the SMAD1/5/8 and SMAD4 complex in the nucleus. Small molecules such as dorsomorphin and LDN-193189 may be useful therapeutic strategies aimed at inhibiting the activation of this pathway. *ACVR1* mutations cosegregate in DIPG with histone H3.1 K27M mutations, which enhance transcription via disruption of trimethylated lysine 27 interactions with the repressive PRC2 complex. This derepression of gene expression may include common targets with SMAD signaling, including *ID1* and *ID2*.

superfamily bind to heteromeric complexes containing two type II receptors and two type I receptors (32). Type II receptors in this complex activate the type I receptors by phosphorylating their intracellular GS domain, which in turn allows the type I receptors to recruit and phosphorylate the substrate proteins SMAD1/5/8 (Fig. 1C; ref. 32). These receptor-associated SMADs then assemble with SMAD4 and migrate to the nucleus where they bind the promoters of BMP target genes, including *ID1-3*, *SMAD6*, *SMAD7*, *SNAIL*, and *HEY1* (32). The crystal structure of the GS and kinase domains of ALK2 in complex with the inhibitory protein FKBP12 shows that the disease mutations will break critical side chain interactions that normally stabilize the inactive conformation of the kinase domain (30). The mutant type I receptor therefore partially escapes the normal mechanisms of regulation by FKBP12 and becomes weakly active in the absence of ligand (30). This activation seems sufficient to drive endothelial-to-mesenchymal transition, potentially explaining the origin of progenitor cells in FOP lesions with a $Tie2^+$ lineage (33). Furthermore, an *Acvr1*^{R206H/+} knock-in mouse displays classical FOP demonstrating that this single mutation is the causative factor (34).

In contrast to FOP, the assessment of the functional significance of *ACVR1* in the context of DIPG has thus far been limited (24–27). The early consensus is of these somatic mutations conferring, to varying degrees, a weakly activated BMP signaling pathway, as assessed by transfecting normal human astrocytes and cultures derived from patients with DIPG *in vitro*, with evidence for increased levels of phospho-SMAD1/5/8 and ID1/ID2 mRNA expression (24–27). It is reported that *ACVR1* mutant-transduced *Tp53*-null mouse astrocytes reimplanted to the mouse pons failed to produce tumors *in vivo*, suggesting that the additional genetic aberrations found in the human disease (e.g., H3.1 K27M, PI3K) may be required for gliomagenesis (26). Importantly, there seem to be no reported cases of DIPG in patients with FOP, although numerous neurologic symptoms such as neuropathic pain are observed (35), as well as imaging lesions that are linked to dysmyelination and delayed oligodendroglial commitment, seen in both patients and *ACVR1* R206H mouse models (36). Hints at a direct oncogenic role are provided by evidence of *ACVR1* mutations conferring an enhanced proliferative capacity (25), and selective ALK2 inhibitors reducing cell viability *in vitro* (24). The specific association of *ACVR1* with mutations in histone H3.1, rather than H3.3, seems to point to the likely differing neurodevelopmental contexts from which these tumors arise. BMPs play a crucial role in brain development (37), and BMP signaling in the context of neural stem cells plays an important role in stem cell maintenance and cell fate and is known to drive progenitor cells toward an astrocytic differentiation (38). DIPGs with predominantly astrocytic features are reported to have an extended survival compared with the remaining subgroup with pronounced oligodendroglial differentiation (19), and this seems likely to be driven by activated BMP signaling *via ACVR1* mutation. Indeed in adult glioblastoma, BMPs have been suggested to act as a prodifferentiation regulator of tumor-initiating, stem-like cells (39). It remains unclear whether the modest pathway activation observed in DIPG cells is playing a similar role in these tumors, or whether

noncanonical roles for these mutations can be found in the context of cancer development.

Targeting ACVR1 in FOP and DIPG

There is a desperate need for effective treatments to manage both FOP and DIPG. Surgery is precluded for both conditions, and therapeutic antibodies seem unsuitable as the activating mutations found in ALK2 affect only the cytoplasmic portion of the receptor. Much effort has therefore focused on small-molecule inhibitors that can target the intracellular kinase activity of the rogue ALK2 protein. The most advanced kinase inhibitors, including DMH1, ML347, LDN-193189, and LDN-212854, share the pyrazolo[1,5-a]pyrimidine scaffold of dorsomorphin, which was first identified as a BMP inhibitor by a phenotypic screen in zebrafish (40) and later cocrystallized as an ATP-mimetic inhibitor of ALK2 (30). These compounds target the BMP receptors ALK2, ALK3, and ALK6 in the low nanomolar range to inhibit SMAD1/5/8 phosphorylation, without affecting the type I TGF β receptor ALK5 and the SMAD2/3 pathway. The 5-quinoline-substituted compound LDN-212854 shows additional selectivity for ALK2 over other BMP receptors and also inhibits heterotopic ossification in mice at a twice daily intraperitoneal dose of 6 mg/kg (41). Improvements in selectivity against the wider kinome have also been observed in a new inhibitor class based on the 2-aminopyridine scaffold of K02288 (42). Further pre-clinical development of both compound series is required to identify ALK2 inhibitors suitable for trials in humans. As a proof of principle, specific silencing of the mutant *ACVR1* c.617A allele has also been demonstrated in FOP patient cell lines using allele-specific siRNA (43).

Other molecular targets may also hold promise for FOP. Retinoic acid receptor γ agonists inhibit chondrogenesis and thereby block heterotopic bone formation in animal models (44). Similar efficacy has also been achieved in mice using the tachykinin NK1 receptor antagonist, RP-67580, suggesting a disease dependence on the neuroinflammatory factor substance P (45). Indeed, prednisolone and NSAIDs are the current standard care for FOP to mitigate swelling during flare-ups. Whether flare-ups spontaneously resolve or ossify remains unpredictable. Therefore, precise natural history studies are also required in the population of patients with FOP to provide a statistical measure of drug efficacy.

Despite the head start afforded by the progress made in FOP, several challenges need to be overcome to move forward with ALK2 inhibitors in DIPG. The first and arguably most critical is to develop small molecules with sufficient central nervous system (CNS) penetration to reach potentially effective doses in these brainstem tumors. Although GBMs frequently show evidence of a disrupted blood–brain barrier, delivery to the pons may represent an additional hurdle, and DIPGs seem to have a relatively intact vasculature (14). With current ALK2 inhibitors lacking the chemical indicators of efficient CNS penetration, novel medicinal chemistry approaches may be necessary to produce a DIPG-specific compound, although specific models mimicking the blood–brain–tumor barrier in humans are currently lacking. Alternative forms of administration such as

convection-enhanced delivery (46) may be required to take advantage of existing chemical series, and clinical trials using this technique in DIPG are still ongoing.

An additional complication is associated with the complicated genetic background present in DIPG compared with the monogenic nature of FOP. The limited *in vitro* preclinical work in DIPG cells has shown only modest sensitivity to ALK2 inhibitors as single agents (24), and combinatorial approaches additionally targeting other cosegregating somatic alterations such as H3.1 K27M mutations and PI3K activation (24–27) will likely be required. Even then, the inherent intratumoral heterogeneity of DIPG represents a major obstacle to targeted therapy due to the subclonal diversity of these tumors providing the substrate for clonal selection and development of resistance according to evolutionary biology principles (47).

Despite these caveats, there remains optimism that a more thorough understanding of the underlying biology of these tumors afforded by genome-wide profiling will provide clinicians with an enhanced armamentarium of drugs with which to combat these tumors. Owing to the continued dire clinical outcome of children with DIPG, there are significant opportunities for rapid testing of promising approaches within first-line clinical trials, though the rarity of the disease will necessitate a coordinated, collaborative approach. With *ACVR1*-mutant tumors representing around 25% of DIPGs, predictive biomarkers will be key in guiding patients to the most suitable therapy. There is evidence of pathway activation even in the absence of *ACVR1* mutation in DIPG (24–27), possibly expanding the patient population who may benefit from ALK2 inhibitors, but also complicating patient stratification. For all potential predictive markers, routine biopsies will likely need to be reintroduced to select patients who will most likely benefit from novel agents, a further challenge for the pediatric neurooncology community, which the unexpected identification of *ACVR1* mutations may help to overcome.

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Conclusions

It is becoming increasingly apparent that pediatric brain tumors have their origins during neurodevelopment and that cross-disciplinary approaches will be necessary to fully understand and leverage the wealth of data from whole-genome sequencing studies into improved outcomes for these patients. The surprising link between the seemingly unrelated diseases of DIPG and FOP suggested by common mutations in *ACVR1* represents a unique opportunity for collaboration between researchers in disparate fields to fast-track drug development for both entities. Although each disease has its own requirements and obstacles, we ought to be optimistic that our shared experiences, insights, and approaches can lead to synergies in tackling the desperate unmet clinical needs of two groups of children.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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