Molecularly Targeted Treatments for NF1-Mutant Diffuse Intrinsic Pontine Glioma

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CASE DESCRIPTIONS

The first patient presented at 2 years of age when an MRI brain/spine revealed a nonenhancing, T2-hyperintense, expanded pons (Fig. 1A). A biopsy revealed a diffuse midline glioma, H3 K27M-mutant (DMG) (Fig. 2A). UW-Oncoplex™, a targeted sequencing platform *(1)*, identified a *H3F3A* p.K28M (i.e., *H3.3* p.K27M) mutation, a novel mutation in *NF1* (*NF1* p.N2071D), and mutations in *PIK3CA* (*PIK3CA* p.E545K and p.G1050S). Without a well-suited clinical trial option, Seattle Children's Hospital (SCH) institutional Pediatric Brain Tumor Board recommended treatment per our Best-Available Therapy (BAT): focal radiation (∼54 Gy) with concurrent temozolomide followed by maintenance bevacizumab, irinotecan, and temozolomide. The patient had near resolution of symptoms until progression 9 months from diagnosis (Fig. 1B).

The second patient presented at 11 years of age when an MRI brain/spine showed a heterogeneously enhancing mass arising from the medulla as well as intracranial/spinal leptomeningeal metastases (Fig. 1D). A biopsy revealed a DMG (Fig. 2B). Cerebrospinal fluid cytology was negative for malignant cells. UW-Oncoplex™ identified mutations in *H3F3A* p.K28M and *NF1* (*NF1* p.K1423E) along with a subclone mutation in *KRAS* (*KRAS* p.G13D). The patient received 36 Gy craniospinal radiation with a 54 Gy boost to the cervicomedullary junction and foci of spinal disease. An MRI 4 weeks following radiation revealed resolution of supratentorial/infratentorial leptomeningeal disease, but persistent medulla T2 signal abnormality. Only 4 weeks later, the patient's weakness worsened. An MRI revealed increased leptomeningeal disease with high spinal T2 signal (Fig. 1, E and F).

After progression of disease in each patient, the families were not interested in the early phase clinical trials for which the patient qualified, but the families did want to continue with a cancer-directed therapy that would minimally impact

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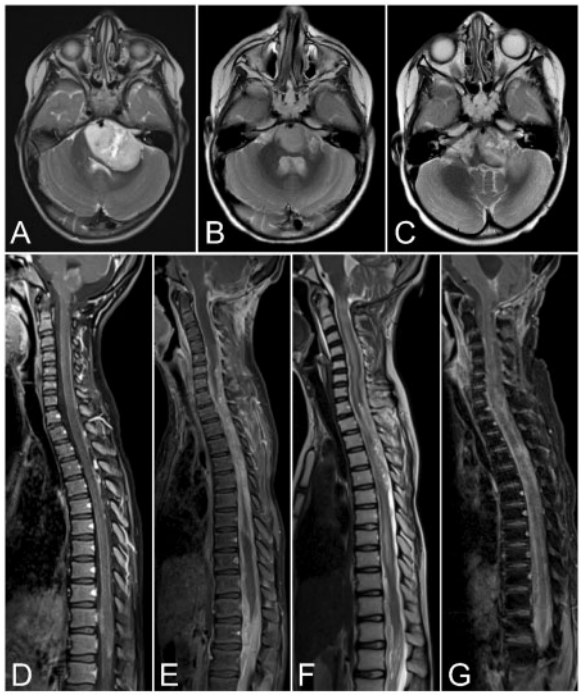

Fig. 1. Patient 1 neuroimaging: A) Axial T2 image at diagnosis. B) Axial T2 image at 9 months after initial diagnosis. C) Axial T2 image at 13.5 months after diagnosis. Patient 2 neuroimaging: D) Sagittal T1 post-contrast at diagnosis. Sagittal E) T1 post-contrast and F) T2 images 8 weeks after radiation. G) Sagittal T1 post-contrast image of the spine 6 weeks after initiating trametinib.

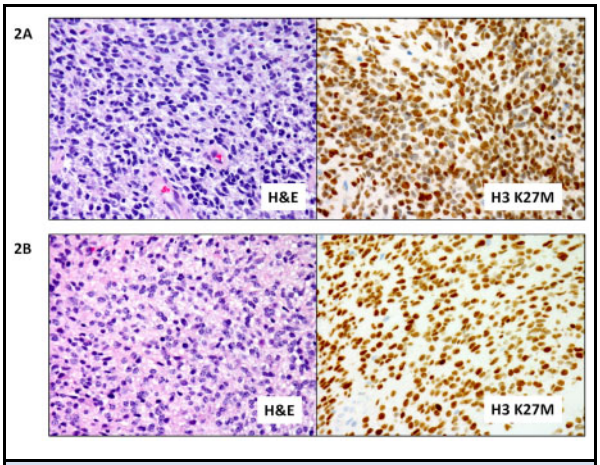

Fig. 2. H&E and H3 K27M mutation immunohistochemistry for Case 1 and Case 2.

quality of life. Therefore, we were tasked with providing a tolerable, potentially disease-stabilizing or life-extending therapy in the setting of progressive DIPG. Considering the potential benefit of MEK inhibition in the setting of *NF1* mutations, trametinib, in combination with other therapy, was prescribed in each case. At progression, the first patient was prescribed focal re-irradiation (~20 Gy) *(2)*along with the MEK inhibitor trametinib (0.025 mg/kg daily). This combination resulted in symptom improvement (Lansky score increasing from 70 to 90) (Fig. 1C). She received trametinib for 5 months with the only adverse effect of grade 1 rash, but died 16 months from diagnosis. At progression, the second patient began trametinib (0.025 mg/kg daily) and oral etoposide (50 mg/m^2 daily, every 14/28-days), after which there was a reduction in back pain and hemiparesis. An MRI 6 weeks later confirmed significant reduction in leptomeningeal disease (Fig. 1G). While she had neurologic improvement during treatment with trametinib and etoposide, she experienced a shunt infection and lower extremity edema. Several weeks later she developed progressive hydrocephalus and died 8 months from diagnosis.

CASE DISCUSSION

DIPG is a fatal pediatric central nervous system (CNS) tumor affecting ~300 children per year in the United States with a median overall survival of 11 months *(3)*. As 85% of DIPG harbor mutations in genes encoding histone 3 also present in other gliomas, the 2016 World Health Organization (WHO) reclassification included a novel diagnosis: diffuse midline glioma, H3 K27M-mutant *(4)*. Other

mutations, such as *ACVR1*, *TP53*, *PDGFRA*, *KIT*, *KDR*, and *MYC*, are not infrequent, although the less common mutations such as in *NF1* or *FGFR1* may represent targetable vulnerabilities *(5)*. *NF1* is a tumor suppressor gene and regulator of the RAS/MAPK pathway. A MEK inhibitor was prescribed for our patients despite multiple targetable members of the pathway, as inhibiting MEK-driven phosphorylation of ERK in *BRAF*- and *NF1*-mutant tumors has been preclinically and clinically efficacious, particularly against low-grade glioma (LGG) *(6)*. Of note, the specific *NF1* mutation, *NF1* p.N2071D, has not previously been described in patients with neurofibromatosis, type 1, or DIPG. While single-agent molecularly-targeted therapies may not be curative in high-grade CNS tumors, they may play a role in multi-agent regimens. The median time from progression to death for children with DIPG is 4.8 months *(3)*. While our first patient survived 6.1 months after progression and our second patient survived 4.6 months after progression, the overall survival benefit of trametinib cannot be assessed in such a small patient cohort.

Our patients highlight several critical factors in the care of patients with DIPG/DMG. DIPG/DMG may disseminate metastatically/leptomeningeally, so patients should undergo an MRI of the spine at diagnosis and then as clinically indicated. We also recommend, when reasonable, to biopsy suspected DIPG/DMG as molecular characterization of these tumors is routine for clinical trials and allows for identification of targetable mutations. We also advocate for obtaining CSF cytology, when clinically reasonable, to evaluate for disseminated disease. Ultimately, CSF-derived circulating tumor DNA (ctDNA) could provide a less invasive molecular characterization of DIPG at diagnosis, as well as a route to track its molecular evolution during treatment via serial CSF monitoring. Critically, ctDNA may also provide an avenue to offer targeted therapies to children clinically unable to undergo biopsy or who are being treated at institutions without that neurosurgical capability *(7)*. Seattle Children's has partnered with Tgen® to investigate serial sequencing of CSF and plasma ctDNA, as well as interrogation of extracellular vesicle cargo to not only quantify but to understand the functional state of DIPG cells during treatment *(8)*. While the discovery of histone mutations amongst DIPG/DMG has been critical, their role in tumor initiation and treatment response remains elusive. While concurrent mutations in *NF1* are less common, they are potentially targetable as MEK inhibition has become routine in Ras-pathway aberrant LGG after selumetinib demonstrated 69 ± 9.8% 2-year progression-free survival *(6)*. Interestingly, there may be a correlation between a high/responsive MEK signature and concurrent Ras mutations *(9)*. The presence of a *KRAS* mutation in our second patient, therefore, may have led to a response-predictive gene expression pattern that could be evaluated in future patients. Along with combining molecular agents with cytotoxic chemotherapies, focal re-irradiation is tolerable and effective *(2)* and could be evaluated with new targeted agents. Recently, another DIPG case series demonstrated the tolerability of histone deacetylase (HDAC) inhibition in conjunction with re-irradiation, supporting the report of novel approaches even when in small series *(10)*. While we believe the best system for evaluating novel therapeutics is within a clinical trial, some disease subsets, such as *NF1*-mutant DIPG, are so rare that even with international collaboration their molecular targeting could not be studied within a clinical trial. Ultimately, whether evaluated in a clinical trial or by experienced clinical teams with careful consideration, molecularly targeted agents may be beneficial in combination with other novel therapies, conventional chemotherapy, or radiation for children with DIPG/DMG.

Nonstandard Abbreviations: BAT, Best available therapy; ctDNA, Circulating tumor DNA; DIPG, Diffuse intrinsic pontine glioma; DMG, Diffuse midline glioma, H3 K27M-mutant; Gy, Gray; HDAC, Histone deacetylase; LGG, Low-grade glioma; SCH, Seattle Children's Hospital; WHO, World Health Organization.

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Human Genes: *ACVR1*, activin A receptor type 1; *H3-3A*, H3.3 histone A; *KDR*, kinase insert domain receptor; *KIT*, KIT proto-oncogene, receptor tyrosine kinase; *KRAS*, KRAS proto-oncogene, GTPase; *NF1*, neurofibromin 1; *PDGFRA*, platelet derived growth factor receptor alpha; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; *TP53*, tumor protein p53; *MYC*, MYC proto-oncogene, bHLH transcription factor; *FGFR1*, fibroblast growth factor receptor 1.

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REFERENCES

1. Pritchard CC, Salipante SJ, Koehler K, Smith C, Scroggins S, Wood B, et al. Validation and implementation of targeted capture and sequencing for the detection of actionable mutation, copy number variation, and gene rearrangement in clinical cancer specimens. J Mol Diagn 2014;16:56–67.
2. Janssens GO, Gandola L, Bolle S, Mandeville H, Ramos-Albiac M, van Beek K, et al. Survival benefit for patients with diffuse intrinsic pontine glioma (DIPG) undergoing re-irradiation at first progression: a matched-cohort analysis on behalf of the SIOP-E-HGG/DIPG working group. Eur J Cancer 2017;73:38–47.
3. Cooney T, Lane A, Bartels U, Bouffet E, Goldman S, Leary SES, et al. Contemporary survival endpoints: an International Diffuse Intrinsic Pontine Glioma Registry study. Neuro-Oncology 2017;19:1279–80.
4. Louis DN, Perry A, Reifenberger G, von Deimling A, Figarella-Branger D, Cavenee WK, et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. Acta Neuropathol 2016;131:803–20.
5. Mackay A, Burford A, Carvalho D, Izquierdo E, Fazal-Salom J, Taylor KR, et al. Integrated molecular meta-analysis of 1,000 pediatric high-grade and diffuse intrinsic pontine glioma. Cancer Cell 2017;32:520–37.
6. Banerjee A, Jakacki RI, Onar-Thomas A, Wu S, Nicolaides T, Young Poussaint T, et al. A phase I trial of the MEK inhibitor selumetinib (AZD6244) in pediatric patients with recurrent or refractory low-grade glioma: a Pediatric Brain Tumor Consortium (PBTC) study. Neuro-oncology 2017;19:1135–44.
7. Huang TY, Piunti A, Lulla RR, Qi J, Horbinski CM, Tomita T, et al. Detection of Histone H3 mutations in cerebrospinal fluid-derived tumor DNA from children with diffuse midline glioma. Acta Neuropathol Commun 2017;5:28.
8. McDonald BR, Contente-Cuomo T, Sammut SJ, Odenheimer-Bergman A, Ernst B, Perdigones N, et al. Personalized circulating tumor DNA analysis to detect residual disease after neoadjuvant therapy in breast cancer. Sci Transl Med 2019;11: eaax7392.
9. Ahn S, Brant R, Sharpe A, Dry JR, Hodgson DR, Kilgour E, et al. Correlation between MEK signature and Ras gene alteration in advanced gastric cancer. Oncotarget 2017;8: 107492–9.
10. Wang ZJ, Ge Y, Altinok D, Poulik J, Sood S, Taub JW, et al. Concomitant use of panobinostat and reirradiation in progressive DIPG: report of 2 cases. J Pediatr Hematol/Oncol 2017;39:e332–5.