The experience of successful treatment of ETV6-NTRK3-positive infant glioblastoma with entrectinib

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In recently published articles, the superior outcome of infant patients with high-grade gliomas (HGGs) when compared with older children was demonstrated. Receptor tyrosine kinase (RTK) gene fusions highlight the distinct biology of infant HGGs and could provide the rationale for utilization of targeted therapy. Here, we describe a case of ETV6-NTRK3-positive infant HGG successfully treated with the TRK/ROS1/ALK inhibitor entrectinib. To date, reports of fusion-targeting therapy in these patients remain sparse.

Our female patient was diagnosed at 4 months of age, presenting initially with increasing head circumference, vomiting, nystagmus, right-sided hemiparesis, and regression of motor skills. An MRI revealed a large (9 x 5.6 x 9.3 cm), heterogenous tumor with solid, cystic, and hemorrhagic components in the left cerebral hemisphere with accompanying hydrocephalus (Figure 1A). No metastasis was noted.

Due to the high risk of intraoperative bleeding, a biopsy was not performed, and chemotherapy with carboplatin and etoposide and subsequent cyclophosphamide and vincristine was initiated. An MRI was performed after 2 cycles of chemotherapy and showed a 32% reduction of the tumor, at which time a biopsy was safely obtained, followed by neuropathological and molecular analysis. Based on morphology and immunophenotype, this tumor was classified as a high-grade glioma. Molecular testing of tumor RNA revealed the expression of the ETV6-NTRK3 fusion transcript, where exon 5 of ETV6 gene was fused in-frame to exon 15 of NTRK3 gene (COSF571) (Figure 1H). The finding was confirmed by reverse-transcription PCR with Sanger sequencing of the amplicon. The integrative diagnosis was stated as infant (receptor tyrosine kinase driven) glioblastoma.

After the biopsy, the patient received 2 additional cycles of chemotherapy with the same regimen. Repeat neuroimaging revealed disease stabilization, although due to worsening seizure activity, a surgical resection was performed and resulted in near-total tumor resection with residual tumor 1.4 cm³ (Figure 1B). The tissue samples from both surgeries had identical pathological features such as cellular and nuclear polymorphism, high mitotic activity, and focal microvascular proliferation, in some areas post-treatment changes were noted as well. These findings were matched with diagnosis of glioblastoma.

Because of clinical deterioration (appearance of seizures), presence of residual tumor mass and potential benefit from targetted therapy, entrectinib 100 mg per day orally was administered as second-line treatment. Entrectinib was obtained from Roche via compassionate use. The neuroimaging performed after 3 months of targeted treatment showed complete response (Figure 1C). Transient neutropenia was the only adverse event requiring a brief (3 day) treatment interruption.

To date, the patient remains stable on entrectinib without evidence of recurrence after 8 months of treatment. The treatment has been exceedingly well tolerated with minimal adverse effects. To our knowledge, sufficient clinical data to suggest a specific duration of therapy have not yet been published. However, because the experience of targeted treatment for malignant gliomas suggests that treatment discontinuation leads to rapid disease progression, we would consider treatment continuation until disease progression or significant toxicity.

Patients with infant HGG harboring NTRK fusions have better outcome comparing to older patients. There are no clinical trials, that provide direct comparison of the effectiveness of chemotherapy and targeted therapy. Moreover, rapid disease progression during chemotherapy has been reported. Stucklin et al. showed a 5-year overall survival of 42.9% in young patients with hemispheric NTRK-fused gliomas, whereas Torre et al. described progression and recurrence in both reported cases of infantile NTRK-fused gliomas.

The first reported case of successful treatment of a patient with ETV6-NTRK3-positive HGG with larotrectinib...
Figure 1. (A) Initial axial T1-weighted contrast brain MR images demonstrating large tumor with contrast enhancement. (B) T1-weighted axial contrast brain MR images performed after second operation. (C) Axial T1-weighted contrast brain MR images after 3 months of entrectinib therapy showing complete tumor regression. (D) Plump neoplastic cells with abundant cytoplasm and moderate nuclear polymorphism. Some mitotic figures are seen. ×400, H&E, x400, scale bar = 40 µm. E. Post-treatment changes are presented as claster of xanthoma cells with central calcification, H&E, ×200, scale bar = 20 µm. (F) Immunostaining reveals tumor tissue positivity for GFAP expression. GFAP, ×100, scale bar = 10 µm. (G) Increased proliferative activity according Ki67 to 15%. Ki67, ×200, scale bar = 20 µm. (H) Schematic representation of the ETV6-NTRK3 gene fusion and chimeric protein structure. Sequence analysis of the ETV6-NTRK3 fusion transcript demonstrates exons 1 to 5 of ETV6 are fused to exons 15 to 20 of NTRK3 preserving the tyrosine kinase domain. In Sanger sequence chromatogram the black vertical line indicates the fusion breakpoint. ENST00000396373.9 and ENST00000360948.6 transcripts were used as reference sequences for ETV6 and NTRK3 genes respectively, genome build GRCh38.p13.
### Table 1. Review of Patients With Infant Glioma, Who Received Targeted Therapy

| Parameter/References | Ziegler et al.3 | Clarke et al. Case 1 | Clarke et al. Case 2 | Alharbi et al.4 | Torre et al.7 | Torre et al.7 | Torre et al.7 | Desai et al.5 | Our Case |
|----------------------|-----------------|----------------------|----------------------|-----------------|--------------|--------------|--------------|--------------|----------|
| **Sex**              | Female          | Female               | No data              | Female          | No data      | No data      | No data      | No data      | Female   |
| **Age at diagnosis** | 5 months        | 36 weeks of gestation| 11 months           | 18 months       | Pediatric patient | Pediatric patient | Infantile patient | No data | 4 months |
| **Tumor location**   | Right lateral ventricle | Frontal lobe | Pons | Frontal lobe | No data | No data | No data | No data | No data | Left cerebral hemisphere |
| **Pathology**        | High-grade glioma | Glioblastoma (WHO grade IV) | Low-grade neuroepithelial neoplasm | Glioblastoma (WHO grade IV) | No data | No data | No data | No data | Unspecified | Glioblastoma (WHO grade IV) |
| **Surgery**          | 1) GTR          | 1) Biopsy            | 2) partial resection in progression | 1) GTR          | GTR          | No data      | No data      | No data      | No data | 1) Biopsy | 2) Subtotal resection |
| **Prior CT**         | Vincristine, cisplatin, cyclophosphamide, etoposide, carboplatin, ifosfamide | Methotrexate, vincristine, etoposide, cyclophosphamide, thiotepa | Vincristine and carboplatin | No data | No data | No data | No data | No data | Carbo platin, etoposide, cyclophosphamide, vincristine |
| **Prior RT**         | 54 Gy to the tumor bed | No | No | No | No data | No data | No data | No data | No | No |
| **NTRK rearrangement** | ETV6:NTRK3 fusion | ETV6:NTRK3 fusion | ETV6:NTRK3 fusion | Unspecified | Unspecified | Unspecified | ETV6:NTRK3 fusion | ETV6:NTRK3 fusion |
| **Inhibitor**        | Larotrectinib   | 1) Crizotinib for 9 months | Larotrectinib | Larotrectinib | Larotrectinib | Larotrectinib | Entrectinib | Entrectinib |
| **Response to treatment** | MRI at 5 months confirmed the response, with resolution of enhancement in the tumor bed and almost all metastatic lesions | No evidence of recurrence after 12 months of treatment | MRI after 8 weeks of therapy showed marked tumor regression | Decrease in tumor burden | Stable disease | Treatment was terminated due to elevated liver function tests | Complete response | Complete response |
| **Side effects**     | No              | No data              | No data              | No data         | No data      | Elevated liver function tests | No data | Transient neutropenia |

GTR, gross total resection; WHO, World Health Organization.
was published in 2018 in a 3-year-old girl with progressive HGG after irradiation and chemotherapy. Among 12 children with ETV6-NTRK3-positive infant HGGs described by Clarke et al., only 2 patients received targeted therapy with larotrectinib and both with significant clinical benefit. One case has also been reported of upfront usage of NTRK inhibition by larotrectinib for a patient with infantile glioblastoma harboring ETV6-NTRK3 fusion. The phase 1/2 STARTRK-NG trial (NCT02650401) of entrectinib demonstrated encouraging results in patients with NTRK fusion-positive CNS tumors (n = 8), including one patient with ETV6-NTRK3-positive high-grade glioma.

There are several cases of successful targeting ETV6-NTRK3-positive infant high-grade glioma, with only one patient receiving entrectinib. Among the reported cases treatment was effective and well-tolerated. The summary of published cases of infant glioma with targeted therapy is given in Table 1. These early data suggest that both available NTRK inhibitors (larotrectinib and entrectinib) could be an effective treatment for patients with ETV6-NTRK3-positive infant HGG.

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**Conflict of interest statement.** The authors have no conflicts to declare.

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