TG02, A NOVEL MULTIKINASE INHIBITOR, IS EFFECTIVE IN PEDIATRIC BRAIN TUMORS, WITH SELECTIVE POTENCY IN THOSE WITH MYC EXPRESSION

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Background:
- Brain tumors account for the most cancer-related deaths among children.
- MYC is one of the key oncogenes implicated in the tumor pathogenesis, and MYC (c-MYC, MYCN, and MYCL) deregulation is common in various types of malignant brain tumors in children, including those with the worst prognoses.
- Subsets of pediatric glioblastoma (GBM), medulloblastoma, diffuse intrinsic pontine glioma (DIPG), and atypical teratoid rhabdoid tumor (ATRT) have been shown to harbor MYC overexpression, amplification, and chromosomal translocation.
- Cyclin-dependent kinase 9 (CDK9) is a key regulator of transcription via its substrate, RNA polymerase II, and with CDK9 inhibition, very short-lived proteins are rapidly depleted, resulting in caspase activation and subsequent apoptosis.
- MYC is a short-lived protein that plays a prominent role in cancer cell survival signaling and has been shown to be depleted with CDK9 inhibition.
- TG02, a novel, orally bioavailable CDK9 inhibitor, which also inhibits other CDKs (1, 2, 5, 7), works at achievable exposures and has demonstrated anti-tumor activity in vitro and in vivo.
- TG02 significantly reduced MYC in cell lines, primary cells, and mouse models of adult glioblastoma.
- TG02 has not yet been evaluated with pediatric brain tumors.

Objective:
- To test the efficacy of CDK inhibitor TG02 against a panel of pediatric brain tumors, both cell lines and primary cells.
- To determine if c-myc expression is related to efficacy

Hypothesis:
- We hypothesize that therapy with TG02 will be effective in pediatric brain tumors. We anticipate that TG02 will be more effective in cell lines harboring c-myc expression.

Methods:
- We interrogated various pediatric brain tumor cell lines using TG02 (Tragara) monotherapy.
- We used a combination of immortalized and primary cell lines. Cell lines include NHA (Normal Human Astrocytes), DBTRG (BRAFV600E-mutant glioblastoma), SF188 (p53-mutant glioblastoma), KNS42 (G34-mutant glioblastoma), MED8a (c-myc amplified, TP53 wildtype medulloblastoma), DAOY (c-myc non-amplified, TP53 mutapt medulloblastoma), SF10067 (medulloblastoma), SF11178 (atypical teratoid rhabdoid tumor).
- Cell Viability Assays were conducted in 10% FBS containing media, and cells were treated for 72 hours.
- Western blots were performed using c-myc antibody in proteins extracted from untreated cells.

Results:
Fig 1. Treatment with TG02 significantly reduced cell viability in various pediatric brain tumor cell lines. Cell viability was measured by WST-1 assay. Cells were treated with TG02 for 72 hours. DMSO was used as control.

Fig 2. MED8a and SF188 showed the highest c-myc expression. IC50 from cell viability assay (Figure 1). C-myc expression from immunoblot (Figure 2).

Table: IC50 and c-myc expression

<table>
<thead>
<tr>
<th>Cell line</th>
<th>IC50</th>
<th>c-myc expression</th>
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<tbody>
<tr>
<td>NHA</td>
<td>0.105</td>
<td>-</td>
</tr>
<tr>
<td>DBTRG</td>
<td>0.5745</td>
<td>-</td>
</tr>
<tr>
<td>SF188</td>
<td>0.2722</td>
<td>+</td>
</tr>
<tr>
<td>KNS42</td>
<td>0.2877</td>
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<tr>
<td>MED8a</td>
<td>0.0819</td>
<td>+</td>
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<tr>
<td>DAOY</td>
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<td>-</td>
</tr>
<tr>
<td>SF10067</td>
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<td>-</td>
</tr>
<tr>
<td>SF11178</td>
<td>0.1302</td>
<td>+</td>
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Conclusions/Implications:
- While treatment with TG02 universally resulted in decreased cell viability, TG02 was selectively more potent in cells with high levels of c-myc expression as compared to those with low levels of expression.
- The greatest efficacy in reduction of cell proliferation with TG02 treatment was shown in c-myc-amplified medulloblastoma.
- This work provides rationale for pursuing in vivo studies testing the efficacy of TG02 in pediatric brain tumors that express MYC.

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