3. Study Methods

3.1 In vitro Studies

- Cells from 8 pediatric GBM PDOX models treated with CT-179 were used.
- Gene expression levels of OLIG2 measured from tumor tissue.
- Higher expression in invasive glioma cells than tumor core.
- Highly expressed in all human diffuse gliomas including GBM and DIPG.

3.2 In vivo Studies

- Determined ideal dose and dosing frequency of CT-179 in mice.
- Assessed if CT-179 is brain penetrant.
- Dose-ranging studies:
  - Same cell number (vol)
  - Same depth
  - Same location
- Normal Brain Intra-cerebral GBM Xenograft (IC-1406 GBM)
- Normal Brain Intra-cerebellar MB Xenograft (ICb-984 MB)
- Orthotopic PDX Models for in vivo Drug Testing

- Intra-cerebral (IC) studies:
  - Same cell number (vol)
  - Same depth
  - Same location
- Brain Tumor Cells:
  - GBM tumor cells harvested from patient-derived orthotopic xenograft (PDOX) models.
  - Cells from 8 pediatric GBM PDOX models treated with CT-179 were used.
  - Gene expression levels of OLIG2 measured from tumor tissue.
- Inhibitor CT-179 in Orthotopic PDX Models of Pediatric Glioblastomas

Table 1: CT-179 lead to in vivo inhibition of cell growth in 4 pediatric GBM models grown as monolayer cells in FBS-based media and as cancer stem cell-enriched neurospheres in FBS-negative media. OLIG2 expression levels varied between GBM models.

4. Results of in vitro studies.

- Figure 1: Representative CCK-8 curves from 5 pediatric GBM PDOX models showing time- and dose-dependent inhibition of cell growth seen when treated with CT-179. GBM cell subtypes directly proportional to cell viability.

5. Discussion and Conclusions

- The combination of CT-179 and XRT increased survival times in some pGBM PDOX models.
- CT-179 effectively penetrated and accumulated in CNS tissues and PDOX tumors.
- CT-179 inhibited cell viability of pGBM cells in vitro.
- Combined with fractionated radiation (2 Gy/day x 5 days).

Table 2: Examination of animal survival times following the treatment with CT-179 alone and in combination with fractionated radiation (2 Gy/day x 5 days).

- The combination of CT-179 and XRT resulted in statistically significant survival benefits.
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