# Novel targets in ependymal tumors

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## Abstract

BACKGROUND: Despite surgery and radiation for ependymomas, dissemination and recurrence occur. The role of chemotherapy is not well-defined. Alternative targeted approaches are desperately needed.

METHODS: Ependymal tumors (total, n=47; cranial, n=30; spinal, n=17; grade-I, n=8; grade-II, n=11; grade-III, n=23) were profiled by Caris Life Sciences using immunohistochemistry, next-generation sequencing, and in-situ hybridization.

RESULTS: The median age was 34, spanning 3-79 years. Cranial tumors occurred in younger patients relative to spinal tumors (median age 30.2 vs. 47; p=0.018). There was a trend for a higher incidence of spinal tumors in males, at 65% vs 37% (p=0.07). While 3%, 23%, and 63% of cranial tumors were of grades-I, -II, and -III, respectively; spinal tumors were of lower grade, with 41%, 24%, and 24% being grades-I, -II, and -III. The DNA excision repair protein ERCC1, which correlates with cisplatin resistance, is frequently expressed in grade-I (80%, 4/5) tumors but is absent in 57% of grade-II (4/7), and grade-III (8/14) tumors. ERCC1 expression is more frequent in spinal tumors (67%; 6/9) than in cranial (42%, 8/19). MGMT-promoter-methylation was seen in 14% of tumors and over-expression was present in 17%. Frequently overexpressed targets included PTEN (80%, 36/45), TOPO1 (62%; 26/42), EGFR (62%, 8/13), TUBB3 (52%; 12/23), and RRM1 (49%; 17/35). Adults were more likely to have PTEN (31/35 vs 5/10; p=0.017) or RRM1 overexpression (17/30 vs 0/5, p=0.045) relative to pediatric patients. TOPO1 expression decreased with grade (I=86%, II=73%, III=55%; p=0.015) while thymidylate synthase expression increased with grade (I=13%, II=18%, III=66%; p=0.058). Among 23 tumors sequenced, PTEN K267fs was seen in one grade-III cranial tumor. An ALK translocation was identified in one grade-III ependymoma.

CONCLUSIONS: If predicated on tumor expression, our data suggest that therapeutics directed toward ERCC1, ALK, PTEN, TOPO1, EGFR, TUBB3, and RRM1 could be considered for conventional treatment-refractory ependymal tumor patients.

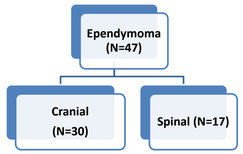
## Background

Ependymomas occur in both the brain and the spine in pediatric and adult populations. Ependymoma is a molecularly heterogeneous disease and have multiple clinico-pathological factors affecting clinical outcome, including age, localization, and extent of tumor resection. In spite of this heterogeneous nature, treatment is mainly based on surgery with or without radiation therapy, and there has not been significant changes in the treatment paradigm in the past 20 years. The use of chemotherapies including temozolomide, platinum agents, epirubicin and etoposide has generated variable results. There is an urgent need for informative biomarkers to tailor the treatment strategy.

As such, the purpose of this study was to analyze a group of ependymoma tumors to explore targetable aberrations that could potentially inform the selection of effective chemotherapeutic regimens, targeted therapies, and immune checkpoint inhibitors.

## Results

**Figure 1: Patient characteristics**




| | | Total |
|---|---|---|
| Gender | Female | N=25 |
| | Male | N=22 |
| Grade | Grade I | N=8 |
| | Grade II | N=11 |
| | Grade III | N=23 |
| | Grade unclear | N=5 |
| | Average (range) | 34.9 |
| Age | Pediatric | N=10 |
| | Adult | N=37 |

- Cranial tumors occurred in younger patients relative to spinal tumors (median age 30.2 vs. 47; p=0.018).
- Cranial tumors are seen with higher grades compared to spinal tumors: 3% (1/30) of cranial tumors are grade I and 63% (19/30) are grade III; whereas 41% (7/17) of spinal tumors are grade I and only 27% (4/17) are grade III (p=0.0019 and 0.0145, respectively).

## Results, continued

**Figure 2: A) Protein expression frequencies in 47 ependymoma tumors; B) Comparison of protein expression frequency in spinal versus cranial tumors.** A star indicates statistically significant difference found between different tumor location. (p<0.05)

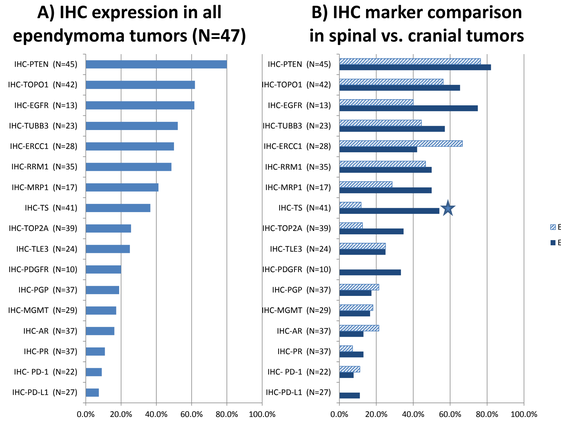
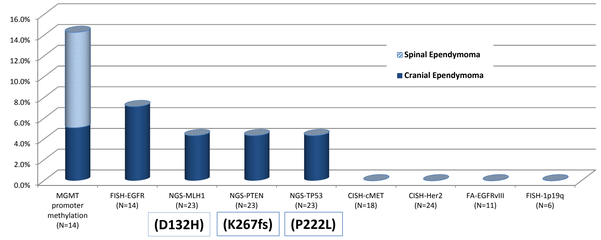
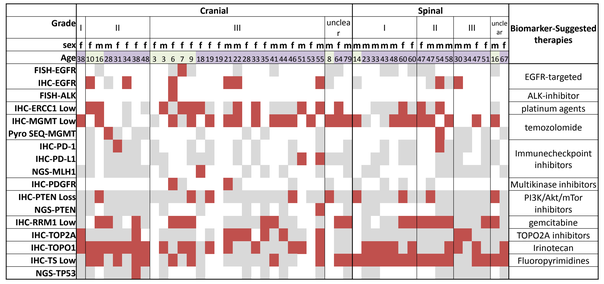



**Figure 3: Molecular aberrations detected by pyrosequencing (MGMT promoter methylation), in-situ hybridization, and Next-Gen sequencing.**




## Results, continued

**Figure 4: Oncoprint of the 47 ependymoma tumors studies and the biomarker-suggested therapies.** Molecular aberrations potentially associated with responses to chemotherapy or targeted therapies are marked red, other results are marked in grey. Blank indicates data not available. For age, pediatric cases are marked green and adult cases are marked purple.




## Conclusions

1. Cranial ependymomas are seen in younger patients and present at higher grades, consistent with its reported aggressive behavior and poor prognosis. Significantly higher expression of TS and a trend of higher TOP2A expression support the clinical observations.

2. Molecular aberrations detected by multiple technologies suggest potential responsiveness of chemotherapies including platinum agents, temozolomide and Top2A inhibitors in a select group of patients carrying a favorable biomarker profile.

3. Biomarkers suggesting therapies targeting EGFR, ALK, PIK3CA/Akt/mTor pathway are identified in the cohort studied. PD-L1 expression and MLH1 mutation seen in grade III cranial tumors suggest the investigation of immune checkpoint inhibitors.

## References

- Lee, CH., C.H., Kim, et al (2016) "Genetic differences on intracranial versus spinal cord ependymal tumors: a meta-analysis of genetic researches" Eur Spine J. 2016 Sep 16. [Epub ahead of print]
- Gramatzki, D., M. Weller, et al (2016) "Chemotherapy for intracranial ependymoma in adults. "BMC Cancer. 16:287.