

results of subjects treated at dose level 1 (DL1; 1 million GD2-CAR T-cells/kg IV). Methods: Four patients (3 DIPG, 1 spinal DMG; ages 4–25; 1M/3F) were enrolled at DL1. Three subjects with H3K27M+ DIPG received 1e6 GD2-CAR T-cells/kg IV on study. One patient with spinal DMG enrolled but became ineligible after manufacturing and was treated on an eIND at DL1. An Ommaya reservoir was placed in all subjects for therapeutic monitoring of intracranial pressure. Subjects underwent lymphodepletion with fludarabine/cyclophosphamide and remained inpatient for at least two weeks post-infusion. Results: All subjects developed cytokine release syndrome (Grade 1–3) manifested by fever, tachycardia and hypotension. Other toxicities included ICANS (Grade 1–2) and neurological symptoms/signs mediated by intratumoral inflammation which we have termed Tumor Inflammation-Associated Neurotoxicity (TIAN). No evidence of on-target, off-tumor toxicity was observed in any patients. No dose-limiting toxicities occurred. CAR T cells trafficked to the CNS and were detected in CSF and blood. 3/4 patients exhibited marked improvement or resolution of neurological deficits and radiographic improvement. The patient treated on an eIND exhibited >90% reduction in spinal DMG volume but progressed by month 3. Re-treatment of this subject via intracerebroventricular administration resulted in a second reduction in spinal DMG volume by ~80%. Conclusions: GD2-CAR T-cells at DL1 demonstrate a tolerable safety profile in patients with H3K27M+ DIPG/DMG with clear signs of T-cell expansion and activity including clinical responses.

#### EPCT-15. RAPID EPIGENOMIC CLASSIFICATION OF BRAIN TUMORS ENABLES INTRAOPERATIVE NEUROSURGICAL RISK MODULATION

Luna Djirackor<sup>1</sup>, Skarphedinn Halldorsson<sup>1</sup>, Pitt Niehusmann<sup>2,3</sup>, Henning Leske<sup>2,3</sup>, Luis P. Kuschel<sup>4</sup>, Jens Pahnke<sup>2,3</sup>, Bernd J. Due-Tønnessen<sup>6</sup>, Iver A. Langmoen<sup>1,6</sup>, Cecilie J. Sandberg<sup>1</sup>, Philipp Euskirchen<sup>4,7</sup>, Einar O. Vik-Mo<sup>1,6</sup>, Vilhelm Magnus Laboratory for Neurosurgical Research, Institute for Surgical Research/ Department of Neurosurgery, Oslo University Hospital, Oslo, Norway, <sup>2</sup>Section of Neuropathology, Department of Pathology, Oslo University Hospital, Oslo, Norway, <sup>3</sup>Institute of Clinical Medicine (KlinMED), Faculty of Medicine, University of Oslo, Oslo, Norway, <sup>4</sup>Department of Neurology, Charité-Universitätsmedizin Berlin, Berlin, Germany, <sup>5</sup>Department of Pharmacology, Faculty of Medicine, University of Latvia, Riga, Latvia, <sup>6</sup>Department of Neurosurgery, Oslo University Hospital, Oslo, Norway, <sup>7</sup>German Cancer Consortium (DKTK), partner site Berlin, German Cancer Research Center (DKFZ), Berlin, Germany

Background: Clear identification of tumor subtype is the main predictor of patient outcome and ultimately what is considered an adequate level of surgical risk. At brain tumor resection, imaging modalities and intraoperative histology often give an ambiguous diagnosis, complicating intraoperative surgical decision-making. Here, we report a nanopore DNA methylation analysis (NDMA) sequencing approach combined with machine learning for classification of tumor entities that could be used intraoperatively. Methods: We analyzed 50 biopsies obtained from biobanked tissue (43, prospective) or sampled at surgery (7, intraoperative) from 20 female and 30 male patients with a median age of 8 years. DNA was extracted using spin columns, quantified on a Qubit fluorometer and assessed for purity using NanoDrop spectrophotometer. DNA was then barcoded with the Rapid Barcoding kit from Oxford Nanopore technologies and loaded onto a MinION flow cell. Sequencing was performed for 3 hours (intraoperative) and 24 hours (prospective). Raw reads were basecalled using the Guppy algorithm, then fed into a snakemake workflow (nanoDx pipeline). This generated a report showing the copy number profile, genome-wide methylation status and subclassification of the tumor according to the Heidelberg reference cohort. Results: Twelve different tumor classes were discovered within our cohort spanning from WHO Grade I to Grade IV. The results generated by NDMA were concordant with standard neuropathological diagnosis in 43 out of 50 cases (86%). Of the discordant cases, six were due to the biological complexity of the tumor and one case was misclassified by the pipeline. NDMA enabled correct subclassification of 6/7 intraop cases within a mean of 129 minutes. Conclusion: NDMA can accurately subclassify tumor entities intraoperatively and guide surgical procedures when preoperative imaging and frozen section evaluation are unclear.

#### EPCT-16. LENALIDOMIDE ACTIVITY IN PILOCYTIC ASTROCYTOMA AND OPTIC PATHWAY GLIOMAS: REPORT ON CHILDREN'S ONCOLOGY GROUP ACNS1022

Katherine Warren<sup>1,2</sup>, Gilbert Vezina<sup>3</sup>, Linda Springer<sup>4</sup>, Allen Buxton<sup>4</sup>, Cody Peer<sup>5</sup>, W. Douglas Figg<sup>5</sup>, Maryam Fouladi<sup>6</sup>, Amar Gajjar<sup>7</sup>, Mark Krailo<sup>8</sup>, Daniel Bowers<sup>9</sup>, Dana Farber Cancer Institute, Boston, MA, USA, <sup>2</sup>Boston Children's Hospital, Boston, MA, USA, <sup>3</sup>Children's National Hospital, Washington, DC, USA, <sup>4</sup>Children's Oncology Group Statistics and Data Center, Arcadia, CA, USA, <sup>5</sup>National Cancer Institute, Bethesda, MD, USA, <sup>6</sup>Nationwide Children's Hospital, Columbus, OH, USA, <sup>7</sup>St. Jude Children's Hospital, Memphis, TN, USA, <sup>8</sup>University of Southern

California, Los Angeles, CA, USA, <sup>9</sup>UT Southwestern Medical Center, Dallas-Ft. Worth, TX, USA

Children with low-grade glioma have excellent survival rates but often suffer from the morbidity of treatment, particularly from cytotoxic chemotherapies. Targeted agents appear to have some activity but the long-term effects of inhibiting normal developmental pathways are unknown. Lenalidomide is an oral immunomodulatory agent with additional properties including anti-angiogenesis. Phase I studies indicated greater tolerability of this agent compared to adults, and a potential dose-response effect. We performed a Phase 2 trial of lenalidomide in children with pilocytic astrocytoma and optic pathway gliomas who failed initial therapy. The primary objective was to determine the objective response rate of children randomized to Regimen A low-dose (20 mg/m<sup>2</sup>/dose) or Regimen B high-dose (115 mg/m<sup>2</sup>/dose) lenalidomide, each administering lenalidomide daily x 21 days of each 28-day course. Secondary objectives included estimation of event-free survival (EFS) in this population and correlation of plasma lenalidomide concentration with toxicity and outcome.

Results: 74 eligible patients were enrolled (n=37 for each arm). The pre-defined activity level of interest was achieved for both arms. Objective responses were observed in both arms, with 4 partial responses in each. A total of n=18 patients completed 26 courses of therapy (Arm A, n=12, Arm B, n=6) The median number of courses on each arm was 14 (range 2–26) for Arm A and 11 for Arm B (range 1–26). Of the 74 eligible patients who received study drug, 30 required a dose reduction for toxicity (Arm A, n=6, Arm B, n=24) and 16 discontinued treatment on protocol due to toxicity (Arm A, n=2, Arm B, n=14). Conclusion: Lenalidomide demonstrates a sufficient level of activity in children with low-grade glioma to warrant further exploration in Phase 3 studies. Low-dose (20 mg/m<sup>2</sup>) lenalidomide appears to have better tolerability.

#### EPCT-17. DEVELOPING EYA PHOSPHATASE INHIBITORS WITH ON-TARGET EFFECTS IN SHH-MEDULLOBLASTOMA

Grace H. Hwang<sup>1,2</sup>, David A. Scott<sup>1,3</sup>, and Rosalind A. Segal<sup>1,2</sup>, <sup>1</sup>Department of Cancer Biology, Dana-Farber Cancer Institute, Boston, MA, USA, <sup>2</sup>Department of Neurobiology, Harvard Medical School, Boston, MA, USA, <sup>3</sup>Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA, USA

Medulloblastoma, one of the most frequent malignant pediatric brain tumors, encompasses four molecularly and clinically distinct cancers. Sonic hedgehog (SHH)-subtype medulloblastoma constitutes about 30% of medulloblastomas, and therapies targeting the SHH pathway can lead to new highly selective treatment. The haloacetic dehalogenase (HAD) phosphatase Eyes Absent 1 (EYA1) is critically involved in the development and progression of SHH-medulloblastoma: *Eya1* is highly expressed in SHH-medulloblastomas, and single cell sequencing indicates that *Eya1* is a consistent feature that can be detected in every individual cancer cell. Inhibition of EYA1 interrupts SHH pathway signaling. During normal development, EYA1 promotes symmetric division of cerebellar granule cell precursors (GCPs), the cells of origin for SHH-subtype medulloblastoma, and reduced levels of EYA1 decrease medulloblastoma mortality rates in mouse models. Therefore, targeting EYA1 may be a novel therapeutic avenue for these pediatric cancers. Benzarone derivatives have been suggested as allosteric EYA-inhibitors, and benzarone provides a promising platform for chemical derivatives. Here, we develop 60 novel benzarone derivatives and assess their efficacy in inhibiting SHH-medulloblastoma growth through the inhibition of EYA1. Several of the new compounds inhibit EYA1 phosphotyrosine phosphatase activity in a cell-based assay, interrupt SHH pathway, and prevent SHH-medulloblastoma growth *in vitro*. Our results show that these novel benzarone derivatives are a new promising avenue for developing therapeutics for pediatric SHH-medulloblastoma via inhibition of EYA phosphatases.

#### EPCT-18. A TWO-PART, PHASE 1 STUDY OF RHENIUM-186 NANOLIPOSOME (186RNL) DELIVERED BY CONVECTION ENHANCED DELIVERY FOR RECURRENT, REFRACTORY, OR PROGRESSIVE EPENDYMOMA AND HIGH-GRADE GLIOMA (HGG) AND NEWLY DIAGNOSED DIFFUSE INTRINSIC PONTINE GLIOMA (DIPG)

Ashley Plant<sup>1,2</sup>, Stewart Goldman<sup>3,4</sup>, Sandi Lam<sup>1,2</sup>, Michael DeCuypere<sup>1,2</sup>, Gregory Stein<sup>5</sup>, and Andrew Brenner<sup>6</sup>; <sup>1</sup>Ann and Robert H. Lurie Children's Hospital, Chicago, IL, USA, <sup>2</sup>Northwestern University Feinberg School of Medicine, Chicago, IL, USA, <sup>3</sup>Phoenix Children's Hospital, Phoenix, AZ, USA, <sup>4</sup>University of Arizona College of Medicine, Phoenix, AZ, USA, <sup>5</sup>Plus Therapeutics, Inc, Austin, TX, USA, <sup>6</sup>University of Texas Health Science Center at San Antonio, San Antonio, TX, USA

Ependymoma, HGG, and DIPG are gliomas that are often difficult to treat, frequently aggressive, and often carry an extremely poor prognosis. While external beam radiation therapy (EBRT) remains a central component of