various grades. Loss of p16 expression was defined as less than 2% IHC positivity in tumor cells.
Results: Based on histologic features, 4 meningiomas were diagnosed as G1, 26 as G2, and 13 as G3. Among the 43 tumors, 7 harbored *CDKN2A* homozygous deletion and 1 harbored a *CDKN2A* frameshift mutation with loss of the remaining wildtype allele. All 8 tumors were histologically G3 and were p16 negative by IHC. Among the remaining 35 tumors with intact/wildtype *CDKN2A* alleles, p16 expression was variably positive in 31 and lost in 4: 1/4 G1 and 3/26 G2 tumors. Of note, p16 expression was generally patchy and weaker in G1 compared to G2 or G3 tumors. Overall, the sensitivity of p16 loss among those with *CDKN2A* homozygous deletion or truncating mutation was 100% (8/8) and the negative predictive value was 100% (31/31).
Conclusion: Loss of p16 expression by IHC is a sensitive marker for *CDKN2A* homozygous deletion or truncating mutation in meningioma. However, occasional meningiomas without detectable *CDKN2A* alterations may show absence of p16 expression by IHC, the significance of which is unclear. These results support p16 IHC as a potential cost-effective screening assay in higher-grade meningiomas to identify cases that would benefit from molecular testing assessing *CDKN2A* status, informing prognostic tumor grading and possible targeted therapeutic treatment for these patients.

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Comprehensive Clinical and Molecular Evaluation of Atypical Meningioma (CNS WHO grade 2) Following Gross Total Resection

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Atypical meningioma, CNS WHO grade 2, (AM2) shows intermediate risk of recurrence/progression, and molecular parameters informing management following gross total resection (GTR) remain to be established.
We evaluated an institutional cohort of 69 patients with AM2 confirmed by central pathology review, all with initial GTR, and 8 receiving adjuvant radiotherapy. All 69 primary tumors were evaluated by Oncoscan chromosomal microarray analysis, 67 by a 50-gene next-generation DNA sequencing panel, and 68 underwent DNA methylation profiling by the Illumina 850K array with subgroup assignment based on the DKFZ classifier (v12.5). RNA sequencing was performed on 24 cases with available frozen primary tissue, including 9 experiencing subsequent recurrence.
With a median follow-up of 5.2 years, 19/69 (28%) patients experienced recurrence, with five-year recurrence-free survival (RFS) of 80%. No clinical, histological, or therapeutic parameter showed statistically significant association with RFS. No mutation was associated with RFS, including *NF2* ($n = 34$), *SMARCB1* ($n = 5$), *AKT1* ($n = 2$), *SUFU* ($n = 2$), or *TERT* promoter ($n = 1$). Univariate analyses revealed losses of 1p ($n = 35$), 7p ($n = 8$), 10q ($n = 15$), 18 ($n = 14$), and gain of 1q ($n = 9$) as significantly associated with decreased RFS (Log-rank, $p < 0.05$). Combined assessment of chromosome 1p and 1q status was associated with RFS on multivariate (Cox, $p < 0.05$) and pairwise comparisons (BH-corrected Log-rank, $p < 0.05$), with chromosome 1-neutral tumors showing 5-year RFS of 100%, compared to 74% for 1p loss alone and 0% for combined 1p loss/1q gain. By DNA methylation analysis, cases spanned benign (33/68, 48.5%) and intermediate (35/68, 51.5%) meningioma subgroups, without significant RFS association (Log-rank $p = 0.45$). Ontology analysis disclosed upregulation of MAPK regulation and leukocyte chemotaxis pathways among cases which recurred.
In conjunction with established clinicopathological parameters, adjunctive chromosomal copy number analysis may best help inform AM2 risk stratification following GTR. Significance of combined 1p loss/ 1q gain in this setting may be further evaluated in larger cohorts.

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Molecular profiling of sporadic meningiomas through targeted genomic and methylation sequencing

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Meningiomas are the most common primary central nervous system tumor. While most are benign, some can impinge on critical neurovascular structures or progress to atypical high-grade tumors that invade local tissue and carry high risk of recurrence and current histological classification of these tumors does not adequately predict patient outcomes. Previous studies have characterized recurrent genetic and epigenetic alterations that predict patient prognosis and open new avenues for therapeutic intervention. Continued efforts to characterize these molecular alterations can enhance the accuracy of diagnostic classifications and aid in the discovery of novel therapeutic targets. Here we describe our approach to characterizing patient meningioma samples through high throughput genomic and methylation sequencing to 17 primary and 2 recurrent meningiomas. We uncover a diverse landscape of genomic variants in meningioma samples including recurrent mutations in the canonical meningioma tumor suppressor *NF2*. In addition to *NF2*, we find variants in *SLX4*, *ARID1A*, *KDM4C*, *ERCC2*, *EP300*, *ERBB2*, *KMT2D*, *LRP1B*, *FANCE*, *CRKL*, and *SPEN* in multiple patients, but no canonical non-*NF2* meningioma mutations. All angiomatous WHO grade I meningioma samples contained variants in the PI3K-AKT signaling pathway previously described to regulate tumor angiogenesis. Analysis of patient-matched primary and recurrent meningioma samples reveals clonal enrichment for mutations in the SWI/SNF complex subunits *ARID1A* and *SMARCA4*. Additionally, we discuss DNA methylation differences across our cohort and its utility in tumor classification. Together, our data provides additional insights into the molecular alterations in sporadic meningiomas.

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Clinical utility of whole genome cell-free tumor DNA mutational signatures for liquid biopsy of pediatric brain tumors

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Introduction: In pediatric brain tumors, targeted liquid biopsy has been unsuccessful due to a low tumor burden in coding regions. Whole genome sequencing (WGS)-derived patient specific mutational signature from a matched tumor-normal WGS can provide a sensitive and specific approach to detect mutations in circulating cell free tumor DNA (ctDNA) and provide blood-based monitoring in pediatric patients with brain tumor.

Methods: Tumor DNA was extracted from pathology tissue and normal germline DNA from the white blood cells, while ctDNA was extracted from 1-2 mL of post-surgery plasma samples. WGS was applied to sequence DNA from matched tumor-normal and plasma samples, coverage 40x for tumor-normal DNA and 20x for ctDNA. Using the C2i assay, we derived a personalized mutational pattern for each tumor and used an AI-based error suppression model for quantification and ultra-sensitive detection of ctDNA in plasma samples. A patient-specific personalized genome-wide compendium of somatic mutations was established and ctDNA tested at 1 to 4 available time points during the therapy or surveillance period. An AI-based error suppression model was implemented to filter out the noise in the cell free DNA (cfDNA). The ctDNA Tumor Fraction (TF) was compared to the clinical status and MR-based imaging.

Results: We profiled 12 pediatric brain tumors, including 4 medulloblastomas, 3 pediatric glioblastomas IDH wild-type, 2 ependymoma PFA subtype, 1 PXA, 1 diffuse leptomeningeal glioneuronal tumor and 1 low grade ganglioglioma. Tumor specific signatures were derived for all patients and correlated with the disease course on imaging at given time points reaching a 10^{-4} minimal residual disease detection sensitivity.

Conclusions: Patient-specific WGS tumor signature in ctDNA can be used for monitoring of pediatric brain tumors from blood and correlates with clinical course and imaging. This provides opportunity for minimally invasive monitoring of pediatric brain tumors.

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Beyond beta-catenin: Genetic alterations of *TP53* and *OTX2* indicate increased risk of relapse in WNT medulloblastomas.

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This genetic analysis of WNT-activated medulloblastomas (WNT-MBs) aimed to re-evaluate the prognostic impact of *TP53* mutations and to identify specific chromosomal aberrations as possible prognostic markers.

In a cohort of 191 patients with WNT-MBs, mutations in *CTNNB1*, *APC* and *TP53* were analyzed by Sanger and/or NGS panel sequencing. Chromosomal copy number aberrations (CNAs) were assessed by high-resolution, genome-wide molecular inversion probe technology (MIP), SNP6 array, and/or 850k methylation bead-array hybridization. Association with prognosis was evaluated in 120 patients with follow-up data from the HIT2000 medulloblastoma trial or HIT registries.

CTNNB1 mutations were present in 92.2% of the samples. *APC* mutations were found in 6.8% (13 samples). One *CTNNB1* wildtype tumor gained WNT-activation due to a homozygous deletion of *FBXW7*. Monosomy 6 was present in 78.6%, and more frequent in children compared to adults. 16.1% of the tumor samples showed *TP53* mutations, of those 60% with nuclear positivity for the p53 protein. A loss of heterozygosity at the *TP53* locus on chromosome 17p13.1 was found in 40.7% (11/27) of *TP53* mutant tumor samples and in 18.5% of the whole cohort (24/130 cases). Patients with tumors harboring *TP53* mutations showed significant worse progression-free survival (PFS; 5-year-PFS *TP53*wt 93% vs. *TP53*mut 68%; $p = 0.001$), and were enriched for chromosomes 17p ($p = 0.001$), 10, and 13 losses. Gains of the *OTX2* locus on chromosome 14q were found in 38.9% of samples, independent of *TP53* mutation status, and also were associated with poor PFS and OS (5-year-PFS 72% vs. 93%, $p = 0.017$; 5-year-OS 83% vs. 97%, $p = 0.006$). Multivariate Cox regression analysis for PFS/OS identified both genetic alterations as independent prognostic markers.

Our data suggest that patients with WNT-MB carrying *TP53* mutations or *OTX2* gains are at higher risk of relapse. Consequences for the clinical management including eligibility of these patients for therapy de-escalation trials need to be debated.

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Evaluating the clinical utility of DNA methylation profiling for choroid plexus tumors

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Introduction: Choroid plexus tumors (CPT) are rare, potentially aggressive CNS tumors with defined histologic criteria for grading. In recent years, several patients within our practice have demonstrated discordance between histological diagnosis and clinical behavior. DNA methylation profiling has emerged as a potential diagnostic adjunct for aiding clinical planning and treatment approach. In this study, we sought to retrospectively evaluate the clinical utility of DNA methylation profiling within our cohort of patients with CPT.

Methods: We performed a retrospective chart review of all patients with choroid plexus tumors treated at Dana-Farber / Boston's Children's Cancer and Blood Disorder Center between 1990-2021, evaluating the histology, treatment approach, and clinical outcome. Available tissue samples were sent to the National Institute of Health for DNA methylation profiling.

Results: Seventeen patients with CPT were identified. Median age at diagnosis was 1.8 years (range: 0.4-27.7). Histologic diagnosis included choroid plexus papilloma (CPP; $n = 4$), atypical choroid plexus papilloma (aCPP; $n = 5$), and choroid plexus carcinoma (CPC, $n = 8$). DNA methylation in an initial subset placed these tumors with the pediatric type A ($n = 5$), pediatric type B ($n = 6$), and adult ($n = 1$) subgroups. For one patient, methylation profiling returned as unclassifiable (possibly representing an alternative diagnosis).

Discrepancies with the histologic grade were noted in several cases: one patient diagnosed with CPP grouped with pediatric type B CPT on methylation analysis, had rapid recurrence, and a diagnosis of CPC was made on a re-resection specimen; another patient with aCPP with concerning features was classified as pediatric type A by methylation, and is without evidence of disease after initial complete resection.

Survival outcomes based on histologic diagnosis and molecular subgroups are compared and reported.

Conclusion: DNA methylation profiling is a useful tool for the diagnosis of CPT and may have the potential to guide clinical planning and management.

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Solitary Fibrous Tumor: Natural History and Prognosis in Accordance with the WHO 2021 Classification of CNS Tumors

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