ETMR-19. SINGLE CELL ANALYSES OF ETMRS REVEAL THAT C19MC+ POPULATION DRIVES CELL CYCLE PROGRESSION AND STEM CELL MAINTENANCE

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Embryonal tumors with multilayered rosettes (ETMRs) are highly fatal diseases characterized by recurrent amplification of *C19MC*, an oncogenic miRNA cluster. While *C19MC* was discovered as a major driver of ETMRs, its direct role in ETMRs remains unknown. As ETMRs exhibit significant heterogeneity in C19MC expression, we employed single cell transcriptomics to investigate features of C19MC+ population. We conducted single-nuclei RNAseq of 23,269 cells from 6 primary and 2 matched recurrent ETMRs. We also conducted single-cell RNAseq of human neural stem cells (hNSC-5miR) and ETMR cell line (A664-5miR) with stable expression of 5 C19MC miRNAs. Bulk RNAseq (n=27), H3K27Ac ChiP-seq (n=5) and ATAC-seq (n=5) corroborated scRNAseq data and identified core transcription factors (TFs) of C19MC+ population. C19MC+ population (24%) mapped to neuro-epithelial cells and exhibited signatures of cell cycle and stem cell maintenance, consistent with bulk-RNAseq data. The C19MC+ population overlaps with MKI67+ cycling (57%) and PROM1+ stem cell population (56%). Interestingly, interrogation of hNSC-5mir and A664-5miR showed a larger MKI67+/PROM1+ population compared to controls. Likewise, hNSC-5miR/A664-5miR *in vitro* and *in vivo* experiments showed increased proliferation/stemness. C19MC+ population is characterized by SHH, WNT, mTOR, Hippo and IGF-signalling and driven by MEIS1, SOX11, ZNF521, RFX4 and NR2F2 TFs. Recurrent ETMRs exhibit a persistent but smaller C19MC+ population. Intriguingly, recurrent tumors were more quiescent with a smaller proliferative population. C19MC is directly involved in driving cell cycle and stemness in ETMRs. Cellular and molecular features of primary and recurrent ETMRs were remarkably different, suggesting that C19MC plays a different role upon recurrence.

ETMR-20. IMPACT OF HIGH DOSE CHEMOTHERAPY WITH AND WITHOUT METHOTREXATE (MTX) ON OUTCOME OF PATIENTS WITH EMBRYONAL TUMORS WITH MULTI-LAYERED ROSETTES (ETMRS): A REPORT FROM CHILDREN'S ONCOLOGY GROUP PHASE III TRIAL ACNS0334

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Infant embryonal brain tumors comprise a spectrum of histologic and molecular entities including medulloblastoma (MB) and tumors collectively called CNS PNET's, including supratentorial PNET (sPNET), pineoblastoma and other less common histologic entities. Non-MB embryonal tumors, historically considered high risk disease, were included in ACNS0334, A Children's Oncology Group prospective phase III trial which compared efficacy of an induction regimen with and without methotrexate combined with high dose chemotherapy and stem cell rescue; no radiation was mandated. Molecular testing performed after ACNS0334 closure identified 14 patients with embryonal tumors with multi-layered rosettes (ETMRs), a new molecular entity previously classified under various diagnostic categories. ETMR patients made up 20% of the molecularly analyzed ACNS0334 cohort and were predominantly females. Tumors were largely non-metastatic (10/14 M0, 1 M1, 3 M2/M3) and originated in the cerebrum (8), cerebellum (3) and pineal gland (3). Gross total tumor resection was achieved in 5/11 patients with M0/M1 disease; 9/14 patients completed full treatment with 5 randomized to MTX induction and 9 to no-MTX. Five of 14 patients progressed on treatment, one had a toxic death. Disease progression was primarily local (88 %). No difference by methotrexate randomization was observed. Four patients are alive without progression 5–10+ years off therapy, none received radiation. No patients received radiation prior to progression. Four were irradiated after progression and died from disease within 3 to 13 months. Our study, a first report on ETMRs prospectively treated on a clinical trial, suggests high dose chemotherapy benefits a portion of ETMR patients.

ETMR-21. META-ANALYSIS OF PINEAL REGION TUMOURS DEMONSTRATES MOLECULAR SUBGROUPS WITH DISTINCT CLINICO-PATHOLOGICAL FEATURES: A CONSENSUS STUDY

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Pineoblastomas (PB) are rare, aggressive pineal gland tumours with poor global OS of 50–70% and only 15–49% OS for patients <4 years, despite intensive treatments. Recently, three independent groups (German Cancer Research Centre, Rare Brain Tumour Consortium/SickKids, St. Jude Children's Research Hospital) collectively analyzed large tumour cohorts and revealed molecular sub-groups of PB. To harmonize and better characterize clinicopathologic associations of these sub-groups, we undertook a meta-analysis of molecular and clinical data of the combined cohorts. Unsupervised consensus cluster analyses of global methylation data from 227 unique cases identified five robust molecular sub-groups of pineal region tumours: PB_miRNA_1, PB_miRNA_2, PB_MYC/FOXR2, and PB_RB, mainly comprised of pediatric WHO grade 4 PBs and PNETs; and a fifth group: named PPTID, comprised of mainly pineal parenchymal tumours of intermediate differentiation, a WHO grade 2–3 tumour common in adults. PB_miRNA_1 and PB_miRNA_2 tumours, primarily arising in children (median ages 7.7, 11.4y, respectively), were characterized by alterations of miRNA biogenesis genes *DICER1*, *DROSHA,* and *DGCR8*. PB_MYC/FOXR2 and PB_RB groups, arising in infants/toddlers (median ages 1.4, 2.0y, respectively), were distinguished by recurrent *MYC* gain/amplification and *RB1* loss, respectively. The PPTID group affected mainly adults (median age 33y) and exhibited limited CNAs. Higher rates of metastasis were observed with PB_miRNA_1 (42%), PB_MYC/FOXR2 (38%), and PB_RB (75%) tumours, compared to PB_miRNA_2 (20%) and PPTID (25%). Results from ongoing integrative survival analyses of this large cohort will provide critical data for design of future clinical trials.