

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

Iqra Mumal^{1,2}, Liming Xu¹, Fupan Yao^{1,2}, Tannu Suwal^{1,2}, Xiaolian Fan¹, Mei Lu¹, and Annie Huang^{1,2}; ¹The Hospital for Sick Children, Toronto, ON, Canada, ²University of Toronto, Toronto, ON, Canada

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

Claire Mazewski^{1,2}, Guolian Kang³, Stewart Kellie⁴, Jeffrey Gossett³, Sarah Leary⁵, Bryan Li^{6,7}, Paul Aridgides⁸, Laura Hayes⁹, Alyssa Reddy¹⁰, Dennis Shaw¹¹, Peter Burger¹², Alexander Judkins¹³, Jeffrey Russell Geyer¹⁴, Maryam Fouladi¹⁵, and Annie Huang^{16,17}; ¹Emory University School of Medicine, Atlanta, Georgia, USA, ²Aflac Cancer and Blood Disorders Center, Children's Healthcare of Atlanta, Atlanta, Georgia, USA, ³Saint Jude Children's Research Hospital, Department of Biostatistics, Memphis, TN, USA, ⁴University of Sydney, Children's Hospital at Westmead, Department of Oncology, Westmead, NSW, Australia, ⁵Seattle Children's Hospital, Department of Pediatric Hematology-Oncology, Seattle, WA, USA, ⁶Hospital for Sick Children, Arthur and Sonia Labatt Brain Tumour Research Centre, Pediatrics Department of Hematology Oncology, Toronto, ON, Canada, ⁷University of Toronto, Toronto, ON, Canada, ⁸SUNY Upstate Medical University, Syracuse, NY, USA, ⁹Nemours Children's Hospital Department of Radiology, Orlando, FL, USA, ¹⁰University of California San Francisco, Department of Neurology, San Francisco, CA, USA, ¹¹Seattle Children's Hospital, Department of Radiology, Seattle, WA, USA, ¹²Johns Hopkins University, Department of Pathology, Neuropathology Division, Baltimore, MD, USA, ¹³Children's Hospital of Los Angeles, Keck School of Medicine, University of Southern California, Pathology and Laboratory Medicine, Los Angeles, CA, USA, ¹⁴Seattle Children's Hospital, Department of Pediatric Hematology Oncology, Seattle, WA, USA, ¹⁵Cincinnati Children's Hospital Medical Center, Pediatric Hematology Oncology, Cincinnati, OH, USA, ¹⁶Hospital for Sick Children, Division of Hematology Oncology, Arthur and Sonia Labatt Brain Tumour Research Centre, Pediatrics, Toronto, ON, Canada, ¹⁷University of Toronto, Laboratory Medicine and Pathology, Toronto, ON, Canada

Infant embryonal brain tumors comprise a spectrum of histologic and molecular entities including medulloblastoma (MB) and tumors collectively called CNS PNETs, 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 co-

hort 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

Bryan K Li^{1,2}, Anthony PY Liu³, Elke Pfaff^{4,5}, Brian Gudenäs⁶, Sivan Gershanov², Christelle Dufour⁷, Christian Aichmüller⁸, Martin Sill^{4,9}, Tong Lin¹⁰, Arzu Onar-Thomas¹⁰, Brent A Orr¹¹, Cynthia Hawkins^{2,12}, David W Ellison¹¹, Matija Snuderl^{13,14}, Annie Laquiere¹⁵, Eugene Hwang¹⁶, Sri Gururangan¹⁷, Matthias A Karajannis¹⁸, Giles W Robinson³, Eric Bouffet¹, Alexandre Vasiljevic^{19,20}, Amar Gajjar², Stefan M Pfister^{4,21}, Paul A Northcott⁶, David TW Jones^{4,5}, and Annie Huang^{1,2}; ¹Division of Hematology/Oncology, Hospital for Sick Children, Toronto, ON, Canada, ²Arthur and Sonia Labatt Brain Tumour Research Centre, Hospital for Sick Children, Toronto, ON, Canada, ³Department of Oncology, St. Jude Children's Research Hospital, Memphis, TN, USA, ⁴Hopp Children's Cancer Center Heidelberg (KiTZ), Heidelberg, Germany, ⁵Pediatric Glioma Research Group, German Cancer Research Center (DKFZ), Heidelberg, Germany, ⁶Department of Developmental Neurobiology, St. Jude Children's Research Hospital, Memphis, TN, USA, ⁷Département de Cancérologie de l'Enfant et de l'Adolescent, Institut Gustave Roussy, Villejuif, Paris, France, ⁸Division of Molecular Genetics, German Cancer Research Center (DKFZ), Heidelberg, Germany, ⁹Division of Pediatric Neurooncology, German Cancer Research Center (DKFZ), Heidelberg, Germany, ¹⁰Department of Biostatistics, St. Jude Children's Research Hospital, Memphis, TN, USA, ¹¹Department of Pathology, St. Jude Children's Research Hospital, Memphis, TN, USA, ¹²Division of Pathology, The Hospital for Sick Children, Toronto, ON, Canada, ¹³Division of Neuropathology, NYU Langone Health, New York, NY, USA, ¹⁴Laura and Isaac Perlmutter Cancer Center, NYU Langone Health, New York, NY, USA, ¹⁵Normandie University, UNIROUEN, Inserm U1245, and Rouen University Hospital, Department of Pathology, F76000, Normandy Center for Genomic and Personalized Medicine, Rouen, France, ¹⁶Department of Oncology, Children's National Medical Center, Washington DC, USA, ¹⁷Preston A, Wells Jr, Center for Brain Tumor Therapy and Department of Pediatrics, UF Health Shands Hospital, University of Florida, Gainesville, FL, USA, ¹⁸Department of Pediatrics, Memorial Sloan Kettering Cancer Center, New York, NY, USA, ¹⁹Faculté de Médecine, Université de Lyon, Lyon, France, ²⁰Service d'Anatomie et Cytologie Pathologiques, CHU de Lyon, Lyon, France, ²¹Department of Pediatric Oncology, Hematology & Immunology, Heidelberg University Hospital, Heidelberg, Germany

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 *RBI* 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.