[5]Department of Pediatrics, University of Tokyo, Tokyo, Japan, [6]Department of Hematology and Oncology, Shizuoka Children's Hospital, Shizuoka, Japan, [7]Department of Pediatrics, St. Marianna University School of Medicine Hospital, Kawasaki, Japan, [8]Division of Neurosurgery, National Center for Child Health and Development, Tokyo, Japan, [9]Division of Surgery Oncology, Children's Cancer Center, National Center for Child Health and Development, Tokyo, Japan, [10]Department of Pathology and Tumor Biology, Graduate School of Medicine, Kyoto University, Kyoto, Japan, [11]Department of Pathology, National Center for Child Health and Development, Tokyo, Japan, [12]Department of Pathology, Keio University School of Medicine, Tokyo, Japan

BACKGROUND: Genetic hallmark of atypical teratoid/rhabdoid tumor (AT/RT) is loss-of-function variants or deletions in *SMARCB1* gene on 22q11.2 chromosome, which is common to extracranial malignant rhabdoid tumors (MRT). Previous studies demonstrated that approximately one-thirds of AT/RT and extracranial MRT patients harbored germline *SMARCB1* variants as the rhabdoid tumor predisposing syndrome. We studied herein intensive analysis of the *SMARCB1* gene in AT/RT and extracranial MRT patients focusing on prevalence of germline genetic variants. PROCEDURE: In total, 16 patients were included. Both tumor-derived DNA and germline DNA were obtained from all patients. First, screening for *SMARCB1* alterations in the tumor specimens was done by direct sequencing, ddPCR and SNP array analysis. Then, analysis of germline DNA samples focusing on the genomic abnormalities detected in the paired tumors in each case was performed. RESULTS: In eight of 16 cases (50%), genomic alterations observed in the tumor-derived DNA were also detected in the germline DNA. It is worth noting that three patients had germline mosaicism. Two of three patients had mosaic deletion, including *SMARCB1* region, and the average copy number of the deleted region in the *SMARCB1* gene in the germline was 1.60 and 1.76. For another patient, the fraction of *SMARCB1* variants in normal cells was as low as 1.7%. CONCLUSIONS: Approximately half the MRT cases in this study had *SMARCB1* germline alterations. Considering the presence of low-frequency mosaicisms which conventional methods might overlook, inherited germline variants in predisposition genes are more important than previously assumed for the pathogenesis of pediatric cancers.

ATRT-13. DIFFERENT CELLS OF ORIGIN PAVE THE WAY FOR MOLECULAR HETEROGENEITY IN RHABDOID TUMORS

Monika Graf[1], Marta Interlandi[2,1], Natalia Moreno[1], Dörthe Holdhof[3], Viktoria Melcher[1], Dennis Kastrati[1], Gerd Meyer zu Hörste[4], Martin Dugas[2], Michael C. Frühwald[5], Thomas K. Albert[1], Ulrich Schüller[3,6], and Kornelius Kerl[1]; [1]Department of Pediatric Hematology and Oncology, University Children's Hospital Münster, Münster, Germany, [2]Institute of Medical Informatics, University of Münster, Münster, Germany, [3]Department of Pediatric Hematology and Oncology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany, [4]Department of Neurology with Institute of Translational Neurology, University Hospital Münster, Münster, Germany, [5]Swabian Children's Cancer Center, University Children's Hospital Augsburg, Augsburg, Germany, [6]Research Institute Children's Cancer Center, 20251 Hamburg, Hamburg, Germany

Rhabdoid tumors (RT) are rare but highly aggressive pediatric neoplasms. These tumors carry homozygous loss-of-function alterations of *SMARCB1* in almost all cases with an otherwise low mutational load. RT arise at different intracranial (ATRT) as well as extracranial (MRT) anatomical sites. Three main molecular subgroups (ATRT-SHH, ATRT-TYR, ATRT-MYC) have been characterized for ATRT which are epigenetically and clinically diverse, while MRT show remarkable similarities with ATRT-MYC distinct from ATRT-SHH and ATRT-TYR. Even though there are hypotheses about various cells of origin among RT subgroups, precursor cells of RT have not yet been identified. Previous studies on the temporal control of *SMARCB1* knockout in genetically engineered mouse models have unveiled a tight vulnerable time frame during embryogenesis with regard to the susceptibility of precursor cells to result in RT. In this study, we employed single-cell RNA sequencing to describe the intra- and intertumoral heterogeneity of murine ATRT-SHH and -MYC as well as extracranial MYC tumor cells. We defined subgroup-specific tumor markers for all RT classes but also observed a notable overlap of gene expression patterns in all MYC subgroups. By comparing these single-cell transcriptomes with available single-cell maps of early embryogenesis, we gained first insights into the cellular origin of RT. Finally, unsupervised clustering of published human RT methylation data and healthy control tissues confirmed the existence of different cells of origin for intracranial SHH tumors and MYC tumors independent of their anatomical localizations.

ATRT-14. MACROPHAGE-TUMOR CELL INTERACTION PROMOTES ATRT PROGRESSION AND CHEMORESISTANCE

Viktoria Melcher[1], Monika Graf[1], Marta Interlandi[1,2], Natalia Moreno[1], Flavia W. de Faria[1], Su Na Kim[1], Dennis Kastrati[1], Sonja Korbanka[1], Amelie Alfert[1], Joachim Gerß[3], Gerd Meyer zu Hörste[4], Wolfgang Hartmann[5], Michael C. Frühwald[6,7], Martin Dugas[2], Ulrich Schüller[8,9], Martin Hasselblatt[10], Thomas K. Albert[1], and Kornelius Kerl[1]; [1]Department of Pediatric Hematology and Oncology, University Children's Hospital Münster, Münster, Germany, [2]Institute of Medical Informatics, University of Münster, Münster, Germany, [3]Institute of Biostatistics and Clinical Research, University of Münster, Münster, Germany, [4]Department of Neurology with Institute of Translational Neurology, Münster, Germany, [5]Gerhard-Domagk-Institute of Pathology, University of Münster, Münster, Germany, [6]Swabian Children's Cancer Center, University Children's Hospital Augsburg, Augsburg, Germany, [7]EU-RHAB Registry Center, Augsburg, Germany, [8]Department of Pediatric Hematology and Oncology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany, [9]Research Institute Children's Cancer Center, Hamburg, Germany, [10]Institute of Neuropathology, University Hospital Münster, Münster, Germany

Atypical teratoid/rhabdoid tumors (ATRT) are pediatric brain neoplasms that are known for their heterogeneity concerning pathophysiology and outcome. The three genetically rather uniform but epigenetically distinct molecular subgroups of ATRT alone do not sufficiently explain the clinical heterogeneity. Therefore, we examined the tumor microenvironment (TME) in the context of tumor diversity. By using multiplex-immunofluorescent staining and single-cell RNA sequencing (scRNA-seq) we unveiled the pan-macrophage marker CD68 as a subgroup-independent negative prognostic marker for survival of ATRT patients. ScRNA-seq analysis of murine ATRT-SHH, ATRT-MYC and extracranial RT (eRT) provide a delineation of the TME, which is predominantly infiltrated by myeloid cells: more specifically a microglia-enriched niche in ATRT-SHH and a bone marrow-derived macrophage infiltration in ATRT-MYC and eRT. Exploring the cell-cell communication of tumor cells with tumor-associated immune cells, we found that Cd68+ tumor-associated macrophages (TAMs) are central to intercellular communication with tumor cells. Moreover, we uncovered distinct tumor phenotypes in murine ATRT-MYC that share genetic traits with TAMs. These intermediary cells considerably increase the intratumoral heterogeneity of ATRT-MYC tumors. *In vitro* co-culture experiments recapitulated the capability of ATRT-MYC cells to interchange cell material with macrophages extensively, in contrast to ATRT-SHH cells. We found that microglia are less involved in the exchange of information with ATRT cells and that direct contact is a prerequisite for incorporation. A relapse xenograft model implied that intermediary cells are involved in the acquisition of chemotherapy resistance. We show evidence that TAM-tumor cell interaction is one mechanism of chemotherapy resistance and relapse in ATRT.

ATRT-15. LY6D – A CANDIDATE FOR NANOPARTICLE-BASED TARGETED THERAPIES OF ATRT

Lea Hagemeier[1], Marthe Sönksen[1], Natalia Moreno[1], Romy Ettlinger[2], Hana Bunzen[2], and Kornelius Kerl[1]; [1]Department of Pediatric Hematology and Oncology, University Children's Hospital Münster, Münster, Germany, [2]Institute of Physics, University Augsburg, Augsburg, Germany

Atypical Teratoid Rhabdoid Tumors (ATRT) are aggressive brain malignancies of the infant. Despite intensive multimodal therapy, the overall prognosis remains poor, making investigations on targeted therapies crucial. Arsenic trioxide (ATO) is known to inhibit cell growth of ATRT *in vitro* and *in vivo* but its efficacy in solid tumors is limited by its adverse effects. We aimed to characterize whether a nanoparticle-based drug delivery could overcome these limitations. Therefore metal-organic frameworks containing ATO (MOF-ATO) were constructed. To improve drug specificity further, we searched for unique proteins on the surface of ATRT, in order to create antibody-drug-conjugates out of MOF-ATO and an ATRT-specific ligand. ATRT are marked by a biallelic loss of *SMARCB1*, which results in an activation of the repressive histone methyltransferase EZH2. After chemical inhibition of EZH2 with GSK126, a mass spectrometric based screening for differentially expressed surface proteins was performed. Treatment with ATO, as well as MOF-ATO and GSK126 each reduces the cell viability of ATRT cell lines. It results in a cell cycle arrest and an induction in apoptosis, being analysed *via* MTT test and flow cytometry. GSK126 treatment causes a significant upregulation of several cell surface proteins, upon them the Lymphocyte antigen 6 family member D (LY6D). Being rarely expressed on other human cells, this protein is an interesting candidate. An antibody-drug-conjugate consisting of MOF-ATO and LY6D-ligands could be a promising approach for future targeted therapies of ATRT.