distinct entity with relatively favorable outcome. Nevertheless, epigenetic similarity with ATRT-MYC and the potential of malignant progression warrants close follow-up examinations. In line with recent developments of WHO nomenclature, we propose to refer to these tumors as "low-grade diffusely infiltrative tumor, SMARCB1-mutant".

**ATRT-08. SMARCB1- AND SMARCA4-DEFICIENT MALIGNANT BRAIN TUMORS WITH COMPLEX COPY NUMBER ALTERATIONS AND *TP53* MUTATIONS MAY REPRESENT THE FIRST CLINICAL MANIFESTATION OF LI-FRAUMENI SYNDROME**

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Atypical teratoid/rhabdoid tumor (AT/RT) is a malignant central nervous system tumor predominantly affecting infants. Mutations of *SMARCB1* or (rarely) *SMARCA4* causing loss of nuclear SMARCB1 or SMARCA4 protein expression are characteristic features, but further recurrent genetic alterations are lacking. Most AT/RTs occur de novo, but secondary AT/RTs arising in other central nervous system tumors have been reported. Malignant gliomas, IDH-wildtype, arising in patients with Li-Fraumeni syndrome typically show somatic mutations of *TP53* as well as complex copy number alterations, but little is known about loss of SMARCB1 or SMARCA4 protein expression in this context. Here we report two children, in whom malignant supratentorial brain tumors with SMARCB1-deficiency, complex copy number alterations and somatic *TP53* mutations lead to the discovery of pathogenic/likely pathogenic *TP53* variants in the germ line. Screening of the molecularneuropathology.org data set for cases with similar genetic and epigenetic alterations yielded another case with SMARCA4-deficiency in a young adult with Li-Fraumeni syndrome. In conclusion, SMARCB1- or SMARCA4-deficient malignant brain tumors with complex copy number alterations and somatic *TP53* mutations in children and young adults may represent the first clinical manifestation of Li-Fraumeni syndrome and should prompt genetic counseling and investigation for *TP53* germline status.

**ATRT-09. OUTCOME AND THERAPEUTIC INTERVENTIONS IN RELAPSED AND REFRACTORY ATRT – THE EU-RHAB PERSPECTIVE**

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Currently an internationally accepted consensus treatment for relapsed/refractory ATRT is missing. Little is known about relapse patterns, prognostic factors and outcome. In a recently published cohort of 143 ATRTs from the EU-RHAB registry, progression on therapy or relapse occurred in 64% (n=91). Previously published strategies for treatment failure have been restricted to individual, mostly clinically guided, attempts or early phase trials with limited sample sizes. We present a cohort of 55 patients with relapsed/refractory ATRT identified between 2015 and 2021 (total ATRT recruited n=147). Median age was 19 months; in 27.3% (n=15) a germline mutation was identified. A total of 43/55 tumors were subgrouped [60.5% SHH (n=26), 14.0% MYC (n=6), 23.3% TYR (n=10), one patient with SHH+TYR]. Salvage therapy was applied to 83.6% (46/55). Sixty therapy attempts with 17 different regimens subclassified into conventional chemotherapy, epigenetic, targeted or metronomic therapy were applied to 40/55 patients. Median overall survival (OS) was 20±1.8 weeks following the first event, median time to progression was 11±1.8 weeks. 12 months OS was 23.1%. No significant differences in survival were noted between different molecular subgroups; neither was germline mutation in SMARCB1 prognostic. Patients <12 months (n=9;16.4%) had a significantly reduced OS compared to older patients. (9±6.0wks vs. 22±3.2wks, $p<0.05$) Those who received therapy according to metronomic approaches such as MEMMAT (8/55;14.5%) survived longer than patients treated with other regimens, including epigenetic and targeted therapy. (72±36.8wks vs. 25±6.2wks, $p<0.05$) Our data provide valuable insights into a vulnerable group of patients deserving evidence based clinical management and access to clinical trials of all phases. Prospectively we aim to merge the results with data from other, international cohorts to generate more robust and valuable results.

**ATRT-10. SINGLE-CELL TRANSCRIPTIONAL PROFILING OF ATRTS REVEALS HETEROGENEOUS SIGNATURES OF TUMOR AND NON-MALIGNANT CELL POPULATIONS**

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Atypical Teratoid/Rhabdoid Tumors (ATRTs) are known for exhibiting high inter-tumor heterogeneity, even though they are almost all characterized by a common loss of SMARCB1 (or rarely SMARCA4). Three subgroups have been identified at bulk methylome and transcriptome level: ATRT-TYR, ATRT-SHH, and ATRT-MYC. To better understand the biology underlying each subgroup and potentially unveil their (different) cell(s) of origin, we performed single-cell transcriptomic analyses in 22 ATRTs using fresh frozen samples and both 10X and Smartseq technology. All data, grouped by technology, underwent quality control and normalization, regressing out the biases introduced by each sample. Tumor microenvironment (TME) and tumor bulk (TB) clusters were characterized by a combination of copy number variant analyses, enrichment in literature lists of marker genes for specific cell populations, and in-depth analysis of differentially enriched (DE) genes. Non-negative Matrix Factorization (NMF) was applied to TB to reveal major transcriptional profiles, which were grouped into

meta-signatures. A total of 71 gene lists were retrieved from NMF (TB) and DE analyses (TME + TB), that gathered into 11 signature groups by Jaccard similarity, with one extra group accounting for unique signatures. Three groups targeted TME, accounting for either microglia, fibroblasts and endothelial cells, or OPCs, oligodendrocytes, astrocytes and neurons. These signatures are enriched in specific clusters across technologies. The remaining eight groups divide into two types, either enriched in clusters predominantly formed by cells of one or two ATRT subgroups or signatures enriched for a particular phenotype, such as cilial, cycling, axonogenesis or EM transition. While the first type is enriched across clusters in a gradient fashion, the second shows enrichment for selected clusters across technologies. Further analyses on the integrated dataset and additional samples are ongoing to validate and refine these 11 signature groups in ATRTs to see how this may lead to new treatment approaches.

**ATRT-11. ANATOMICO-BIOLOGICAL CORRELATIONS DEFINE A NEW LAYER FOR ATRT MOLECULAR SUBGROUPS POINTING TO POTENTIAL LINEAGES OF ORIGIN**

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Atypical teratoid rhabdoid tumors (ATRT) are divided in three molecular subgroups, the so-called MYC, TYR and SHH subgroups. This heterogeneity suggests some diversity in the cells of origin, which remain hypothetical thus far. A careful radiological review of 55 MRI at diagnosis was performed in parallel with a careful analysis of mouse tumor origin in the Rosa26-CreERT2::Smarcbflox/flox model. Methylation, bulk RNAseq and scRNAseq analyses were integrated to these anatomic information to highlight potential origin for each molecularly and anatomically defined subgroups. We demonstrated that mouse Myc-ATRTs derive from extra-parenchymal meningeal areas, a finding consistant with many human MYC ATRT being clearly of intra-cranial extra-axial origin. Although this finding could point to a neural crest origin, transcriptomic features fail to unravel any lineage-specific signature. We also defined a distinct supra-tentorial SHH ATRT subgroup, characterized both in mouse and Humans by neural features pointing to the ganglionic eminence progenitors as the candidate origin. Finally we identified a distinct infra-tentorial SHH ATRT subgroup, not observed in mice, with hindbrain/midbrain boundary progenitor signature. scRNAseq from human SHH infra-tentorial tumors consistently suggest a dedifferentiation process involving the Notch pathway in the oncogenic transformation of hindbrain/midbrain neural progenitors.

**ATRT-12. LIN28A EXPRESSION CORRELATES WITH POOR PROGNOSIS AND THE MYC SUBGROUP IN AT/RTS**

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Atypical teratoid/rhabdoid tumors (AT/RTs) are malignant embryonal tumors of the central nervous system, which mainly affect young children. These tumors are defined by loss of SMARCB1 (or SMARCA4 in rare cases) and can be categorized into three main DNA methylation subgroups (i.e. –MYC, -SHH, -TYR). AT/RTs commonly display heterogeneous expression of LIN28A, an RNA-binding protein, which regulates pluripotency and plays critical roles during embryonic development. The biological impact and clinical significance of LIN28A expression in AT/RTs remains unknown. In this study, we investigated 80 samples of molecularly and clinically characterized AT/RTs for LIN28A expression using immunohistochemistry. Staining signal of tumor tissue was assessed and scored via semi-automated digital image analysis. Global LIN28A expression intensity and heterogeneity were tested for correlation with DNA methylation subtype, tumor localization, as well as patient age, gender and overall survival. LIN28A was found with strongly varying staining patterns and intensities across our cohort of AT/RTs, with significantly elevated expression in the MYC subgroup. Moreover, we identified strong global and focal LIN28A expression as an independent negative prognostic factor in AT/RTs. In summary, we show that AT/RT-MYC tumors display significantly increased LIN28A expression in comparison to the other DNA methylation subgroups, suggesting a subgroup-specific intratumoral role of LIN28A. Furthermore, we demonstrate an impact of LIN28A expression on survival in AT/RTs. Further investigations on the functions of LIN28A in AT/RTs in vitro and in vivo are ongoing and aim to uncover potential therapeutic implications in these tumors.

**ATRT-13. AN INTEGRATIVE ANALYSIS OF THE ATRT PROTEOME UNRAVELS NOVEL DRUG TARGETS AND REFINES MOLECULAR SUBGROUPING**

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INTRODUCTION: Atypical teratoid/rhabdoid tumors (ATRT) represent frequent brain tumors in infants. In recent years, large-scale landscaping efforts on the epigenome and transcriptome of these tumors have unravelled a high degree of heterogeneity and three major molecular subgroups, termed ATRT-TYR, ATRT-SHH,ATRT-MYC, have been identified. The ATRT-proteome, in turn, still represents a largely unchartered territory. METHODS: We have performed a peptide-based screening approach to characterize the proteome of 40 ATRTs and six ATRT cell-lines. All of these samples had matching methylation data available and 28 also corresponding gene expression data. RESULTS: Unsupervised clustering recapitulated the previously described ATRT groups, revealing also a clear split of the SHH-subgroup in a supratentorial (SHH_1) and an infratentorial subgroup (SHH_2). Overall, we identified 7265 proteins, of which 1320 were differentially expressed between the groups, with an enrichment of spliceosome associated terms in SHH_1 and integrins/cell adhesions molecules in SHH_2. ATRT-MYC displayed an overrepresentation of immune cell markers and the TYR subgroup an enrichment of PI3K- as well as mTOR-signaling. Particularly, genes that have previously been described as signature genes for the ATRT-groups such as FABP7 in ATRT-SHH and OTX2 and MITF in ATRT-TYR were among the highly correlating genes that were both expressed in the proteome and the gene expression datasets. On top of this, our analysis revealed highly differentially expressed drug targets such as the tyrosine-kinase MARCKS (overexpressed in ATRT-TYR) not previously identified in ATRT transcriptome data, which warrant investigation by in vitro drug tests. CONCLUSION: Our data reveal the importance of previously described regulatory hubs in the ATRT subgroups, but additionally highlight novel drug targets that merit further exploration. Currently, drug treatment experiments in ATRT cell lines are ongoing to validate these proteins as drug targets, ultimately aiming to establish new therapeutic strategies in this deadly disease.

**ATRT-14. MALIGNANT RHABDOID TUMORS OF CRANIAL NERVES – ATRT OR EXTRACRANIAL RHABDOID TUMOR?**

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