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