well as alterations in DNA damage response pathways, cell cycle checkpoints, miRNA transcription, and numerous proliferative factors. THZ2 penetrates the blood brain barrier (BBB), is well tolerated, and results in prolonged survival in murine xenograft models of AT/RT. CDK7 inhibition also synergizes with a number of currently-approved oncology drugs, as well as with ionizing radiation, in order to inhibit AT/RT growth and viability.

ATRT-21. RHABDOID PREDISPOSITION SYNDROME: REPORT OF MOLECULAR PROFILES AND TREATMENT APPROACH IN THREE CHILDREN WITH SYNCHRONOUS ATYPICAL TERATOID/ RHABDOID TUMOR AND MALIGNANT RHABDOID TUMOR

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BACKGROUND: Rhabdoid predisposition syndrome is characterized by germline alterations in *SMARCB1* or *SMARCA4*, leading to synchronous or metachronous central nervous system (CNS) and extra-CNS rhabdoid tumors. Rare survivors have been reported to date. METHODS: We describe the molecular profiling and treatment regimen of three patients with synchronous atypical teratoid/rhabdoid tumor (ATRT) and malignant rhabdoid tumor of the kidney (MRT-K). All patients underwent radical nephrectomy of the kidney, and gross total resection of the primary CNS tumor was achieved for two patients. An intensive chemotherapy regimen was administered; an induction phase based on the modified Third Intergroup Rhabdomyosarcoma Study (IRS-III) for ATRT followed by a consolidation phase with three cycles of high-dose chemotherapy and autologous hematopoietic progenitor cell rescue, without irradiation. All three patients were enrolled on an institutional comprehensive genomic profiling protocol. RESULTS: A germline focal 22q deletion, including *SMARCB1,* was detected in two patients, while the third patient had a maternally-inherited heterozygous frameshift variant in *SMARCB1*. Somatic loss of heterozygosity of 22q was identified in all patients, resulting in biallelic inactivation of *SMARCB1*. Divergent tumor subgroups were described using DNA methylation. The three MRT-K samples were classified as MYC subtype. One ATRT was classified as SHH while the other as TYR. One patient is currently three years off-therapy without evidence of disease, while the other two patients have completed the consolidation phase without recurrent disease. CONCLUSION: Molecular profiling of CNS and extra-CNS rhabdoid tumors revealed different epigenetic subgroups. An intensive multimodal therapeutic approach without irradiation may achieve prolonged survival.

ATRT-22. HIGH-THROUGHPUT DRUG SCREENING OF FDA-APPROVED CANCER DRUGS REVEALS POTENTIAL THERAPEUTIC APPROACHES FOR ATYPICAL TERATOID RHABDOID TUMOUR

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Atypical teratoid/rhabdoid tumors (ATRT), are the most common brain tumor in children under the age of 1 year with an overall survival of ~17%. Like extracranial rhabdoid tumors, ATRT is exclusively characterized by bi-allelic loss of *SMARCB1*, a critical subunit of the SWI/SNF chromatin remodeling complex, implicating epigenetic deregulation in the pathogenesis of disease. We have previously shown the ability of the histone deacetylase inhibitor, panobinostat, to mimic SMARCB1-mediated SWI/SNF functions in extracranial rhabdoid tumors to inhibit tumor growth by driving multi-lineage differentiation *in vitro* and *in vivo*. Whether this also applies to ATRT is unknown. Using a panel of human-derived ATRT cell lines, representing defined molecular subgroups, we have shown that prolonged treatment with panobinostat at nanomolar concentrations results in markedly reduced clonogenicity, and increased senescence, preceded by increased H3K27 acetylation, decreased H3K27 trimethylation and EZH2 expression. To determine potentially synergistic therapies, we performed high-throughput drug screening of 622 compounds already in advanced clinical trials or FDA-approved for other indications, across our panel of ATRT models and identified 30 common compounds, which decrease cell viability by >50%, with no effect on neural stem cell controls and 12 compounds which demonstrated subgroup specificity, highlighting the necessity to consider therapies in the molecular context. In addition to HDACi, consistent with our panobinostat in vitro findings, inhibitors of CDK, survivin and PI3K were the top hits. *In vitro* and *in vivo* validation of these compounds alone, and in combination with panobinostat is ongoing.

ATRT-23. THE DUAL MTORC1/2 INHIBITOR SAPANISERTIB DISRUPTS THE NRF2-MEDIATED STRESS RESPONSE AND COMBINES SYNERGISTICALLY WITH THE BH3 MIMETIC OBATOCLAX TO EXTEND AT/RT SURVIVAL

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Atypical teratoid/rhabdoid tumors are aggressive infantile tumors highly resistant to intensive therapies. We aim to identify and target critical factors driving this therapy resistance to improve AT/RT survival. Analysis of publically available RNASeq on 32 AT/RT identified elevated expression of NRF2 (median expression 40.78, normal brain 18.81). NRF2 is a master regulator of cell's stress response whose expression is correlated with therapy resistance and poor survival. NRF2 activation is sensitive to mTOR activity and is a biomarker predicting response to the dual mTORC1/2 inhibitor, Sapanisertib (TAK228, INK128). We performed RNASeq on 4 human-derived AT/RT cell models after Sapanisertib treatment. Pathway analysis reveals disruption of the NRF2-mediated stress response (-log p value 0.39, Z-score 1.0). As a result, Sapanisertib decreases ROS scavengers like glutathione (Metabolite analysis UHPLC-MS, *t*-test $p<0.05$) and induces a pro-death phenotype (decreased MCL-1 expression, western blot; gene-expression analysis, RNASeq). The brain-penetrant BH3 mimetic Obatoclax increases ROS generation and induces apoptosis (MUSE oxidative stress and ANNEXIN V assays, *t*-test $p<0.05$). These complementary mechanisms of action synergize to induce high rates of cell death (MUSE ANNEXIN V assay, ANOVA $p<0.05$, C-PARP western blot, Compusyn Synergy analysis CI<1.0) and slow cell growth (MUSE Cell viability, ANOVA $p<0.05$). Once-weekly treatments of Sapanisertib combined with Obatoclax in orthotopic mouse models of AT/RT are well tolerated, slow tumor growth (bioluminescence imaging) and significantly extend median survival from 35 to 55 days (Log-rank $p<0.05$). These findings support a new clinical trial aimed at improving AT/RT survival.

ATRT-24. CELL SURFACE PROTEOME ANALYSIS OF ATRT IDENTIFIES TARGETS FOR IMMUNOTHERAPY

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Atypical teratoid rhabdoid tumor (ATRT) is a rare and fast-growing childhood tumor of the brain and spinal cord. While the recent advances in DNA and RNA sequencing have given deep insights into the biology of cancer, about 90% of ATRTs harbor a single deletion which leads to uncontrolled tumor growth. The lack of targetable genetic abnormalities in ATRT makes it a tough target for therapy and hence radical new approaches are required to develop a treatment. In many cases, the gene expression profile alone DOES NOT represent the presence of the gene product on the surface and cannot detect post-translational modifications such as the addition of sugars which are essential for the interaction of surface proteins with the tumor microenvironment. The ability to escape from surveillance by the immune system is regarded as one of the essential hallmarks of cancer cells. Here we carried out a comprehensive unbiased large-scale surface receptor profiling using high throughput multicolor flow cytometry on surgically resected ATRT patient samples, primary ATRT cell lines, and patient-derived xenograft models. By multiplexing primary samples with antibodies for CD31, CD45, CD11b, CCR2, Cx3cr1, and CD4, and CD8 we eliminated endothelial and immune cells from analysis while also identifying immune populations within the tumor. We identified increased surface expression of CD44, CD146, CD59, CD151, and CD276. These were validated in our screening of primary tumor samples. A combination of CAR-T cell and function-blocking monoclonal antibody approaches have been tested to verify the proof of principle of this approach.

ATRT-25. INTEGRATED QUANTITATIVE SWATH-MS PROTEOMICS ANALYSIS OF ATRTS UNCOVERS NEW THERAPEUTIC OPPORTUNITIES

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The consequences of *SMARCB1* loss in Atypical Teratoid Rhabdoid Tumors (ATRTs) have been extensively characterized at the epigenetic/transcriptomic level. In this study we detail the functional effect of *SMARCB1* mutation on the MRT proteome, its relationship with RNA deregulation or lack thereof. We performed unlabeled discovery proteomics using MS-SWATH on MRT cells in which SMARCB1 was forcibly re-expressed (5 cell lines, +/-SMARCB1); analyzing changes in protein abundance within 3 fractions (total, membrane, nuclear). We generated a custom spectral library, covering >8,000 proteins, for analysis of the ATRT proteome using a pH fractionated pool of each cellular subfraction. This SMARCB1-dependent ATRT spectral library constitutes a powerful tool for profiling proteins of potentially therapeutic relevance in both model systems and primary ATRT samples. We show that whilst gene expression and protein abundance are significantly related there are many instances whereby expression changes do not reliably predict protein abundances. Several hundred proteins show significantly increased abundance ($p<0.01$) with no concomitant change by RNA-seq. *SMARCB1* mutation is able to invoke critical changes in post-transcriptional/translational regulation as well as sub-cellular localization. By integration with whole-genome CRISPR/cas9 screening we describe functionally essential SMARCB1 dependent pathway/membrane biomarkers, evident at the protein but not the RNA level. We describe several which are druggable and suggest certain therapeutic modalities e.g. specific combinations of RTKs, RNA-binding proteins/splicing factors (SpliceosomeA, U4:U5:U6 tri-snRNP complexes). Our analysis links, for the first time in ATRT, genome-wide transcriptomic and proteome aberrations and reveals broad mechanisms underlying the effect of *SMARCB1* mutation.

ATRT-26. META-ANALYSIS OF TREATMENT MODALITIES IN METASTATIC ATYPICAL TERATOID/RHABDOID TUMORS IN CHILDREN

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BACKGROUND: Metastatic atypical teratoid/rhabdoid tumors (AT/RT) are aggressive central nervous system tumors that present during infancy and are associated with dismal outcomes. Patients receive multimodal treatment including surgical resection, systemic chemotherapy and one or more of intrathecal chemotherapy (IT), marrow-ablative chemotherapy with autologous hematopoietic cell rescue (AuHCR) and radiation therapy (XRT). While data regarding treatment modalities for AT/RT patients exist, no comprehensive data have been published regarding the metastatic patient population. METHODS: We performed a meta-analysis of 1,578 articles published through September 2018, including 44 studies with a total of 123 subjects. Additionally, seven patients were incorporated through chart review of patients treated at Nationwide Children's Hospital. RESULTS: Analysis of 130 patients revealed a 3-year overall survival (OS) of 25%. Age at diagnosis had a significant impact on survival ($p=0.0355$); 3-year OS for infants < 18 months was 21%; 18–36 months was 26%; and > 36 months was 36%. Location of the primary tumor, metastatic stage and extent of surgical resection did not have significant impact on OS. On univariate analysis, XRT ($p<0.0001$), IT ($p=0.01$) and AuHCR ($p<0.0001$) were found to significantly improve survival. The most substantial effect was noted in patients who received AuHCR (3-year OS of 60% *versus* 9% in those who did not). On multivariable analysis XRT ($p=0.0006$), IT ($p=0.0124$) and AuHCR ($p<0.0001$) were independently associated with reduced risk of death.

ATRT-27. COST-EFFECTIVE ASSAYS TO SUBGROUP ATRT IN THE DAILY ROUTINE

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Three atypical teratoid rhabdoid tumors (ATRT) molecular subgroups with different bio-clinical characteristics have been reported (TYR, SHH and MYC). Molecular subgrouping relies on either methylation profiling (reference methods), or expression profiling. However, the cost-effectiveness of such pangenomic screening is questionable. This work aims to study the reliability of alternative techniques for subgroup classification in the daily routine. Illumina EPIC-arrays were performed on 46 samples. Among those cases, expression profiling were analysed by RNAseq (n=30). We designed a 26-gene panel to assess expression profiling using the Nanostring technology; this was applied to 35 tumors. Immunohistochemistry (IHC) was used for 20 samples; it relied on the expression of MITF, TYR, OTX2 and MYC. We first assessed the concordance between DNA methylation and RNAseq based profilings; then, between RNAseq and Nanostring and, finally, between methylation profiling and Nanostring or IHC, the two rapidest and cheapest tools. The concordance between the two expression-based profiling was 19/21. EPIC-arrays and RNAseq or Nanostring were concordant in 26/30 and 30/35 samples, respectively. The concordance was perfect for methylation-defined MYC subtype. Finally, 17/20 tumor samples were classified in the same subgroup by EPIC-arrays and IHC; the 3/20 misclassified tumors were SHH by methylation but consistently MYC by IHC, Nanostring and RNAseq. There was 90–100% of concordance for TYR subgroup (all techniques). We have designed a gene panel-based expression signature that shows promising concordance with RNAseq and methylation profiling. Nanostring assay and IHC well predict ATRT subgroup classification for MYC and TYR subclass, but less so for methylation-defined SHH ones.

ATRT-28. SINGLE NUCLEI SEQUENCING REVEALS THE DIFFERENT PHENOTYPIC COMPOSITION OF THE ATRT SUBGROUPS

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Atypical teratoid/rhabdoid tumors (ATRT) represents a genomically homogeneous disease characterized by loss of SMARCB1 protein in the vast majority of cases. In recent years, it has become clear that these tumors display a high degree of intertumoral heterogeneity with three molecularly distinct subgroups. However, the degree of intratumoral heterogeneity and the information on cellular subpopulations currently remains largely an uncharted territory. To explore the transcriptomic composition of ATRTs, we performed single nuclei RNA sequencing for 16 ATRTs representing all three molecular subgroups (5 ATRT-TYR, 7 ATRT-SHH, 4 ATRT-MYC). By performing tSNE cluster analyses of all the single cell data (~50.000 cells have been sequenced), we were able to gain unprecedented insights into the phenotypic composition of ATRTs and unravelled substantial differences between the three subgroups. Integrating transcriptomic information from non-neoplastic brain cells and the data derived from single nuclei sequencing, we found an OPC like gene signature in ATRT-SHH. In contrast, ATRT-TYR subpopulations overexpressed more markers of neuronal stem cells suggesting a larger fraction of undifferentiated cells in this subgroup. We also identified a subpopulation of cells with a clear overexpression of cell cycle associated genes (CDK4, CDKN3), predominantly present in ATRT-MYC samples, a finding which may harbour important consequences for a targeted therapy with e.g. CDK inhibitors. In summary, our analyses reveal different cellular compartments in ATRT and provide important insights into the cellular differentiation of the three ATRT-subgroups. Further analyses to achieve a specific mapping of ATRT to its physiological cell of origin are currently being pursued.

ATRT-30. RETROSPECTIVE ANALYSIS OF CHILDREN WITH ATYPICAL TERATOID RHABDOID TUMOR TREATED ACCORDING TO ACNS0333

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Atypical teratoid rhabdoid tumor (ATRT) is a central nervous system tumor with poor outcome. ACNS0333, a Children's Oncology Group phase 3 trial, enrolled 65 evaluable patients who received two cycles of induction chemotherapy, three cycles of consolidative high-dose chemotherapy (HDCT), and focal radiation therapy (RT) pre- or post-consolidation. Craniospinal irradiation (CSI) was left to clinician discretion. We retrospectively analyzed medical records of 27 children treated at our institutions according to ACNS0333. Median age at diagnosis was 14 months (range 4–165); 13 (48%) were male. M-stage was M0, M2, and M3 for 18 (66%), 5 (19%), and 4 (15%), respectively. Tumor location was supratentorial (n=14, 52%), infratentorial (n=12, 44%), or both (n=1, 4%). Complete resection was achieved for 17 (63%). All but one completed induction. Of 13 (51%)