

NEURO-ONCOLOGY

ABSTRACTS

ABSTRACTS FROM THE 2021 SOCIETY FOR NEURO-ONCOLOGY PEDIATRIC NEURO-ONCOLOGY RESEARCH CONFERENCE

Submission Categories and Abbreviations

ATRT – ATYPICAL TERATOID RHABDOID TUMORS
BIOL – BASIC BIOLOGY
EMBR – EMBRYONAL TUMORS
EPEN – EPENDYMOMA
GERM – GERM CELL TUMORS
HGG – HIGH GRADE GLIOMA
IMMU – IMMUNOLOGY/IMMUNOTHERAPY
LGG – LOW GRADE GLIOMAS
TMOD – MODELS
OMIC – OMICS
RARE – RARE TUMORS/OTHER
EPCT – TRANSLATIONAL/EARLY PHASE CLINICAL TRIALS

ATRT

ATRT-01. IDENTIFICATION OF MICRORNA-BASED PROGNOSTIC BIOMARKERS AND CANDIDATE THERAPEUTIC AGENTS FOR ATYPICAL TERATOID/RHABDOID TUMOR

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Background: MicroRNA (miRNA) has been found to be involved in development of many malignant pediatric brain tumors, including atypical teratoid/rhabdoid tumor (AT/RT) that is highly aggressive and carries a dismal prognosis. The current study investigated the potential value of miRNAs and pivotal genes associated with AT/RT using bioinformatics analysis, aiming to identify new prognostic biomarkers and candidate drugs for AT/RT patients. Methods: Differentially expressed miRNAs (DEMs) and genes (DEGs) between AT/RT and normal control samples were obtained from GEO database. The target genes of DEMs were predicted via TargetScanHuman7.2 and miRDB, and then intersected with DEGs. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes analyses of overlapping genes were conducted, followed by construction of protein-protein interaction network. Hub genes were determined by Cytoscape software, and their prognostic values were evaluated using Kaplan-Meier analysis. Connectivity Map database was used to identify latent therapeutic agents. Results: A total of 11 DEMs (hsa-miR-1224-5p, hsa-miR-128-3p, hsa-miR-17-5p, hsa-miR-18b-5p, hsa-miR-29c-5p, hsa-miR-329-3p, hsa-miR-379-5p, hsa-miR-433-3p, hsa-miR-488-5p, hsa-miR-656-3p and hsa-miR-885-5p) were screened. By intersecting 3275 predicted target genes and 925 DEGs, we finally identified 226 overlapping genes that were enriched in pathways in cancer and MAPK signaling pathway. Four hub genes (GRIA2, NRXN1, SLC6A1 and SYT1) were significantly associated with the overall survival of AT/RT patients. Candidate drugs included histone deacetylase inhibitor (givinostat), DNA synthesis inhibitor (floxuridine), cyclin-dependent kinase inhibitor (purvalanol) and janus kinase inhibitor (lestaurtinib). Conclusion: In summary, this study systematically analyzed AT/RT-related miRNAs and pivotal genes to provide novel prognostic biomarkers and potential therapeutic agents.

ATRT-02. THE DUAL MTORC1/2 INHIBITOR, TAK-228 COMBINES SYNERGISTICALLY WITH THE BH3 MIMETIC, OBATOCLAX TO IMPROVE SURVIVAL IN MICE BEARING ORTHOTOPIC XENOGRAFTS OF AT/RT

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mTOR activation drives tumorigenicity by regulating transcription factor expression and downstream growth and survival pathways. We have previously shown that mTORC1 and mTORC2 are highly activated in AT/RT and the dual mTORC1/2 inhibitor, TAK-228 (Sapanisertib) improves survival in mice bearing orthotopic xenografts of AT/RT. To design a rational combination therapy that enhances TAK-228's efficacy and durability, we performed RNASeq 4 hours after TAK-228 treatment of AT/RT cell models.

Pathway analysis revealed disruption of the NRF2-mediated stress response. NRF2 is a cap'n'collar leucine zipper transcription factor that regulates expression of genes involved in redox homeostasis, energy metabolism, cell proliferation, and survival. Analysis of publicly available RNASeq data on 32 human tumors identified elevated expression of NRF2 in AT/RT (median expression 40.78, normal brain 18.81). Short-hairpin knockdown of NRF2 decreased the expression of NRF2 as well as the anti-apoptotic proteins MCL-1, BCL-xL, and BCL-2 (western blot), and intracellular concentrations of reduced glutathione ($p < 0.005$, t -test). TAK-228 similarly decreased expression of NRF2, MCL-1, and glutathione ($p < 0.005$, t -test) demonstrating that TAK-228 compromises AT/RT defenses against oxidative stress and cell death. The brain-penetrant BH3 mimetic, Obatoclax increases oxidative stress and induces apoptosis in AT/RT (MUSE oxidative stress, cPARP western blot, t -test $p < 0.05$). These complementary mechanisms of action synergize to slow AT/RT cell growth (MUSE Cell viability assay, ANOVA $p < 0.05$) and induce high rates of cell death (MUSE ANNEXIN V assay, ANOVA $p < 0.05$, Western blot for cPARP, Compusyn Synergy analysis $CI < 1.0$). Once-weekly treatments of TAK-228 combined with Obatoclax in orthotopic mouse models of AT/RT is well tolerated, slows tumor growth (bioluminescence imaging, ANOVA $p < 0.05$) and significantly extends 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-04. CORRELATION OF CLINICOPATHOLOGIC FEATURES AND CUMULATIVE INCIDENCE OF RELAPSE FOR PATIENTS WITH ATYPICAL TERATOID RHABDOID TUMOR ON ACNS0333: A REPORT FROM THE CHILDREN'S ONCOLOGY GROUP

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Purpose: Intensive multi-modal regimens have improved survival for patients with atypical teratoid rhabdoid tumor, however relapse rates remain high. A better understanding of clinical and pathologic features associated with tumor relapse is critical to risk-stratifying patients. Patients and Methods: ACNS0333 treatment consisted of multi-agent chemotherapy, high-dose chemotherapy, and radiation therapy, lasting approximately 6 months. Variables including patient age, sex, tumor location, M-stage, degree of resection, order of therapy, germline status, and molecular subgroup were analyzed. Cumulative incidence (CI) of event free survival due to relapse was evaluated for each variable. Results: Thirty-three of 65 evaluable patients had tumor relapse. For the entire cohort, the CI of relapse was 21.8% at 6 months, 40.6% at one year and 50.3% at 4 years. For patients with infratentorial tumors, CI of relapse was 26.3%, 34.2% and 37.2%, at 6 months, 1 and 4 years respectively compared to 15.3%, 49.9%, and 69.7% for those with supratentorial tumors ($p = 0.051$). Patients with SHH subtype had no relapses in the first 6 months and CI of relapse of 37.5% at 4 years, while those with TYR and MYC subgroups had CI of relapse of 33.3% and 26.7% at 6 months and 46.3% and 73.3% at 4 years respectively ($p = 0.088$). Patients with germline mutations had a cumulative incidence of relapse of 20% at 6 months and 60% at 12 months compared to 22.6% and 37.7% respectively for those without. No obvious trends were noted based on other analyzed variables. Conclusions: ACNS0333 was not powered to determine prognostic indicators of relapse, however, this data suggest interesting trends based on tumor location, subtype and germline status. Infratentorial location and SHH subtype maybe associated with lower risk of relapse. Larger data sets must be compiled to further investigate these variables, perform multivariate analyses and inform risk-stratification on future trials.

ATRT-05. REPURPOSED ANTI-MALARIAL QUINACRINE ACTIVATES P53 AND INHIBITS ATRT TUMORIGENICITY

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Atypical teratoid rhabdoid tumors (ATRTs) are fatal pediatric brain tumors that warrant improved therapies urgently. ATRTs are characterized by loss of INI1, a subunit of the SWI/SNF chromatin-remodeling complex. ATRTs grow aggressively despite majority of primary tumors expressing

p53, suggesting inactivation of this tumor suppressor pathway. Reactivation of p53 could be a potential therapeutic strategy for inhibiting ATRT growth. Our laboratory specializes in researching mechanisms contributing to ATRT pathogenesis and potential therapies. In line with this, we investigated an anti-malarial drug called quinacrine that has been safely used in children for decades and can induce p53 in renal cell carcinoma. We used 5 patient-derived ATRT cell lines (BT-37, BT-12, CHLA-06, CHLA-266, CHLA-05) for our studies. We show that ATRT cell lines treated with quinacrine for 6 hours show increased expression of p53, suggesting its activation. Treatment of ATRT cell lines with increasing doses of quinacrine for 24 hours showed dose-dependent decrease in cell growth and proliferation (assessed by MTS assay and BrdU incorporation, $P < 0.05$) and increase in apoptotic cell death (CC-3 and cleaved PARP expression). Nude mice harboring flank tumors of ATRT cell lines and treated with quinacrine for 3 weeks showed significant reduction in tumor growth compared to control animals ($P < 0.05$). Since quinacrine is a substrate for the drug-efflux proteins P-gp/BCRP, we used quinacrine in combination with elacridar (Pgp/BCRP inhibitor) in our intracranial xenograft experiments to increase quinacrine's retention in the brain. Mice harboring intracranial xenografts of ATRT cells showed increased survival when treated with quinacrine and elacridar (median survival 46 days) compared to control animals (median survival 25 days). These results suggest that quinacrine inhibits ATRT growth, partly by activating p53. Our studies are the first to show quinacrine's effect on ATRTs and our current experiments include further investigation of quinacrine's mechanism.

ATRT-06. RESULTS OF MULTICENTER TRIAL CONCERNING THE TREATMENT OF CHILDREN WITH ATYPICAL TERATOID RHABDOID TUMORS OF THE CENTRAL NERVOUS SYSTEM UNDER 3 YEARS OLD

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Objective: To evaluate the prognostic factors in children with AT/RT aged under 3 years. **Patients and methods:** The prognostic factors were analyzed in 106 patients under 3 years who got treatment and follow-up from 2008 to 2020. There were 41 children younger than 12 months and 65 patients older than 12 months. The location of the tumor was infratentorial in 58 patients, supratentorial in 46, and spinal cord in 2. There were 54 boys and 52 girls. Among the patients, 57 had stage M0, 36 had stage M+ or a multifocal tumor, and 13 had stage Mx. All the patients had undergone surgical treatment: total tumor removal in 27, subtotal-33, partial-42, biopsy - 4; 67 patients had got chemoradiotherapy according to the ATRT-2006 (IRS III) protocol; 15, MUV-ATRT protocol; 3, CWS protocol; 9, EU-RHAB protocol; 6, HIT-SKK protocol; and 6 according to individual treatment schemes. 12 patients received HDC with AuHCR. Results: 47 are alive, 1 was lost to follow-up,

and 58 died: 52 of progressive disease, 6 of chemotherapy complications. The five-year PFS was 0.27; the five-year OS was 0.40. The PFS was significantly better in patients older than 12 months old compared to patients under 12 months: 0.33 and 0.17, respectively; $p = 0.0047$. The PFS after total resection was higher than after subtotal resection, partial resection, and tumor biopsy: 0.51, 0.29, 0.09, and 0%, respectively ($p = 0.025$). The PFS after radiotherapy was markedly higher compared to patients without radiotherapy: 0.63 and 0.0, respectively ($p < 0.001$). The tumor location, stage, and gender did not affect the PFS. The survival rate was statistically significantly higher among the patients who had got treatment according to the ATRT-2006 protocol compared to MUV-ATRT, EU-RHAB, individual therapeutic regimens, CWS, and HIT-SKK: 0.33, 0.26, 0.11, 0.30, and 0.0, respectively; $p = 0.0020$. The PFS was higher among the patients who had got intraventricular/intrathecal chemotherapy; $p = 0.0002$. HDC with AuHCR did not statistically affect the PFS; $p = 0.0546$. In multivariate analysis, the PFS was influenced by the age, tumor location, extent of surgery, radiotherapy, regional chemotherapy, HDC with AuHCR. **Conclusions:** The survival of patients with CNS AT/RT aged under 3 years significantly depended on the patients' age, extent of surgery, chemotherapy protocol, radiotherapy, and regional administration of chemotherapeutic drugs.

ATRT-07. DEFINING LOST AND GAINED TRANSCRIPTIONAL REGULATORY NETWORKS IN ATYPICAL TERATOID RHABDOID TUMOR

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Atypical teratoid rhabdoid tumor (ATRT) is a central nervous system cancer of infancy and early childhood that may occur anywhere along the neuraxis and is associated with a high rate of mortality. While contemporary multimodal therapeutic approaches have significantly improved overall survival, targeted therapy remains elusive, and treatment is often associated with significant morbidity. ATRT is unique in its genomic stability, with the only recurrent genetic abnormality being bi-allelic loss of the *SMARCB1* gene, which encodes a core subunit of the BAF chromatin remodeling complex. The epigenetic mechanisms by which *SMARCB1* loss leads to tumorigenesis are not yet well-defined and addressing this gap in understanding is necessary for creating efficacious, targeted therapeutics. To better understand the epigenetic features gained and lost in ATRT, we re-expressed *SMARCB1* in a library of patient-derived and established ATRT cell lines of multiple molecular subtypes. *SMARCB1* restoration significantly reduced or eliminated the proliferative and clonogenic capacity of each cell line. We performed assay for transposase-accessible chromatin with high-throughput sequencing (ATAC-Seq) and RNA sequencing (RNA-Seq) to define putative transcriptional regulatory networks that are gained and lost in ATRT. *SMARCB1* restoration was associated with global changes in chromatin openness consistent with the creation of new regulatory elements throughout the genome, and these were associated with induction of a diverse developmental transcriptional signature. Motif enrichment analysis of regions with increased accessibility defined a small but consistent number of centrally enriched transcription factor motifs across cell lines indicative of putative pioneer factors whose functions may be lost in ATRT. Pertinent chromatin immunoprecipitation with sequencing (ChIP-Seq) data will be discussed in the context of lost and gained transcriptional regulatory networks in ATRT and normal cellular development.

ATRT-08. TARGETING THE TP53-MDM2 ENHANCES RADIATION SENSITIVITY IN ATRT

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Atypical Teratoid Rhabdoid Tumor is a highly aggressive pediatric brain tumor. Despite radiation, aggressive chemotherapy and autologous stem cell rescue, the children usually have poor survival. A functional genomic screen identified the TP53-MDM2 axis as a therapeutic vulnerability in ATRT. Gene expression demonstrates that all ATRT subgroups have high level of MDM2 a negative regulator of p53. We demonstrate that MDM2 inhibition by shRNA or with small-molecule drugs, Nutlin3 and Idasanutlin resulted in decreased ATRT cell growth, inhibition of clonogenic potential and induction of apoptosis in vitro and in vivo. MRI imaging of intracranial tumors shows that Apparent Diffusion Coefficient (ADC), a good marker for successful treatment, significantly increased with Idasanutlin treatment showing tumor necrosis. Moreover, Idasanutlin significantly decreased growth of intracranial orthotopic ATRT brain tumors as evaluated by T2 MRI imaging and prolonged survival compared to control animals. Further idasanutlin potentiated radiation induced DNA damage and increased sensitivity to radiation of ATRT cells. These findings highlight the TP53-MDM2 axis as a rational therapeutic target in ATRT.