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Abstracts

ATYPICAL TERATOID RHABDOID TUMOR (ATRT)

AT-01. PITFALLS IN THE DIAGNOSIS OF ATYPICAL TERATOID/RHABDOID TUMORS (AT/RT): EXPERIENCES FROM THE EUROPEAN RHABDOID TUMOR REGISTRY (EURHAB) AND THE INTERNATIONAL CHOROID PLEXUS TUMOR REGISTRY CPT-SIOP

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BACKGROUND: The diagnosis of atypical teratoid/rhabdoid tumor (AT/RT) may pose difficulties. We therefore aim to share our experiences as reference pathologists within the European Rhabdoid Tumor Registry (EURHAB) and the International Choroid Plexus Tumor Registry CPT-SIOP. METHODS: Evaluation of clinical, histological and molecular genetic features of AT/RT diagnosed at the Institute of Neuropathology, University Hospital Münster. RESULTS: In the vast majority of brain tumors (46/47) seen in the context of the European Rhabdoid Tumor Registry (EURHAB), the diagnosis of AT/RT could be confirmed. The median age of the patients was 1 year (interquartile range 1-3), 59% of tumors were located supratentorially. Loss of nuclear SMARCB1/INI1 staining was encountered in 44 tumors (96%), but SMARCB1/INI1 staining was retained in two cases. In one of those, loss of protein expression of another SWI/SNF chromatin-remodeling complex member, the ATPase subunit SMARCA4/BRG1 due to a homozygous mutation could be demonstrated. A number of 14 CPT-SIOP cases, which had been suspected to represent choroid plexus carcinoma, also turned out to be AT/RT. The median age of the patients was 1 year (interquartile range 1-3), only 29% of tumors were of supratentorial location. Twelve tumors showed loss of nuclear SMARCB1/INI1 staining (86%), but SMARCB1/INI1 staining was retained in two cases, one showing loss of SMARCA4/BRG1 expression due to a homozygous mutation. CONCLUSIONS: In the majority of cases seen within EURHAB, the diagnosis of AT/RT was confirmed. The high number of AT/RT encountered within CPT-SIOP highlights the importance of considering AT/RT as a differential diagnosis in cases suspected to be choroid plexus carcinoma. Importantly, retained SMARCB1/INI1 staining does not rule out the possibility of AT/RT and genetic alterations of other members of the SWI/SNF complex, namely SMARCA4/BRG1, need to be considered. SUPPORT: MH and WP are supported by IZKF Münster (Ha3/016/11) and Deutsche Krebshilfe (DK 108263).

AT-02. GENOME WIDE COPY NUMBER ANALYSIS OF ATYPICAL TERATOID/RHABDOID TUMORS USING FORMALIN-FIXED TISSUES

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BACKGROUND: Atypical teratoid/rhabdoid tumor (AT/RT) is characterized by genetic alterations affecting the *SMARCB1* locus on 22q leading to loss of SMARCB1/INI1 protein expression. Identification of additional genetic alterations might add to a better understanding of the biology of these rare tumors, but fresh-frozen tissue is scarce. METHODS: Using a Molecular Inversion Probe Single Nucleotide Polymorphism (MIP SNP) assay, high resolution genome-wide copy number analysis as well as screening for known somatic mutations was performed on DNA isolated from formalin-fixed paraffin-embedded (FFPE) tissues. RESULTS: Of 18 AT/RT samples, 16 met quality control requirements of the MIP SNP assay (MAPD value ≤ 0.6) and were further processed. Median age of the 8 boys and 8 girls was <1 year. All tumors showed rhabdoid morphology and loss of SMARCB1/INI1 expression on immunohistochemistry. *SMARCB1* deletions were detected in 7 cases (FISH), while sequencing revealed *SMARCB1* mutations in 7 cases. Using the MIP SNP assay, significant clustering of allelic losses affecting 22q was obvious. Genetic alterations affecting the *SMARCB1* locus were encountered in 15/16 tumors (94%) and involved copy number losses (11 cases) and LOH (4 cases). The frequency of alterations involving other chromosomes was remarkably low. Furthermore, screening for 438 somatic mutations involved in cancer biology did not yield a significant number of mutations, while in one case the presence of a *SMARCB1* mutation (SMARCB1_pR158X_c472C_T) already encountered on sequencing was confirmed. CONCLUSIONS: These data confirm the pivotal role of genetic alterations affecting the *SMARCB1* locus in AT/RT and encourage the use of MIP SNP arrays on FFPE samples of rare pediatric brain tumors. SUPPORT: MH and WP are supported by IZKF Münster (Ha3/016/11) and Deutsche Krebshilfe (DK 108263).

AT-03. HIGH-DOSE CHEMOTHERAPY (HDCT) WITH AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION (APBSCT) IN CHILDREN WITH ATYPICAL TERATOID/RHABDOID TUMORS (AT/RT): A REPORT FROM THE EUROPEAN RHABDOID REGISTRY (EU-RHAB)

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PURPOSE: Long-term survival has been reported following intensive multimodality treatment with and without HDCT in patients with AT/RT. The question as to whether conventional or HDCT offers the best results remains unsettled. A retrospective database analysis was performed to describe the outcome of HDCT in children with AT/RT registered within the EU-RHAB. PATIENTS AND METHODS: A total of thirteen patients (male, n = 10; median age at diagnosis 11 months) were identified. Tumor location was infratentorial in 7 and supratentorial in 6 patients. Five patients (38.5%) had metastatic disease at diagnosis. A partial or subtotal resection was performed in 9 and a total resection in 2 patients. The remaining two patients underwent a biopsy only. Twelve patients received conventional chemotherapy prior to HDCT. Radiotherapy was performed in nine patients. Conditioning included carboplatin (n = 12), thiotepa (n = 12), etoposide (n = 5), cyclophosphamide (n = 3) and melphalan (n = 1). Disease status before HDCT was complete remission (CR) in 4, partial remission (PR) in 2, stable disease (SD) in 3, and progressive disease (PD) in 3 patients. In one patient data on the pretransplant remission status is missing. RESULTS: With a median follow-up of 15 (range, 8-70) months 11 patients (85%) progressed. Estimated progression-free and overall survival at 2 years was 15.4% and 38.5%. Median estimated time to progression was 11.3 months. Three patients are alive in CR 29, 32 and 70 months following diagnosis. In two of them tumors were located in the posterior fossa. Eight patients died of PD within 8-16 months after diagnosis. Two patients are alive with disease. CONCLUSIONS: Despite HDCT with APBSCT prognosis is poor in AT/RT. Selected patients, however, might benefit from HDCT. The definitive impact of this treatment modality has to be evaluated prospectively in a larger cohort of patients. This will allow defining prognostic parameters in patients with favorable/unfavorable outcome.

AT-04. SOME INFANTS WITH CNS ATRT CAN BE CURED WITHOUT IRRADIATION.

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BACKGROUND: CNS ATRT are extremely aggressive brain tumors that mostly affect infants and young children. Encouraging results are emerging with the use of multimodality approach combining maximal surgical resection, intensive chemotherapy (without or with stem cell support) and radiotherapy. Because of the very young age of the majority of the patients, some centers favor a non radiation strategy in order to preserve the neurocognitive outcome of this vulnerable group of patients. PATIENTS AND METHODS: We report 16 cases of children with CNS ATRT treated with high dose chemotherapy and omission of radiation. All diagnoses were centrally reviewed. RESULTS: The chart of 16 patients (9 female), diagnosed between 2001 and 2010 were reviewed. The median age at diagnosis was 16.1 months (range 4.2 to 43.3). The median duration of symptoms before diagnosis was 3 weeks (0.5-6). Tumors were evenly distributed in supra and infratentorial compartments (50%). Six patients (37.5%) had metastatic disease at presentation. Five of the ten patients tested for INI1 germline mutation and were found to be positive. A greater than subtotal resection was achieved in 9 patients (87.5%). All patient received conventional chemotherapy followed by high dose chemotherapy and stem cell rescue (Carboplatin Thiotepa)x3 or (Carboplatin, Thiotepa, Etoposide)x1. In addition to HDC, 5 patients (31%) received IT chemotherapy. Seven patients progressed or relapsed at a median time of 5 months (range 2.3-14.2). Six patients died of disease and one of HDC related toxicity. At a median follow up time of 33.5 months (7.8-96.0), 9(56.2%) patients were alive without evidence of disease. Three of them harbored a germline mutation. CONCLUSION: Some patients with CNS ATRT, including patients metastatic at presentation, can be successfully cured without radiation. Because no clear clinical factor can yet differentiate these patients from those who may require radiation, refined molecular analysis is underway.

AT-05. DESCRIPTIVE EPIDEMIOLOGY OF CNS ATYPICAL TERATOID/RHABDOID TUMORS: AN ANALYSIS USING TWO U.S. POPULATION-BASED DATA SETS (CBTRUS AND SEER)

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Atypical teratoid/rhabdoid tumor (ATRT) was first identified as a unique clinical and neuropathological entity in the 1980s and 1990s. ATRT was added to the World Health Organization Classification of Tumours of the Central Nervous System in 2000. Many hospital-based and observational studies on ATRT have been published, but few population-based statistics are available prompting this evaluation of brain tumor data on cases diagnosed in children ages 0-19 years from two large U.S. cancer registries. ATRT was defined by ICD-O-3 histology code: 9508 of the brain/CNS (ICD-O-3 primary sites: C70.0-C72.9, C75.1-C75.3). The Central Brain Tumor Registry of the United States (CBTRUS), 21 state population-based cancer registries analytic data set, 2004-2008, was used to estimate ATRT incidence rates. The Surveillance, Epidemiology, and End Results (SEER) Program 17 registry research data file, 2000-2008, was used to determine survival patterns for ATRT. All analyses were conducted using SEER*Stat v. 7.0.5. Incidence rates are expressed per 100,000 person-years. Survival estimates were made using the Kaplan-Meier method. CBTRUS incidence data: 114 cases were identified. Cases occurred primarily among children of white race. Most cases (90%) were among children ages 0-5 years. The highest incidence rate was observed in the infants 0 year age group (0.60/100,000) followed by children in the 1-4 year age group (0.20/100,000). Incidence rates for boys and girls were similar. Survival analysis: 132 cases were followed. The one-year relative survival rate was 47.6%. The five-year relative survival rate was 27.3%. This study provides insight into the incidence and survival of a rare childhood tumor, ATRT, at the population level.

AT-06. SYNERGISTIC EFFECTS OF A COMBINATION OF HDAC INHIBITORS WITH EZH2 OR CYCLIND INHIBITORS ON RHABDOID TUMOR CELL GROWTH

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INTRODUCTION: Rhabdoid tumors rarely respond in a lasting manner to conventional chemotherapy: Nevertheless recent approaches using epigenetic compounds such as histone deacetylase (HDAC)-inhibitors have demonstrated efficient inhibition of tumor cell growth *in vitro*. METHODS: Rhabdoid tumor cell lines (G401, A204, BT16) were treated with HDAC inhibitor (HDI) (e.g. SAHA) alone and in combination with the CyclinD inhibitors Fenretinide and 4OH-Tamoxifen or with the EZH2 inhibitor DZNep. Effects on cell proliferation, cell cycle and apoptosis were analyzed by using XTT, DAPI and annexin/propidiumiodid. RNA Microarrays were performed to detect effected pathways by HDI in BT16 cell lines. RESULTS: Inhibition of HDACs by small molecular compounds such as suberoylanilidehydroxamic acid (SAHA) and by selective HDAC inhibitors resulted in G1-arrest and induction of apoptosis in the rhabdoid tumor cell lines. HDAC1, HDAC2, HDAC3 and HDAC8 were found highly expressed in primary rhabdoid tumors and rhabdoid tumor cell lines. Using microarray analysis we identified multiple mechanisms, such as chromosomal condensation, which may be responsible for cell death induced by HDI. Furthermore we demonstrated that SAHA also activates pathways and specific genes associated with cell cycle progression (e.g. Rb, stem cell program). Targeting these activated pro-proliferative genes by combined therapy (SAHA plus EZH2 inhibitors, SAHA plus CyclinD inhibitors) demonstrated strong synergistic inhibitory effects on tumor cell growth. In addition, HDAC inhibition was shown to sensitize rhabdoid tumor cell lines to chemotherapeutic-induced cell death. CONCLUSION: Chromatin-based HDI treatment in combination with EZH2-inhibitors, CyclinD-inhibitors or conventional chemotherapy is a promising tool for the treatment of these often fatal embryonal tumors. This work was supported by the "Innovative Medical Research" fund of the University of Muenster Medical Faculty and the Sonja Wasowicz Stiftung Germany, Sitfterverband; Germany

AT-07. ABSENCE OF ONCOGENE AND TUMOR SUPPRESSOR GENE MUTATIONS IN ATYPICAL TERATOID/RHABDOID TUMORS (AT/RT)

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BACKGROUND: Atypical Teratoid Rhabdoid Tumor (AT/RT) is a highly malignant central nervous system embryonal tumor of infants. While the genetic defect underlying AT/RT has been well demonstrated in pre-clinical models and primary human tumor material (loss or mutation of the INI1/SNF5/SMARCB1 subunit of the SWI/SNF chromatin complex), appropriate targets for therapeutic intervention have not been clearly elucidated. METHOD: We performed OncoMap, a high-throughput, mass spectrometry genotyping platform of archival (formalin-fixed, paraffin-embedded) tissue focused on the subset of oncogenes and tumor suppressor genes that are responsible for activating events in most human cancers. Samples selected were patients with classic AT/RT defined by the presence of the SMARCB1 mutation. RESULTS: Twenty-five purified FFPE samples were submitted for OncoMap analysis, 24 passed all of the quality assurance methods and underwent full analysis. A total of 983 unique mutations in 115 oncogenes or tumor suppressor genes were tested. No mutations in any of the oncogenes or tumor suppressor genes, with the exception of the known driver mutations in SMARCB1 and a single example of an NRAS mutation were identified. All positive and negative controls were appropriate and concurrent analysis of 20 diffuse intrinsic pontine glioma (DIPG) samples identified both previously known mutations in these tumors (p53) and novel mutations in PI3K (published in Grill et al, Pediatr Blood Cancer. 2011 Dec 20). DISCUSSION: The remarkably low rate of mutation in ATRT contrasts with other aggressive cancers. In concert with the published gene expression profile for AT/RT and lack of significant genomic instability identified in these tumors (Jackson et al., Clin Cancer Res. 2009 Mar 15;15(6):1923-30; McKenna et al, Mol Cell Biol. 2008 Oct;28(20):6223-33), the need to focus on other mechanisms of cell control dysregulation such as epigenetic regulated events is being undertaken.

AT-08. THE MOLECULAR BIOLOGICAL COMPARISONS BETWEEN ORIGINAL, RECURRENT TUMORS, AND PRIMARY CULTURE CELLS FROM ATYPICAL TERATOID / RHABDOID TUMOR

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BACKGROUND: Atypical teratoid/rhabdoid tumor (AT/RT) is a highly malignant central nervous system neoplasm in early childhood. In this report,

we used high throughput array as a platform to identify the difference between original tumor and recurrent tumor with the profiling of expression genes. Furthermore, we tried to identify the tumor stem cell character of these tumor using primary culture cells from the tumor. METHODS: We extracted the DNA from paraffin embedded tumors those recurred after chemotherapy in the same patient. We also have succeeded to sustain the growth of tumor cells in culture condition obtained from tumors of this infant with AT/RT in previously reported method. Then, we performed high throughput study and compared the expression data from these tumors and cells using a commercially utilized SNP array system. RESULTS: The comparison between original tumor and recurrent tumor detected several differences in the regulations of genes. The cells in serum free medium formed sphere and showed some stem cell characters. CONCLUSIONS: Our comparison may reveal the mechanism of the drug sensitivity patterns among this tumor. The analysis of the primary cell culture derived from the tumor would provide an effective way to study the biology of AT/RT and to identify potential targets for future therapeutics for this tumor.

AT-09. INHIBITION OF THE POLYCOMB GROUP GENE EZH2 SUPPRESSES GROWTH AND RADIO-SENSITIZES ATRT CELLS BY PROMOTING SENESCENCE AND INHIBITING THE CYCIND-E2F1 AXIS.

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The polycomb group gene Enhancer of Zeste 2 (EZH2) is a critical regulator of global gene transcription and its function is associated with maintaining cells in an undifferentiated state. Over expression of EZH2 occurs in many malignancies including brain tumors such as glioblastoma multiforme and medulloblastoma. EZH2 specifically methylates Histone 3 Lysine 27, resulting in target gene silencing. Recent evidence suggests EZH2 may also have a role in rhabdoid tumors. Atypical teratoid/rhabdoid tumor (ATRT) is a rare, high-grade embryonal brain tumor that occurs most commonly in children and carries a very poor overall survival. ATRTs are characterized by absence of the chromatin remodelingprotein SMARCB1. Given the role of EZH2 in regulating epigenetic changes we investigated the role of EZH2 in ATRT. Genomic analysis shows that an EZH2 reponsive gene expression signature is associated with tumors from patients with short survival. Targeted disruption of EZH2 by RNAi or pharmacologic inhibition strongly impairs ATRT cell growth, induces apoptosis, and potently sensitizes these cells to radiation. Using functional analysis of transcription factor activity we found the cyclin D1-E2F axis is repressed upon EZH2 depletion in ATRT cells. Taken together, our observations provide evidence that EZH2 regulates cell cycle progression by targeting the G1-S transition machinery and may be an important new therapeutic target, particularly in combination with radiation, in ATRT.

AT-10. RHABDOID 2007 - 5 YEAR FOLLOW-UP OF A CONSENSUS TREATMENT REGIMEN IN 29 GERMAN CHILDREN WITH AT/RT

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Thus far only one prospective trial specifically designed for children with AT/RT has been published (Chi et al. 2009). Primary purpose of Rhabdoid 2007 was to collect data on German children with AT/RT treated according to a rhabdoid-centered consensus protocol registered in a central data base. Up to 2009, 29 patients (11 f, 18 m) were registered into Rhabdoid 2007 and treated using 9 cycles of alternating VCD and ICE with intrathecal MTX. Median age at diagnosis was 18 months (n = 11 <12 months, n = 10 12-36 m, n = 8 >36 m). Reference pathology including immunohistochemistry for INI-1 was available in 100% (n = 1 INI-1 positive AT/RT). Genetic evaluation was performed in 13 patients with germ line mutations detected in 2 of them (15%). Total resection was performed in n= 8 (28%), partial resection in n = 20 (69%) and biopsy only in 1 patient (3%). All received chemotherapy, of these 18 (62%) were given i.th. MTX at least once. 7 (24%) patients received HDCT, 20 patients (69%) were treated by RT. 5 year follow-up shows an EFS of 41% and OS 48%. Statistical analysis detected a significantly better OS ($p<0.05$) for patients over 3 years at diagnosis and those who were treated with radiotherapy. Evaluation of prognostic factors reveals radiotherapy and age > 3 years as positive prognostic factors for OS. The 5 year follow-up of Rhabdoid 2007 shows promising results for EFS and OS. After successful data collection for Rhabdoid 2007 a registry - EURHAB - for rhabdoid tumors of all locations and a protocol in consensus with European countries has been opened in 2009. Data of Rhabdoid 2007 and EURHAB will be relevant for the future concept of a European phase II study in AT/RT and other rhabdoid tumors. Supported by the Deutsche Kinderkrebsstiftung

AT-11. SMARCB1 DEFICIENCY IN TUMOURS FROM THE PERIPHERAL NERVOUS SYSTEM: A LINK BETWEEN SCWHANNOMAS AND RHADBOID TUMOURS?

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Inactivation of *SMARCB1* tumor suppressor gene was originally described as highly specific for rhabdoid tumours (RTs). Nevertheless, recent reports have illustrated that *SMARCB1* alterations also characterize other tumours. In particular, some familial schwannomatosis and epithelioid malignant peripheral nerve sheath tumours, both from peripheral nervous system (PNS) origin, lack BAF47 expression. To document the putative role of *SMARCB1* in PNS, we reviewed peripheral nervous tumours referred to our institution for a molecular analysis of *SMARCB1* because of histological features compatible with *RT*. METHODS: Clinicopathological, radiological and molecular characteristics were detailed for the 12 cases showing *SMARCB1* loss of expression and/or biallelic inactivation. The status of *NF2* gene, likely to synergize with *SMARCB1* in PNS tumours, was also analysed. RESULTS: Patients' age ranged from 0 to 45 years (median age 6.6 years). Neurological symptoms were observed in 7/12 cases with radiological features evoking a neuroblastic tumour in 6 cases and a peripheral nerve tumour in 4 cases. The mean delay prior to diagnosis was 3 months. Histological examination revealed rhabdoid features in 11/12 tumours. All tumours showed a complete loss of *SMARCB1* expression. Interestingly, adjacent nervous proliferation resembling neurofibromas were observed in 3 cases, suggesting a multi-step transformation. Three tumours harboured a hemizygous deletion at *NF2* locus but all *NF2* sequences were normal. CONCLUSION: We report the first series of PNS rhabdoid tumour. In patients with aggressive PNS tumours, RT should be suggested and anti-SMARCB1 immunohistochemistry performed. *SMARCB1* inactivation, occasionally associated with *NF2* deletion, might have oncogenic effects in peripheral nerves.

AT-12. ATYPICAL TERATOID RHABDOID TUMOR - REPORT FROM A CHILDREN'S CANCER CENTRE IN SINGAPORE

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BACKGROUND: Atypical teratoid rhabdoid tumor (ATRT) of the central nervous system (CNS) is a rare and highly malignant neoplasm usually seen in very young children. We describe our experience from a centre in South East Asia. METHODOLOGY: We retrospectively reviewed hospital database and chart records between 2000 and 2010. RESULTS: There were 6 patients diagnosed with ATRT in the review period. The patients were all of Asian ethnicities. There were more boys (67%) than girls (33%). The age at diagnosis ranged from 1.1 to 14.8 years (median 3.1). The top presenting complaints were increasing head circumference, pain (headache / back pain) and unsteady walking. All except one patient had cerebral primaries - 2 supratentorial; 2 posterior fossa; 1 had both supratentorial and posterior

fossa tumors; 1 spinal. The patient with spinal primary presented with back pain and cord compression. One patient (with brain primary) had drop metastases to the spine (cauda equina). None had extraneural ATRT. Most of the tumors had characteristic rhabdoid cells. However INI-1 staining was not done in earlier cases. Most patients received multimodality therapy - surgery, multiagent systemic chemotherapy and radiation. We had only 2 survivors. One patient died soon after presentation from tumor hemorrhage, before any treatment could be given. One succumbed to disease recurrence 27 months from diagnosis. Of note we had 2 deaths from sepsis - one during induction chemotherapy (3 months from diagnosis), and the other during treatment for disease recurrence (2.7 years from diagnosis). DISCUSSION: The disease may be under-diagnosed in the previous years when INI-1 staining was not routinely used. Despite being a highly aggressive disease, some patients can be cured using intensive multi-modality treatment. However, current chemotherapy regimens used for ATRT have significant toxicities, and may not be suitable when good supportive care is not available.

AT-13. PRECLINICAL EVALUATION OF COMBINATION EPIGENETIC THERAPY FOR ATYPICAL TERATOID/ RHABDOID TUMOR

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BACKGROUND: Despite intensive multimodality therapy, outcomes for atypical teratoid/rhabdoid tumor (ATRT) remain poor. Histone deacetylase inhibitors (HDACi) and DNA methyltransferase inhibitors (DNMTi) function as epigenetic modifiers and lead to re-expression of inappropriately repressed genes and cellular functions. Due to epigenetic alterations in ATRT resulting from SMARCB1 loss, HDACi and DNMTi may offer a novel approach to this tumor. METHODS: Primary tissue was obtained from 19 ATRT patients, mRNA was isolated for Affymetrix gene chip hybridization, and analysis of HDAC and DNMT expression versus control tissue was performed. For proliferation, apoptosis, cell cycle, and gene array evaluations of combination epigenetic therapy, BT-12 and BT-16 ATRT cells were treated with the DNMTi decitabine and the HDACi SNDX-275. Synergy was determined by the Bliss additivity model. Neurosphere formation was performed in neurobasal media with UPN737 (primary ATRT patient) cells undergoing combination therapy. Genomic alterations after drug treatment (control, monotherapy, and combination) of BT-12 and BT-16 cells were evaluated by Affymetrix mRNA array analysis (BT-12, BT-16 cells) and subsequent DAVID and Ingenuity Pathway Analysis. RESULTS: Microarray analysis of ATRT patient samples demonstrated upregulation of HDAC1, HDAC2, DNMT3A, and DNMT3B. Combination decitabine and SNDX-275 treatment synergistically decreased ATRT cell proliferation in both MTS and clonogenic assays compared to either treatment alone, increased early and late apoptotic fractions, and resulted in cell cycle arrest. Additionally, neurosphere size was significantly decreased by combination therapy. Pathway analyses of RNA isolated after combination therapy demonstrated upregulation in immune response pathways and downregulation in functional clusters most notably associated with cell cycle progression at the G2-M checkpoint. CONCLUSIONS: Class I HDACs and DNMT3A/3B are upregulated in ATRT. Inhibition of these enzymes by SNDX-275 and decitabine results in decreased proliferation, increased apoptosis, cell cycle arrest, decreased neurosphere size, and an antiproliferative genomic phenotype. Further preclinical evaluation of these agents is warranted.

AT-14. EFFICACY OF MULTIMODAL THERAPY WITH IT CHEMOTHERAPY FOR INI-1 NEGATIVE CNS TUMORS : FOUR CASES REPORT IN OSAKA CITY GENERAL HOSPITAL

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INTRODUCTION: INI-1 negative CNS tumors are highly malignant pediatric brain tumors. Usually younger children are affected. The outcome was dismal and effective therapy has not been established. Recently, it was reported multimodal therapy including intrathecal (IT) chemotherapy and radiotherapy may improve outcome. Here, we describe four cases treated with multimodal therapy including IT chemotherapy in our institute. PATIENTS: From 2006 to 2009, we experienced four cases with INI-1 negative CNS tumors. One was choroid plexus carcinoma and the others were atypical teratoid rhabdoid tumor. The median age was 28 months (range: 11m to 8y). Metastatic status at diagnosis was M1 in one and M0 in three patients. After surgical resection (three: GTR, one: biopsy), four or five courses of chemotherapy concomitant with IT chemotherapy (MTX 12 mg + DEX 6.6 mg) were administered, Two cases were treated with a regimen for medulloblastoma; CDDP 90 mg/m^2 D1, ETP 100 mg/m^2 D1-5, VCR 1.5 mg/m^2 D1, CPA 1000 mg/m^2 D1,3,5, the other two were treated with a regimen for rabdomyosarcoma; CDDP 70 mg/m^2, THP-ADR 25 mg/m^2 D1-2, VCR 1.5 mg/m^2 D8, 15, AMD 0.045 mg/m^2 D1. A Double-conditioning high-dose chemotherapy (TEPA 800 mg/m^2/total + LAPM 280 mg/m^2/total) with peripheral blood stem cell rescue (PBSCR) was performed as consolidation. On the first day of high-dose-chemotherapy, IT chemotherapy was added. Delayed local radiotherapy (45-54Gy) was performed in all cases after completion of chemotherapy. 3D-conformal radiotherapy and proton therapy were used in three and one cases, respectively. 24Gy craniospinal irradiation was added in an 8-yr-old patient. Recurrence was observed in the anterior horn of lateral ventricle in a patient 58 months after diagnosis and he is currently disease-free following stereotactic radiosurgery and intraventricular chemotherapy. The others are in continuous remission (38, 45, 63 mo). CONCLUSION: Multimodal therapy may improve clinical outcome of patients with INI-1 negative CNS tumors.

AT-15. AT/RTS: UNUSUAL CLINICAL VARIANTS MANIFEST TYPICAL MICROARRAY PROFILES

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INTRODUCTION: AT/RTs have been well characterized in their manner of presentation, demographics, genetics and histology. Some differences have been described in their gene expression profiles which may have prognostic significance. We wondered whether patients with unusual clinical features might have different profiles than routine AT/RTs. METHODS: From among AT/RTs treated here over 11 years, 3 patients were noted with very unusual clinical histories. Among the 17 for who both was available, the pathology and gene expression microarray data were compared to the others in our series. RESULTS: Two patients were females who presented at birth, one with macrocephaly and the other with untoward and unremitting irritability. One had a massive posterior fossa tumor and diffuse leptomeningeal disease throughout the spine, and the other, a massive posterior fossa tumor with another large lateral ventricular mass. Both showed typical loss of INI-1 protein. In both, support was withdrawn and they died rapidly. The third was a 21 month old with cleft lip, profound developmental delays, and a history of pathologic left handedness. Her massive tumor involved the third and fourth ventricles, and showed loss of INI-1 protein in tumor; she was found to have a germ line ring 22 chromosome rather than the more common monosomy 22. She failed to respond favorably to therapy and care was withdrawn. In all three cases, the mRNA using Affymetrix technology failed to reveal remarkable differences from the rest of our series. CONCLUSION: These cases of AT/RTs, while exceptional clinically, show gene expression profiles similar to other AT/RTs.

AT-16. URINARY BLADDER MYOEPITHELIOMA IN A LONG-TERM SURVIVOR OF ATYPICAL TERATOID RHABDOID TUMOR WITH A GERMLINE SMARCB1 MUTATION

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Germline mutations and deletions of SMARCB1/INI1 in chromosome band 22q11.2 predispose patients to rhabdoid tumors and schwannomatosis. The authors report a case of the rhabdoid tumor predisposition syndrome (RTPS) secondary to a germline SMARCB1/INI-1 mutation. The girl was diagnosed at the age of 38 months with a posterior fossa tumor and supratentorial hydrocephalus. The tumor was nearly completely resected and originally classified as a medulloblastoma, cytogenetic analysis have shown monosomy of 22 chromosome in the tumor. She was then treated with craniospinal irradiation and four cycles of submyeloablative chemotherapy with PBSC support and finished the treatment in complete remission. During a follow-up period she presented with macroscopic hematuria and urinary bladder tumor was diagnosed 56 months after completion of brain tumor treatment. The biopsy revealed malignant myoepithelioma, the tumor was

completely resected and six courses of chemotherapy were administered. The occurrence of unusual bladder tumor, and known 22 monosomy led to re-evaluation of brain tumor histology. Although tumor morphology resembled classic medulloblastoma, INI-1 staining negativity prompted genetic analysis. Mutational screening of the SMARCB1 gene by direct sequence analysis and multiplex ligation-dependent probe amplification (MLPA) detected germ line exon 7 deletion. Parents were tested negative. The girl is disease free 24 months after completion of treatment. This is the first case of urinary bladder myoepithelioma in ATRT survivor illustrating that young patients with RTPS are at increased risk for the development of various malignancies.

AT-17. RHABDOID PROTOCOL IS A PROMISING TREATMENT FOR CNS ATYPICAL TERATOID RHABDOID TUMORS

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Atypical teratoid/rabdoid tumor (AT/RT) of the CNS is characterized with aggressive behavior. We evaluated the results of treatment of 30 pts (15 girls and 15 boys) with AT/RT, treated from 1997 to 2011. 20 pts were under 3 y.o. Median age – 1 year 9 months (range 7 months to 15 ys). 15 pts were with M0 stage, 9 – M + , 6-Mx and 23 pts with partial resection. Chemotherapy regimes were not standardized: 14 pts were treated with Rhabdoid protocol with ith. CHT (MTX, ARA-C, PRED), 8 pts – with protocol CWS with ith. CHT (MTX, ARA-C, PRED), 3 pts – with HIT-SKK-92 (with ith. MTX), 4 pts nonprotocol CHT and one – without CHT, but with local RT. 4 pts received CSI 35Gy and boost to tumor bed/metastasis up to 55 Gy, 9 pts – local RT, 17 pts were without any RT according to the age <3 y.o. 3-years PFS for all group was 0,29 ± 0,09, OS 0,13 ± 0,1. PFS of pts, who received Rhabdoid protocol was 0,54, CWS protocol – 0,25, HIT-SKK'92 or nonprotocol therapy – 0 ($p = 0,031$). Pts without CHT – died in 6 mths with PD. PFS of pts, who treated with ith MTX + ARA-C + PRED was 0,44 and without – 0 ($p = 0,007$). PFS of pts under 3 y.o. was 0,16, older 3 y.o. 0,54 ($p = 0,02$). Pts with M0 stage had better PFS – 0,52 than pts with M+ - 0,1 ($p = 0,02$). PFS of pts with total resection was 0,43, with subtotal – 0,28 and partial – 0 ($p = 0,63$). PFS pts who received CSI with tumor/MTS boost was 0,32, with local RT 0,75, without RT 0,12 ($p = 0,06$). Median time of survival was 13 mths, of follow up 10 mths (range, 2 to 76 mths). Pts with M0 stage, older 3 y.o. and who received Rhabdoid protocol had better outcome.

AT-18. COMPARATIVE ANALYSIS OF THERAPEUTIC TARGETS IN AT/RTS AND KRTS BASED ON GENE EXPRESSION

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INTRODUCTION: Atypical Teratoid / Rhabdoid Tumors (AT/RTs) and Kidney Rhabdoid Tumors (KRTs) are highly aggressive malignancies that occur primarily in very young children. Average survival times remain poor even with extremely aggressive treatment. As expanding numbers of therapies are developed that target specific biological processes or pathways, the question of whether these therapies may be effective for all rhabdoid tumors (RTs) or may be limited to a subset of RTs, is of critical importance for the design of new clinical trials and analysis of outcomes. This study provides some initial answers to this question, based on comprehensive analysis of gene expression in AT/RTs and in KRTs. METHODS: Using Affymetrix gene chips, gene expression of 18 AT/RTs was compared to 13 non-tumor brain control samples. 10 KRTs were compared to 14 non-tumor kidney samples. Potential therapeutic targets were defined as known oncogenes, kinases, or genes with currently available clinically relevant treatments that were significantly up-regulated in the tumor samples (FDR <0.01 and fold change >= 2). RESULTS: 264 potential therapeutic targets were identified in AT/RTs and 245 in KRTs; approximately 50% were common to both RTs. Among those with currently available treatments, TOP2A, RRM1, RRM2, CDKs, AURKA/AURKB, FGFR1, HDAC1 and HDAC2 were common to both RTs. Those unique to AT/RTs included MUC1, WEE1, CHEK1, CDK6, and VEGFA. Those unique to KRTs included CDK19, CDK7, CDK8, HSP90AA1, and TOP2B. Flavopiridol and gemcitabine targeted the most numbers of genes overall. CONCLUSIONS: Based on gene expression, numerous therapeutic targets are common to both AT/RTs and KRTs, however there are also many targetable genes that show up-regulated expression in only one or the other. The findings from this study provide a basis for rational testing of therapeutic strategies in preclinical models, and suggest that both AT/RT and KRT models should be evaluated.

AT-19. RHABDOID TUMOUR SUPPRESSOR SMARCB1: EVIDENCE FOR A ROLE IN THE POST-TRANSCRIPTIONAL REGULATION OF NF2

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Malignant rhabdoid tumours of the kidney (KRT) and brain (AT/RT) are biologically complex diseases occurring in early childhood. Inactivation of the SMARCB1 locus is common to KRT and AT/RT. As a component of the SWI/SNF complex, SMARCB1 loss has widespread effects on gene expression and epigenetic regulation. Targeted treatment approaches intersecting SMARCB1 loss, will therefore be a challenge. Given the association of SMARCB1 mutation with schwannoma, we hypothesized NF2 might also play a role in rhabdoid tumour (RT). Although there have been no previous reports of NF2 inactivation in RT, other than the loss of one allele in tumours with 22q deletion, we hypothesized homozygous loss of SMARCB1 might affect NF2 post-transcriptionally in some tumours. To test this, we examined NF2 expression in human embryonic kidney cells (HEK293T) following SMARCB1 knockdown with siRNA. We identified short, novel NF2 transcripts appearing 72 hours after first SMARCB1 siRNA treatment, coincident with loss of full-length NF2. The appearance of short NF2 coincided with SMARCB1 levels approximately 50% those at the outset of the experiment. We then showed that full length NF2 could be restored in a recombinant RT G401 cell line containing an inducible double vector system expressing SMARCB1, which when activated, coincided with the disappearance of shorter forms of NF2. We identified numerous short forms of NF2 in other RT cell lines and tumours, and either very low or negligible levels of full-length NF2. Thus far we have identified several NF2 transcripts distinct from the alternatively spliced NF2 variants deposited in the UCSC database and we predict they are either non-functional or alternatively, dominant oncogenic forms of NF2. Further characterization is in progress. Our results demonstrate another level of complexity to SMARCB1 and provide an explanation for the observed up-regulation of oncogenic signalling downstream of NF2 in RT.

AT-20. CASE REPORT: LONG-TERM SURVIVAL IN AN INFANT SYNDROMIC PATIENT AFFECTED BY ATYPICAL THERATOID-RHABDOID TUMOR

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Atypical theratoid rhabdoid tumor (ATRT) is an aggressive neoplasm of infancy with a dismal prognosis, particularly in patients affected by rhabdoid tumor predisposition syndrome, that presents less than 20% overall survival at 1 year from diagnosis. We here describe an unusual case of ATRT in a 2-years old patient with a mosaicism 47, XXY[14]/46,XY[65] and affected by rhabdoid tumor predisposition syndrome caused by constitutional SMARCB1 heterozygous mutation c.118C > T (Arg40X). Patient's primary tumor presented a cumbersome differential diagnosis and was finally treated in 2003 as a less-than-3-years metastatic medulloblastoma. At the onset of a second spinal lesion seven years later, both tumors were pathologically and molecularly evaluated at the national central pathology review board and both tumors were defined as ATRT in a syndromic patient, with strong evidence of a clonal origin of the two lesions. Both lesions presented loss of the entire second allele, the same pattern of 22q loss of heterozigosity as detected by microsatellite analysis and identical sequence polymorphisms in mitocondrial DNA hypervariable regions. The patient underwent further treatment of the metastatic spinal lesion and is now alive without evidence of disease, over one year after the detection of metastatic disease and at seven years from the original diagnosis. The report underscores the current utility of multiple comprehensive approaches for the correct diagnosis and clinical management of patients affected by rare and atypical brain neoplasms, and also evidences that successful local control

of disease and achievement of long-term survival is possible in ATRT patients even in the setting of rhabdoid tumor predisposition syndrome, thus justifying the efforts for the management of this severe condition.

AT-21. BRAF V600E IN PEDIATRIC BRAIN TUMORS: TREATING THE MUTATION

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BRAF V600E mutations occur in approximately 7% of human malignancies including 60% of melanomas. Vemurafenib (PLX4032) is an FDA-approved drug that specifically targets the mutation and has demonstrated an unprecedented rate of success in treatment of late-stage melanoma. V600E has been shown to occur in a subset of pediatric brain tumors, predominantly gangliogliomas, pleomorphic xanthoastrocytomas, and pilocytic astrocytomas and sporadically in atypical teratoid/rhabdoid tumor (ATRT) and glioblastoma. The aim of this study was to establish whether V600E could be targeted by vemurafenib in a pediatric brain tumor setting. We found V600E in a ganglioglioma that progressed to ATRT, from which a cell line was established. The ganglioglioma, the ATRT, and the cell line all harbor V600E. Only the ATRT and the cell line showed deletion of *hSNF5/INI1/SMARCB1*, the defining feature of ATRTs. V600E and wild-type (WT) ATRT and melanoma cell lines were treated with vemurafenib, and differential sensitivity to the drug was established based on mutational status. Cell viability assays showed that ATRT harboring V600E was >300-fold more sensitive to vemurafenib than WT. V600E ATRT and melanoma lines also exhibited rapid MAPK pathway inhibition upon treatment as shown by Western blot, similar to what has been previously described in melanoma V600E studies. Conversely, WT ATRT and melanoma lines exhibit post-treatment upregulation of activated MAPK, highlighting the specificity of the drug and the paradoxical enhancement of growth seen in WT lines upon treatment. Cell cycle analysis revealed similar G0/G1 arrest upon treatment in V600E ATRT and melanoma. Future studies will assess the sensitivity of other pediatric brain tumors harboring V600E to vemurafenib, specifically short-term cultures of gangliogliomas and pilocytic astrocytomas. Sensitivity of V600E ATRT to vemurafenib provides rationale for new treatment protocols that focus on treating specific mutations rather than specific cancer types.

AT-22. EIGHTEEN MONTH OLD WITH MULTI-FOCAL ATYPICAL TERATOID/RHABDOID TUMOR (AT/RT) OF THE BRAIN AND THE NEED FOR EARLY INVOLVEMENT OF GENETIC SPECIALIST

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Atypical teratoid/rhabdoid tumors (ATRT) are rare and typically develop in the central nervous system of infants and young children. These are highly aggressive malignancies with poor prognosis. The majority of children succumb despite multi-modal therapy. We report the case of an eighteen month old with a three-day history of clumsiness and rapid progression of ataxia. His brain MRI was notable for 3 separate lesions including a large minimally enhancing mass in the right lateral ventricle, a second mass with prominent contrast enhancement located at the left cerebellopontine angle, and a third smaller lesion in the right basal ganglia. The pathologic diagnosis was atypical teratoid/rhabdoid tumor with absence of INI-1 antibody staining. The metastatic evaluation noted presence of tumor cells in the cerebral spinal fluid. Prior to chemotherapy initiation, he experienced a rapid neurological decline with apnea and bradycardia. A repeat MRI was remarkable for extensive extra-axial fluid collections and progressive enhancing lesions consistent with diffuse subarachnoid spread. Given his precarious clinical state, the family agreed to comfort measures only and he died 21 days after his initial MRI. Prior to death, a genetics consultation was obtained and testing revealed a heterozygosity for a paternally derived variant (c.233-43A > T) of uncertain significance in intron 2 of the SMARCB1 gene in blood lymphocytes. Loss of heterozygosity (LOH) was demonstrated in tumor tissue, with loss of the c.233-43A wild type allele. Further microsatellite analysis showed this LOH to be copy neutral. Our case is unusual because of the tumors' very distinct radiographic characteristics. Although this case was not successful, it also calls attention to the need for genetic testing for these patients to determine if a germline mutation exists for appropriate counseling of family members. Given the rapid progression the majority of these patients experience early involvement of a genetic specialist is recommended.

AT-23. INITIAL TESTING OF THE DOUBLE DELETED VACCINIA VIRUS FOR ONCOLYTIC ACTIVITY AGAINST PEDIATRIC CNS AT/RT CELLS: IN VITRO CYTOTOXICITY, DRUG SYNERGY AND TUMOR GROWTH INHIBITION IN XENOGRAFT MODELS

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Despite aggressive treatment approaches, primary CNS atypical teratoid/rhabdoid tumor (CNS AT/RT) remains one of the most difficult to treat cancers in children. Vaccinia virus is a lytic DNA virus that has several advantages as an oncolytic agent; it can replicate and spread rapidly but is incapable of integrating into human DNA. Its safety has been proven in small pox virus eradication programs. METHODS: The original strain of a mutant, "double deleted" Vaccinia virus (vvDD) with deletions in thymidine kinase and vaccinia growth factor genes for enhanced safety was provided by Dr. John Bell. Three AT/RT cell lines, BT12, BT16 and KCCF1, were cultured with increasing concentrations of vvDD and cell viability was quantified by automated microscopy. In drug combination assays, a panel of 129 small-molecule targeted agents were evaluated. GFP labelled AT/RT cells were inoculated intracranially in CD-1 Nude mice and after allowing time for the tumors to establish, Renilla luciferase and RFP expressing vvDD suspensions were injected intravenously. Tumor growth inhibition and viral replication at the tumor site were quantified by Xenogen imaging techniques. RESULTS: Significant cytotoxicity was seen in all cell lines with IC50 less than 0.1MOI for BT12 and BT16 and less than 0.01 for KCCF1 cells. Ten MOI of vvDD induced complete cell death within 72hr. Among the agents tested, three showed significant synergy with viral cytolysis. These were; BMS-599626(pan-HER Kinase Inhibitor), AZD05030 (Src inhibitor) and RDEA119(Mek inhibitor). Imaging studies showed a clear loss of tumor volume in four of the five mice treated with vvDD with co-localizing viral replication. CONCLUSIONS: Our studies provide evidence for effective antitumor activity of a Vaccinia virus based treatment approach. Drug combination studies provide new knowledge on growth regulatory pathways that intersect with viral activity and can be used to enhance the efficacy of such treatment approaches in the future.