ATRT-16. MODELLING ATRT THROUGH SWI/SNF COMPLEX DEFICIENCY IN GENETICALLY-ENGINEERED MOUSE MODELS

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Atypical Teratoid/Rhabdoid Tumours (ATRT) are highly malignant neoplasms arising primarily in the CNS of children. They are defined by loss of function mutations in *smarcb1*, a gene serving a vital role in neurogenesis and differentiation. In order to recapitulate ATRT in the mouse, we used a Cre-Lox recombination system to conditionally knockout *smarcb1* in specific cell compartments. Loss of *smarcb1* in BLBP-expressing cells of the developing brain led to severe neurologic defects. Mice exhibited seizures, ataxia, and median 12-day survival. Histological analysis revealed severe thinning of the cerebral cortex and cerebellum. Temporally-targeted *smarcb1* loss in BLBP/Nestin-expressing embryonic compartments did not result in tumour formation. Similarly, BLBP-expressing, *smarcb1*-deficient neural stem/progenitor cells (NSC/NPCs) were isolated and allografted but did not form tumours. These cells demonstrated decreased proliferation, higher apoptosis, and upregulation of p53, CDKN1A, and CDKN2A. In contrast, ubiquitous *smarcb1* loss at the earlier embryonic day 6.5 produced widespread tumorigenicity in the forebrain, hindbrain, skullbase, and spine; each with unique phenotypes, survival, and morphology. We employed a clinically-relevant Nanostring gene-panel screen to stratify tumours into genetically distinct subgroups. Our findings indicate that *smarcb1* plays an important role in CNS development. Loss of *smarcb1* in NSC/NPCs is lethal, and its developmental context influences cell fate. Targeted *smarcb1* loss likely plays a tumorigenic role at an earlier developmental stage than previously determined, in a diverse array of primitive stem cells. These data support the generation of a murine ATRT model capable of producing distinct tumour entities that recapitulate the human disease.

ATRT-17. TARGETING GLUTAMINE METABOLISM LOWERS METHYLATION POTENCIALS IN AT/RT AND SYNERGIZE WITH TAZEMETOSTAT

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Atypical teratoid/rhabdoid tumors (AT/RT) have a single recurring genetic mutation in *SMARCB1*. This deletion leads to an abnormal SWI/SNF chromatin remodeling complex and the constitutive activation of *EZH2*. S-adenosyl-L-methionine (SAM) donates a methyl group to EZH2 which then methylates DNA and histones leading to the abnormal gene expression responsible for AT/RT's aggressive phenotype. We have previously shown that glutamine metabolic inhibition with 6-diazo-5-oxo-L-norleucine (DON) confers a survival advantage in AT/RT. In this study, we identified with ultra-high performance liquid chromatography mass spectrometry that DON treatment lowered the methylation potential in AT/RT (Decreased SAM:SAH ratio, t-test in 5 AT/RT human-derived cell models comparing DON treatment to DMSO control, $p<0.05$). AT/RT cell lines grown in glutamine deplete media compared to normal growth conditions also had a reduced methylation potential (decreased SAM:SAH, t-test, $p<0.05$). DON treatment over 5 days decreased histone methylation (as determined by western blot for H3K27me3). Tazemetostat is a small molecule inhibitor that blocks the SAM methyl donor site on EZH2. We find that DON combines synergistically with Tazemetostat to slow AT/RT cell growth (MTS assay, $p<0.01$ t-test; MUSE viability assay, $p<0.01$ ANOVA) and enhances cytotoxicity (MUSE Annexin-V, $p<0.01$ by ANOVA). Synergies were especially pronounced at low concentrations of Tazemetostat which is significant given that Tazemetostat's efficacy in AT/RT has been limited by poor CNS penetration. These studies identify a novel treatment strategy that has potential to improve survival in AT/RT.

ATRT-18. SHH-SUBTYPE ATYPICAL TERATOID/RHABDOID TUMORS ARE SELECTIVELY SENSITIVE TO GEMCITABINE TREATMENT

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Atypical Teratoid Rhabdoid Tumors (ATRT) are highly malignant embryonal tumors of the central nervous system with a dismal prognosis. ATRT can be divided into three molecular subgroups of which the Sonic Hedgehog (SHH) subgroup is most prevalent. In this study, we developed and validated a novel patient-derived ATRT model, which we used along a panel of other primary ATRT models for large scale drug discovery assays. We found that ATRTs are selectively sensitive to the nucleoside analogue gemcitabine, with SHH-subtype ATRTs being the most sensitive subgroup. Gene expression profiles and protein analysis indicated that gemcitabine treatment causes degradation of Sirtuin 1 (SIRT1), which causes ATRT specific cell-death through NF-kB and p53 activation. Furthermore, we found that this gemcitabine induced loss of SIRT1 results in a nucleus-to-cytoplasm shift of the SHH signaling activator Gli, explaining the additional gemcitabine sensitivity in SHH-subtype ATRT. Treatment of SHH-subgroup ATRT xenograft-bearing mice resulted in a >40% increase in median survival ($p<0.01$, log-rank test) and long-term survivors in two independent models. To prepare translation of our findings to the clinic, we investigated potential gemcitabine induced resistance mechanisms by conducting kinome-wide CRISPR/Cas9 knockout screens in primary ATRT cells. Through these experiments we found that low-dose gemcitabine treatment combined with inhibition of protein kinase C zeta (PKCζ) prevents regrowth of resistant ATRT subclones. Together, these findings show that ATRT are highly sensitive to gemcitabine treatment; and as such we suggest that gemcitabine may be rapidly incorporated into future treatment regimens for SHH-ATRT.

ATRT-19. EPIGENETIC REPROGRAMMING LEADS TO INNATE IMMUNE PATHWAY ACTIVATION IN AT/RT

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BACKGROUND: Atypical teratoid/rhabdoid tumors (AT/RT) are highly aggressive brain tumors affecting early childhood and are characterized by bi-allelic inactivation of the *SMARCB1* gene. Though patients benefit from multimodal therapy, there is no improvement in overall survival necessitating exploration of alternative approaches including innate-based immune therapy and epigenetic therapy, which have shown promise in treating adult brain tumors and other cancers. Though reconstitution of SMARCB1 in *SMARCB1*-deficient cells leads to activation of interferon-stimulated genes, the role of innate immune signaling has not been investigated in AT/RTs. METHODS: Our data from a panel of AT/RT cell lines indicates loss of expression of key innate signaling components, like RIG-I, MDA-5, cGAS and STING that are required for sensing extracellular dsRNA and dsDNA. These cell lines also do not respond to dsDNA-based or dsRNA-based innate agonists. However, co-treatment of the BT-16 cell line with two epigenetic drugs, panobinostat and 5-azacytidine leads to re-expression of STING and RIG-I. Panobinostat/5-azacytidine co-treatment followed by either genomic DNA (dsDNA agonist) or poly(I:C) (dsRNA agonist) treatment results in induction of innate responses, measured by STAT1 phosphorylation and production of ISG-15 and IFIT-1. CONCLUSION: Our data suggests that AT/RT cell lines are unresponsive to innate agonists possibly due to the loss of expression of key innate immune components. However, these pathways can be reactivated by epigenetic drugs and further potentiated by dsDNA/dsRNA-based innate agonists. Combined epigenetic reprogramming and innate pathway stimulation may serve as a potential therapy option for treating AT/RT.

ATRT-20. CDK7 INHIBITION IN AT/RT

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Atypical teratoid/rhabdoid tumors (AT/RT) are characterized by loss-of-function mutations in the SMARCB1 component (and less commonly SMARCA4) of the SWI/SNF chromatin-remodeling complex. AT/RT demonstrate an overall silent genomic landscape with epigenetic dysregulation of the genome. CDK7 is a key transcriptional regulator that preferentially phosphorylates the Ser5 and Ser7 positions on RNA Polymerase C terminal domain and is involved early in transcription. In tumor cells, CDK7 is enriched at super enhancers which preferentially regulate genes involved in cell transformation, and expressed at significantly higher levels in transformed tissues than the surrounding normal brain. Our preliminary data shows that CDK7 is expressed in a number of AT/RT tumor cell lines and patient-derived tumor cultures, and that loss of CDK7 function though exposure to the novel CDK7 inhibitor THZ2 results in lack of proliferation at lower doses, and caspase-mediated apoptosis at higher concentrations. shRNA-based inhibition confirms that this effect is due specifically to loss of CDK7. RNA sequencing of cells treated with lower doses of THZ2 show significant alterations in transcript expression consistent with altered balance between antagonistic SWI/SNF and PRC2 chromatin-modeling complex activities, as

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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