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BOOK OF ABSTRACTS

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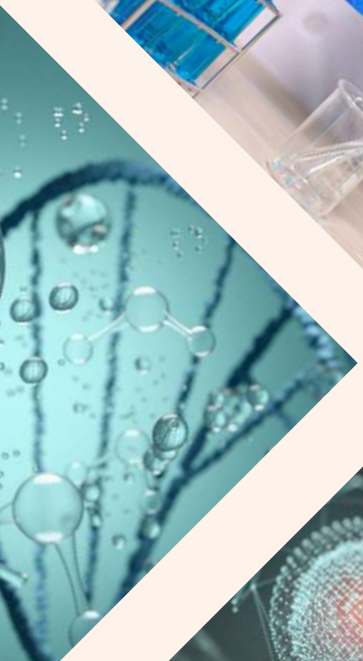

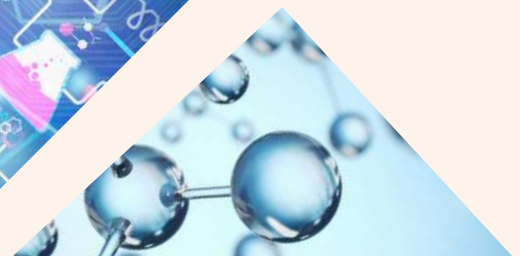

“EPTRI Paediatric Biomarkers & Biosamples TRP”

SPEAKER SECTION

SPEAKER: GIOVANNI CAZZANIGA

Genetic Characterization at Diagnosis of Patients with Acute Lymphoblastic Leukemia Enrolled in Italy in the AIEOP-BFM ALL 2017 Protocol.

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The genetic characterization of pediatric patients with acute lymphoblastic leukemia (ALL) enrolled in AIEOP protocols is conducted in Monza, and it includes a combined analysis of fusion transcripts (whole transcriptome, RNA-seq), identification of gene copy number alterations (digital MLPA), and minimal residual disease (MRD) assessment through IG/TR rearrangement monitoring (NGS and RQ-PCR).

Transcriptomic analysis using NGS (RNA-seq) has led to significant advancements in the genetic characterization of acute lymphoblastic leukemia (ALL). Children newly diagnosed with ALL and enrolled in Italy in the AIEOP-BFM ALL2017 protocol are prospectively analyzed by RNA-seq, with the primary goal of identifying fusion genes.

During the first 28 months of enrollment, transcriptomic analysis was evaluable in 599 out of 613 consecutive B-cell precursor ALL (BCP-ALL) cases (97.7%). At least one fusion gene associated with BCP-ALL was identified in 49.6% (297/599) of cases and validated by RT-PCR or FISH. Among these fusion genes, 116 (39% of identified fusions and 19.4% of the total cases) would not have been detected using conventional multiplex RT-PCR screening performed in parallel. Seven patients presented tyrosine kinase-involving fusions associated with the BCR::ABL1-like subgroup, classified as ABL-class (1.2%), making them eligible for specific therapy and enrollment in the EsPhALL2017/COGAALL1631 protocol. The PAX5 gene was identified as a fusion partner in 23 patients (3.8%), while CRLF2 rearrangement was observed in 23 patients (3.8%). Fifteen patients tested positive for the IGH::DUX4 fusion (2.7%), and eight patients showed other IGH rearrangements (1.3%) with different gene partners, including CEBP (n=4), MYC (n=2), BCL2 (n=1), and EPOR (n=1). Thirteen patients had rearrangements involving ZNF384 (2.2%), and eight involved MEF2D (1.3%). Fusion genes involving ETV6 (n=5, 0.8%), RUNX1 (n=3, 0.5%), and KMT2A (n=3, 0.5%) with non-canonical partners were also identified. Seven patients (1.2%) presented fusion genes classified as "others."

Regarding T-cell precursor ALL (T-ALL), during the first 28 months of enrollment, transcriptomic analysis was evaluable in 137 out of 144 consecutive cases (95.1%). At least one fusion gene associated with T-ALL was identified in 46/137 (33.5%) cases and validated by RT-PCR. Eleven patients carried the STIL::TAL1 fusion gene (8.0%), while nine were classified as KMT2A-rearranged (KMT2Ar, 6.5%), eight as MLLT10-class (5.8%), eight as ABL-class (5.8%), three as ETV6-class (2.1%),

and two as CDK6-class. Additionally, two patients carried the NPM1::ALK fusion, one SET::NUP214, one HOXA10::TRB, and one STMN1::SPI1. Among the KMT2Ar patients, two carried KMT2A::MLLT1, two KMT2A::MLLT4, two KMT2A::ELL, two KMT2A::AFDN, and one KMT2A::CBL. Among MLLT10-class patients, seven had PICALM::MLLT10 rearrangements and one DDX3X::MLLT10. Among ABL-class patients, five carried NUP214::ABL1, one ETV6::ABL1, one TNRCSB::ABL1, and one SEPT9::ABL1. Among ETV6-class patients, two had ETV6::NCOA2 and one ETV6::BCL2L14, while the CDK6-class patients carried CDK6::EVX1 (one case) and CDK6::HOXA11 (one case).

The incidence of fusion genes in the T-ALL cohort was surprisingly high (33.5%), enabling the identification of potential targets for precision therapies, such as tyrosine kinase inhibitors (TKIs) in ABL-class patients, currently being evaluated in an international study.

In the near future, correlation analyses between identified fusions, clinical characteristics at diagnosis, and treatment response will be necessary.

SPEAKER: SHAMIMA RAHMAN

Advancing Mitochondrial Medicine: An Integrative Genomics Approach to Gene Discovery and Therapeutic Innovation

Primary mitochondrial disease are a group of more than 400 monogenic disorders characterised by mitochondrial dysfunction presenting with a bewildering array of clinical features, ranging from brain malformations and congenital lactic acidosis to late onset progressive external ophthalmoplegia, myopathy, parkinsonism and cognitive decline. These disorders share difficulties in clinical recognition and establishing a definitive genetic diagnosis, and intractability to therapeutic interventions. In this talk, I will review my group's work in identifying and curating mitochondrial disease genes, developing computational resources to aid diagnosis, and exploratory work in gene therapy. I will also highlight the importance of global collaboration to achieve optimal outcomes for this group of rare, frequently multisystem and often devastating disorders.

POSTER SECTION

Preliminary Results of Humoral Response Targeting Specific Epitopes of Human Endogenous Retroviruses in Kawasaki Disease and MIS-C.

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Background: Human endogenous retroviruses (HERVs) are relics of ancestral germline infections by exogenous retroviruses, resulting in proviruses transmitted to offspring and integrated in the DNA. HERVs trigger the expression of inflammatory effectors, like cytokines and inflammatory effectors could, in turn, increase HERVs activation. Aberrant expression of two different families of HERVs (i.e. HERV-W and HERV-K) in blood of KD and MIS -C patients vs healthy controls has been demonstrated. The immune response against HERVs in MIS-C and KD have not previously been evaluated.

Objectives: To evaluate the prevalence and magnitude of the immune humoral response against HERV-W and HERV-K epitopes and interferon regulatory factor 5 (IRF5) in patients with KD and MIS-C. To determine associations of clinical features, presentation, laboratory values and coronary involvement (CALs) with humoral response to HERVs and IRF5.

Methods: Study period: October 2020 to June 2021. Population: contemporaneous KD, MIS-C and COVID-19 patients from 2 sites. KD defined by AHA guidelines and MIS-C by CDC criteria. Demographic, laboratory and echocardiograpic data were performed to all KD and MIS-C patients. The reactivity (IgG) against envelope epitopes of HERV-H, HERV-K, HERV-W and IRF5 was tested by indirect ELISA and mesured as Ab optical density (OD) in patients serum blood samples before treatment and compared to healthy controls (HCs). Correlations between clinical and lab data AND Ab against HERVs and IRF5 were investigated. The study was approved by IRB.

Results: 8 KD, 16 MIS-C and 7 COVID-19 (COV) patients and 41 age- and sex-matched healthy controls (HC) were enrolled. Ab anti Hervs W were significantly different in KD vs COVID (p=0.43) and KD vs HCs (p=0.012), Ab antiHervs H were different KD vs HCs (p=0.008), MIS-C vs HCs (p=0.009), Ab anti Hervs K were different in KD vs HCs (p=0.006) , KD vs HCs (p=0.006), MIS-C vs HCs (p=<0.001), COVID vs HCs (p=0.014), MIS-C vs HCs (p=<0.001) and COVID vs HCs (p=0.014); Ab anti IRF-5 were different among grousps as follows: KD vs COVID (p=0.039), KD vs HCs (p=0.014), MIS-C vs HCs (p=<0.001) and MIS-C vs COVID (p=0.012).

Conclusions: In KD and MIS-C the humoral response targeting specific epitopes of HERVs seems to partially contribute to immune response. We found higher humoral response against HERV-W in KD vs COVID and controls, while lower against HERV-K and IRF5 in controls vs KD and MIS-C. Ab against IRF5 are associated with % of lymphocytes, total days of fever and days before treatment. The elevation of IgG response to HERVs and IRF5 might suggest that exposition to these factors causes a secondary antigenic driven immune response in KD. Larger cohorts are needed to further investigate the associations with inflammation to shed a light into the pathogenesis of KD, and to define whether they can represent biomarkers for diagnosis and prognosis.

Metabolomics by liquid chromatography - mass spectrometry for the study of newborn and paediatric diseases.

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Metabolomics refers to the quali-quantitative study of small molecules, i.e. metabolites or hormones, contained in a defined biological sample or system. It is a valuable tool for understanding metabolic changes associated with physiological and pathological conditions, leading to the identification and quantitation of specific biomarkers. Metabolomics also allows the characterization of metabolic alterations induced by treatments, which can provide a more tailored and individualized treatment plan.

Modern liquid chromatography – mass spectrometry (LC-MS) technologies allow for comprehensive metabolomic studies by means of targeted or untargeted approaches. The Center for Applied Biomedical Research of the University of Bologna has more than 15 years' experience in developing and validating LC-MS methods and standard operative procedure for sample collection, storage and processing. Targeted applications are available on the API 4000 Q-Trap (Sciex) triple quadrupole platform for the sensitive and accurate quantitation of panels of steroid hormones, amino acids, biogenic amines, tricarboxylic acids, acylcarnitines, sphingomyelins, glycerophospholipids, endocannabinoids, arachidonic acid derivatives, indoles, nucleotides, endocrine disruptors and antiepileptic drugs in several biological fluids such as serum, plasma, saliva and dried blood spots (DBS). Untargeted metabolomics is available on the Orbitrap Exploris 240 high resolution platform (Thermo Scientific). Methods for expanded metabolite profiling in plasma, serum and urine were developed, operating in polarity switching and data-dependent acquisition mode for achieving larger possible information. A workstation for raw data processing is also available, equipped with Compound Discoverer 3.3 software, referencing to m/z Cloud and ChemSpider spectral libraries for metabolite identification. The untargeted workflow enables the accurate identification and relative quantification of hundreds of metabolites from biofluids, both of human and microbiome origin as well as exogenous chemicals from environmental exposure. All methods are provided with optimized and validated sample extraction procedures including protein precipitation, liquid-liquid extraction, on-line and off-line solid phase extraction. Our LC-MS methods overall demonstrate high throughput and robustness, making them suitable for large-scale clinical metabolomic studies.

First applications of our LC-MS metabolomic facility will focus on the host-microbiome interactions in the frame of bronchiolitis, complications of premature birth, hematologic diseases of childhood and stem cell transplantation, as well as in the longitudinal evolution of endocrinological diseases from paediatric to adult age, including obesity, type 1 and 2 diabetes, adrenal diseases and deficits of steroidogenesis, Turner syndrome and others. Biobanks for these purposes are currently under development. Most of the mentioned studies will benefit from the possibility to use micro-sampling, such as DBS, or non-invasive fluids, such as saliva or urine, which are particularly suited for paediatric populations. Methods dedicated to faecal matter analyses are also under development.

In conclusion, we created a workflow encompassing LC-MS analytics, microbiome characterization, and specialistic clinical experience, allowing to design and conduct studies purposely addressing the study of metabolic derangement caused by several diseases and the influence of microbiome in their evolution.

High-throughput drug screening as drug repurposing strategy for poor outcome BCP-ALL subgroups.

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Background: 15% of pediatric ALL patients are unresponsive to conventional chemotherapy and relapse, raising the need for novel therapeutic schemes. Preclinical high-through put (HTP) drug screening enables monitoring of personalized responses to a collection of clinically approved and novel agents. This approach effectively suggests drug repurposing opportunities concerning single and drug combinations both specific to patient subgroups and across multiple groups.

Aim: Purpose of this study is to apply HTP drug screening to identify effective drugs (as a single agent or in combination) for three subgroups of poor prognosis pediatric BCP-ALL: CRLF2r Down Syndrome (DS), PAX5r, KMT2Ar.

Methods: Primary cells from 34 BCP-ALL patients of three distinct ALL subgroups (9 CRLF2r DS, 15 PAX5r, 10 KMT2Ar) expanded as Patient-derived Xenografts (PDX), leukemic cell lines and healthy controls were seeded in plates pre-coated with a library of 174 compounds under a 6-point concentration range (8nM-25uM) and CellTiter-Glo assay evaluated viability upon a 3-day culture. Additionally, the CRLF2-r BCP-ALL cell line MHH-CALL-4 was pre-treated for 6 hours with vehicle or Givinostat, an HDAC inhibitor with proven high efficacy against CRLF2r ALL to dissect compounds standing as synergistic partner for combination targeting in CRLF2r cases with or without DS. In either case, a quantitative drug sensitivity score (DSS) for each drug was computed and selected efficient compounds statistically identified by Mann Whitney U-test were further interrogated for their apoptotic potential using Annexin/7AAD cytofluorometric approach.

Results: With this approach, we were able to identify 9 compounds with a statistically significant profound anti-leukemic action for all ALL subgroups tested (DSS>50, p-value<0.05), accompanied by a minimum effect on healthy cells (DSS<10). These consist of the Bcl-2 inhibitor ABT-199 (Venetoclax), the HSP90 inhibitors AUY922 (Luminespib), EC144, PF-04929113, NVP-HSP990, the BET bromodomain inhibitor JQ1, the microtubule polymer stabilizer Paclitaxel, as well as two agents of the classical chemotherapy for BCP-ALL, the glucocorticoid Dexamethasone and the antimitotic Vincristine. ABT-199 (Venetoclax) was revealed as the most promising among them, not affecting healthy hematopoietic stem cells and already approved for clinical use for other haematological settings. Further in vitro validation in our ALL samples confirmed its potency in nanomolar concentrations. Interestingly, we observed an NF-kB inhibitor to selectively target DS-ALL cases irrespective of additional leukemia characteristics (mean rank difference 13.26, p-value<0.0001). In the combination setting, we managed to couple Givinostat, our previously established compound active for CRLF2r ALL cases, with Trametinib (ZIP synergy 7.04 and 16.83 for MUTZ-5 and MHH-CALL-4 respectively) or Venetoclax (ZIP synergy 9.23 and 5.03), thus providing a successful synergistic targeting further confirmed in CRLF2r ALL blasts, whose synergistic mechanism of action is currently investigated.

Genetic subgroup-specific candidate drugs were further explored based on stringent efficacy and toxicity cutoffs, and selected for further validation in vitro and in PDX in vivo models. Whilst two multi-target receptor tyrosine kinases (RTKs) inhibitors (Dovitinib and Foretinib) were identified as novel therapeutic candidates for KMT2Ar ALL, gilteritinib, a second-generation FLT3 inhibitor, demonstrated promising efficacy and toxicity profile in PAX5r PDX blasts.

Conclusion: This study has highlighted the emerging benefit of HTP drug screening applications guiding the early design of therapies for multiple or specific patient subgroups in an approach of repurposing drugs available in the pharmacological landscape. Further studies are needed to confirm drugs efficacy in high risk ALL subgroup, when combined to standard chemotherapy.

Urinary miRNA signatures as potential biomarkers of patent ductus arteriosus and therapeutic response to ibuprofen in preterm infants.

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BACKGROUND: Patent ductus arteriosus (PDA) is one of the most common complications in preterm infants (1). The decision to treat PDA is usually based on the echocardiographic diagnosis of a hemodynamically significant PDA (hsPDA) that is associated with higher rates of mortality and multiorgan comorbidities. Ibuprofen is the first line therapy; however, it is ineffective in approximately 30% of patients (2) and may cause side effects. Biomarkers are increasingly recognized as key tools in the identification of patient sub-populations most likely to benefit from treatment, and the development of well-characterized, validated biomarkers for neonatal care has been recently advocated (3). Unfortunately, to date, there are no biomarkers useful to identify infants at high risk of developing hsPDA and/or not responders to pharmacological treatment. In recent years, microRNA (miRNAs) profiling was applied to several pediatric conditions (4) to investigate potential biomarkers of disease, for early diagnosis and/or therapeutic management. However, their potential as predictive tools for the pharmacological management of PDA is still an unexplored path.

AIM: The aim of this study is to assess potential associations between urinary miRNAs signatures and the risk of developing hsPDA and it is refractory to pharmacological treatment in preterm infants.

METHODS: 50 infants with 23+0-29+0 weeks of gestational age or with a birth weight less than 1500 g will be consecutively enrolled at the Neonatal Intensive Care Unit of Careggi Hospital of Florence. Urine samples will be collected on the first 24 h of life and after 3 days of ibuprofen/paracetamol treatment, for the measurement of the entire miRNome. Echocardiography for hsPDA diagnosis will be performed between 24 and 72 h of life to diagnose hsPDA and its response to the first cycle of ibuprofen (10-5-5 mg/kg/day). The entire miRnome will be analysed by the Agilent SurePrint human miRNA microarray.

PRELIMINARY RESULTS: An exploratory miRNome analysis has been conducted in pooled samples from 3 neonates without PDA, 3 neonates whose PDA successfully closed after ibuprofen and 3 neonates whose hsPDA failed to close. Real time validation showed that mir-6089 and miR-137 expression increased upon physiological closure in controls and in neonates who were successfully treated with ibuprofen but not in those who did not achieve pharmacological PDA closure.

CONCLUSIONS: This preliminary analysis suggests that miR-137 and miR-6089 may be potential biomarkers of hsPDA and treatment response that deserve validation in extended cohort of patients. Moreover, functional studies into their role may also disclose new mechanisms underlying PDA development and therapeutic targets.

Vaginal miR-210-3p as a potential biomarker for early fetal growth restriction: a proof-of-concept case-control study.

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Fetal growth restriction (FGR) is a condition in which biometric and functional parameters of the fetus pathologically deviates from the expected growth trajectory, resulting in low birth weight and impaired organ function. FGR globally affects 10–15% of all pregnancies, reaching 30% in low-income countries, and it is associated with increased risk for both acute and long-term multi-organ adverse consequences. Furthermore, this condition is the second leading cause of perinatal morbidity and mortality, behind preterm delivery. Unfortunately, up to 50% of FGR are undetected until the third trimester or at birth and undiagnosed FGR have 2–9 times higher risk of perinatal death and complications to those diagnosed prenatally. The identification of FGR markers could improve the monitoring and surveillance of pregnancies, support in the identification of patients eligible for prophylactic pharmacotherapy and offer mechanistic insights into the pathophysiology and into new pharmacological targets and treatments.

MicroRNAs (miRNAs) play an important role in regulating utero-placental vascular function, placental and fetal development and a series of differentially expressed miRNAs were reported in the placentas from pregnancies complicated by preeclampsia (PE) and FGR. Among those, miR-210 is a well-known hypoxia-inducible miRNA expressed in the villous and extra villous trophoblasts of the placenta and several in vitro evidence pointed out for its prominent role in modulating trophoblasts invasion and migration and mitochondrial activity. miR-210 up-regulation is also crucial for endothelial cell proliferation, migration and angiogenic response to hypoxia. In this proof-of-concept study, we hypothesized that defective placentation might lead to altered miRNA release from gestational tissues into vaginal fluid (VF). In this study we explored the ability of 210-3p measured in VF samples to identify early cases of FGR and its correlations with neonatal outcomes.

Twenty-nine women with pregnancies complicated by early FGR diagnosis and 25 controls matched for gestational age were enrolled and their VF and plasma were collected. MiR-210-3p was measured by RT-qPCR and their targets were identified by in-silico analysis.

VF miR-210-3p levels were significantly lower in early FGR cases compared to controls ($p<0.05$), particularly in more severe cases and in women who later developed preeclampsia ($p<0.05$). Furthermore, VF miR-210-3p levels were correlated with lower birth weight, preterm birth and severe birth complications ($p<0.05$). miR-210-3p was not detectable in plasma samples.

In silico analyses identified HIF-1α, HIF-3α, BDNF, IGFBP3, RAD52 and TWIST-1 as experimentally validated targets of miR-210-3p. Among the predicted biological pathways controlled by miR-210-3p, we found hypoxia-responsive signaling such as autophagy, oxidative stress and metabolic pathways.

Although validation is needed, these results suggest that measuring miR-210-3p levels in vaginal fluid can identify early FGR; future mechanistic studies will investigate whether pharmacological strategies based on miR-210-3p or its targets may be useful for controlling FGR.

Rare premature aging diseases in children: how to face diagnostic challenges through specific biomarkers.

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Premature aging syndromes are congenital or ealy onset diseases featuring one ore more aging phenotypes. Among these pathologies, Werner syndrome, Bloom syndrome and Rothmund-Thomson syndrome, but play also a role in Ataxia-telangiectasia, Cockayne syndrome, Nijmegen breakage syndrome, Seckel syndrome and xeroderma pigmentosum, are associated with mutations in DNA repair enzymes, while Hutchinson-Gilford Progeria, atypical Werner syndrome and Mandibuloacral dysplasia are due to mutations in genes of the nuclear envelope. Although genetic screening allows precise diagnosis of those diseases, the diagnostic path is often complicated by lack of biomarkers allowing a pre-screening. We are proposing molecular markers of disease for some of the above-mentioned premature aging syndromes, namely nuclear envelope linked progeroid forms and xeroderma pigmentosum.

Epigenetic Rerogramming by Decitabine in Retinoblastoma.

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Background: Retinoblastoma (Rb) is a rare cancer, yet it is the most common eye tumor in children. It can occur as either a familial or sporadic form, with the sporadic variant being more prevalent, though its downstream effects on epigenetic markers remain largely unclear. Treatment for retinoblastoma typically involves aggressive chemotherapy and surgical resection. The identification of specific epigenetic characteristics of non-hereditary (sporadic) Rb has led to the development of advanced, high-throughput methods to explore its epigenetic profile. Our previous research showed that treatment with the demethylating agent 5-Aza-2'-deoxycytidine (decitabine; DAC) induced cell cycle arrest and apoptosis in retinoblastoma in a well characterized Retinoblastoma model (WERI-Rb1). Our analysis of time-dependent gene expression in WERI-Rb1 cells following DAC exposure led to the development of testable hypotheses for further investigating the epigenetic impact on the initiation and progression of retinoblastoma tumors.

Methods: Gene expression analysis of publicly available patients' primary tumors and normal retina datasets have been compared with those found in WERI-Rb-1 cells to assess the relevancy of DAC-driven genes as markers of primary retinoblastoma tumors. The effect of DAC treatment has been evaluated in vivo, both in subcutaneous xenografts, and in orthotopic models. qPCR analysis of gene expression, and Methylation-Specific PCR (MSP) has been performed.

Results: Our network maps' analysis of differentially expressed genes in primary tumors compared with DAC-driven genes identified 15 hub/driver genes that could have a pivotal role in the genesis and progression of retinoblastoma. DAC treatment induced significant tumor growth arrest in vivo in both subcutaneous and orthotopic xenograft retinoblastoma models. This was associated with gene expression changes either by direct switching-on of epigenetically locked genes, or by an indirect regulation of linked genes, suggesting the possible use of DAC as an epigenetic anti-cancer drug for the treatment of retinoblastoma patients.

Conclusion: There is a pressing need to develop innovative treatments for retinoblastoma. Our research revealed that DAC can effectively suppress the growth and progression of retinoblastoma in in vivo models, offering a potential new therapeutic approach to battle this destructive disease. This discovery highlights the impact of this epigenetic modifying therapy in reprogramming tumor dynamics, and thus its potential to preserve both the vision, and lives, of affected children.