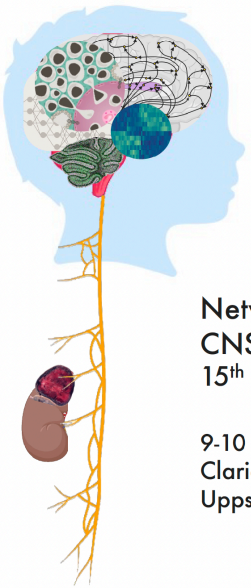

NBCNS 2026UPPSALA

Network for Neuroblastoma and CNS Tumor Research in Children
15th Annual Meeting

9-10 November, 2026
Clarion Hotel Gillet
Uppsala, Sweden

BARNCANCER FONDEN

Conference Programme

Monday November 9

10.30 – 12.30	Registration
11.30 – 12.30	Lunch
12.30 – 12.40	**Welcome note;** Fredrik Swartling, Uppsala University and Kleopatra Georgantzi, Karolinska Institutet

Session 1:
Chairs: Klas Blomgren, Karolinska Institutet and Per Nyman, Linköping University.

12.40 – 13.25	**The Future of Medulloblastoma Care: Increasing Survival and Decreasing Toxicity** Keynote speaker 1: Giles Robinson St. Jude Children's Research Hospital, Memphis
13.25 – 13.45	**Parent/family perspective** Karin Mellström
13.45 – 14.05	**Patient perspective** Elsa Mellström
14.05 – 14.25	**Coffee**

Session 2:
Chairs: Verónica Rendo, Uppsala University and Fredrik Swartling, Uppsala University

14.25 – 15.10	**Decoding the Epigenome: Uncovering Vulnerabilities & Advancing Early Detection in Pediatric Brain Tumors** Keynote speaker 2: Paul Northcott, St. Jude Children's Research Hospital, Memphis
15.10 – 15.25	**Spatio-temporal organization of C19MC in Embryonal Tumors with Multilayered Rosettes** Sander Lambo, Karolinska Institutet
15.25 – 15.40	**Using non-coding constraint mutations to find novel candidate genes in medulloblastoma: The story of FGR** Camille Cabrolier, Uppsala University

Poster sessions

15.40 – 16.10	Poster set up and check-in

16.10 – 16.30	Rapid fire oral poster presentations
16.30 – 17.20	Poster viewing part 1 (presenters at posters with odd numbers at place)
17.20 – 18.10	Poster viewing part 2 (presenters at posters with even numbers at place)
18.10 – 19.00	Poster viewing part 3 with open discussions
19.00– 19.15	Transport/walk to dinner
19:15	Dinner at Katalin Disco, networking, & bar (at your own expense) open to 24:00

Tuesday, November 10

7.00 – 8.30 Breakfast

Session 3: Novel treatment strategies

Chairs: Torben Ek, Gothenburg University and Kleopatra Georgantzi, Karolinska Institutet (Giles Robinson CNS clinical session, Sally George neuroblastoma clinical session)

8.30 – 10.00	**Clinical case studies (parallel sessions)** A. Neuroblastoma B: Pediatric CNS tumors
10.00 – 10.30	**Coffee and checkout**

Session 4:

Chairs: Malin Wickström, Karolinska Institutet and Ruth Palmer, Gothenburg University

10.30 – 11.15	**Neuroblastoma immune evasion in the metastatic niche - from mechanisms to new therapeutic concepts** Keynote speaker 3: Sabine Taschner-Mandl, St. Anna Children's Cancer Research Institute, Vienna
11.15 – 11.30	**Longitudinal profiling of neuroblastoma reveals tumor and microenvironmental adaptation to chemotherapy and relapse** Erick Muciño Olmos, Lund University

11.30 – 11.45	**Improving neuroblastoma differentiation therapy using combinatorial single-cell CRISPR screens** Myra Almén, Karolinska Institutet
11.45 – 12.00	**CAR T-induced vascular alterations in diffuse intrinsic pontine glioma (DIPG)** The Swedish Childhood Cancer Fund young researcher of the year 2025. Lars Karlsson, Gothenburg University
12.00 - 13.00	**Lunch**

Session 5: Emerging targets
Chairs: Daniel Bexell, Lund University and Torben Ek, Gothenburg University

13.00 – 13.45	**Precision medicine for relapsed and refractory cancers of childhood** Keynote speaker 4: Sally George, The Institute of Cancer Research, London
13.45 – 14.00	**O-mannosylation and protein maturation checkpoints represent therapeutic opportunities in BRAF fusion protein oncogenesis** Anna Borgenvik, Uppsala University
14.00 – 14.10	**Strengthening Pediatric Cancer Research Through Patient and Family Involvement** Monika Ehnman, research coordinator, The Swedish Childhood Cancer Fund

Session 6:
Chairs: Johan Holmberg, Umeå University and Helena Carén, Gothenburg University

14.10 – 14.55	**Understanding the Biology of H3K27M to Find New Ways to Treat Childhood Brain Tumours** **Keynote speaker 5:** Adrian Bracken, Trinity College, Dublin
14.55 – 15.10	**Prizes & concluding remarks** Fredrik Swartling, Kleopatra Georgantzi and Johan Holmberg
15.10	**Coffee and departure**

Invited International Speakers

Giles Robinson, St. Judes Children´s Research Hospital, Memphis

Dr. Robinson's lab is focused on improved survival and reduced morbidity of children with medullosblastoma and other central nervous system tumors using genomic analysis.

Paul Northcott, St. Judes Children´s Research Hospital, Memphis

Dr. Northcott's lab studies genetics and preclinical models for treatment development, and uses translational genomics for improving outcomes of children with malignant brain tumors.

Sabine Taschner-Mandl, St. Anna Children's Cancer Research Institute, Vienna

Dr. Tascher-Mandl's lab tackles unresolved questions of neuroblastoma pathogenesis and develops new diagnostic and therapeutic approaches to facilitate precision medicine for children with malignant tumors.

Sally George, The Institute of Cancer Research, London

Dr George is a Paediatric Oncologist and a CRUK Clinician Scientist with an interest in paediatric solid tumours, specifically the common, poor outcome childhood tumour, neuroblastoma.

Adrian Bracken, Trinity College, Dublin

Dr. Bracken's lab is focused on understanding the molecular mechanisms of how Polycomb group proteins regulate cell-fate decisions and cancer.

1. Retrospective study of low-grade gliomas and evaluation of NGS methods for fusion gene detection in formalin-fixed tumors

Authors:

Elinor Einarsson[2], **Lily Deland**[1], Eddie Vuong[2], Tim Eckerström[2], Bart Edelbroek[2,3], Carolina Barros[2,3], Magnus Sabel[4,5], Thomas Olsson Bontell[6,7], Frida Abel[1,2]

Affiliations:

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Abstract:

Pediatric low-grade glioma (pLGG) is the largest CNS tumor group in children, with 90-95% harboring genetic alterations that activate the MAPK pathway. About 80% are driven by fusion genes, 10-15% by point mutations, and 5-15% by unknown drivers. Most clinics use FFPE tissue for tumor analysis and diagnostics. Identifying genetic drivers is key for diagnosis and targeted therapy.

This study retrospectively examined 89 low-grade glioma tumors from patients diagnosed between 1996 and 2003 at Sahlgrenska University Hospital, Gothenburg. It included 80 pLGG patients (ages 0-19) and 9 adult pilocytic astrocytomas (ages 20-51). The goal was to evaluate two commercial NGS assays, the Archer FusionPlex Pan Solid Tumor or Oncology Research kit, and the Oncomine Childhood Cancer Research Assay (OCCRA), for detecting molecular drivers in FFPE material. Results were compared with standard diagnostic methods: BRAF rearrangements by FISH and classification by methylation array.

Of 89 cases, 56 were analyzed by FP; 39 were also analyzed by OCCRA, with 33 showing BRAF rearrangements by FISH. FP identified 20 KIAA1549::BRAF, 2 BRAF p.V600E, 3 FGFR1::TACC1, 1 FGFR1 p.K656E, and 6 non-BRAF fusions; WASF2::NTRK2, KIF21B::NTRK1, RAF1::MBNL1, TPR::ROS1, FGFR2::OPTN, and FGFR2::CTNNA3. OCCRA detected 9 KIAA1549::BRAF, 2 BRAF mutations, and 2 non-BRAF mutations, adding 3 cases. An oncogenic driver was found in 52 of 56 cases using FP, OCCRA, and BRAF FISH.

Conclusion: Archer FusionPlex detects common and novel fusion events in FFPE tumor tissue but may miss lowly expressed KIAA1549::BRAF fusions. Recognizing MAPK driver variants improves diagnostics and guides targeted therapy.

2. Spliceosome inhibition exposes vulnerabilities in MYCN-amplified neuroblastoma

Authors: **Antoniou Eirini**[1], Caroline Lindehell[1], Subhamita Maitra[1], Leonie Maria Halbe[1], Olof Jönsson[1], Laura Elaine Teerenstra[1], Emma Fredin[1], Yao Shi[2], Juan Yuan[2], Johan Holmberg[1]

Affiliations: 1. Department of Molecular Biology, Umeå University. 2. CMB, Karolinska Institutet

Abstract:

Neuroblastoma is the most common extracranial solid childhood cancer, with a high degree of clinical heterogeneity. Dysregulation of RNA splicing, an essential process in gene regulation, contributes to tumorigenesis, and mis-splicing events have been reported in many human cancers. We aimed to address the functional role of aberrant splicing in high-risk neuroblastoma and to investigate how spliceosome inhibition affects isoform expression and tumor properties in neuroblastoma. We used Nanopore long-read sequencing to detect global and transcript-specific effects of spliceosome inhibition in MYCN-amplified LAN-1 neuroblastoma xenograft tumors. In parallel, we performed acute in vitro spliceosome inhibition experiments using LAN-1 neuroblastoma cells and normal human astrocytes (NHA). To enable integrative analysis across our different experimental models, we developed an interactive data exploration and analysis atlas. We identified widespread downregulation of cell cycle genes and increased expression of novel isoforms with higher exon inclusion. Among these, AURKA and CHK1 emerged as candidate genes showing significant isoform switching. Disrupting splicing of these genes in neuroblastoma may affect MYCN stability and DNA repair mechanisms, leading to replication stress and impaired tumor growth. With this approach, we further want to investigate the functional consequences of isoform switching in genes involved in critical cancer-related cellular processes, highlighting novel therapeutic opportunities.

3. Nanopore-based genome-wide detection of allele-specific DNA methylation differences in paediatric medulloblastoma

Authers:

Philip Ekberg[1], Helena Carén[1]

Affiliations:

Sahlgrenska Center for Cancer Research, Department of Laboratory Medicine, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden.

Abstract:

Although methylation arrays support medulloblastoma diagnosis, their unphased bulk signal can mask allele-specific methylation (ASM) differences with possible importance to tumor biology.

We used long-read whole-genome sequencing to map ASM, compare tumors with neural controls, characterize imprinting changes, and identify cancer-relevant allele-specific differentially methylated regions (aDMRs).

Seven paediatric medulloblastomas and three neural controls underwent Nanopore whole-genome sequencing. Reads were aligned and phased to generate haplotype-resolved methylation profiles, enabling genome-wide ASM detection, consensus and subgroup analyses, benchmarking against 20 canonical imprinting control regions, and quantification of allele-specific gains and losses of imprinting. Top aDMRs were further evaluated for known cancer relevance.

ASM was widespread in both medulloblastomas and controls. Tumors shared a set of ASM regions not observed in controls. Canonical imprinting was broadly maintained but showed locus-specific gains and losses of imprinting strength rather than uniformed erosion.

At the consensus DMR level tens of thousands of regions showed increased or decreased imprinting strength, most often driven by changes in a single allele. Several candidate DMRs mapped near cancer-relevant pathways.

These findings demonstrate that haplotype-resolved long-read sequencing can be used to profile ASM and imprinting in paediatric medulloblastoma. Tumors appear to retain a developmental ASM-backbone while acquiring additional tumor-associated aDMRs, with ASM locally redistributed rather than globally lost. The allele-level patterns and gene-level overlaps provide proof-of-concept and nominate candidate regions for targeted follow-up in larger studies.

4. Single-cell transcriptomic profiling reveals systemic immune alterations in pediatric brain tumor patients

Authors:

Elina Välkesalmi[1], Amanda Holmström[1], Markus Talka[2], Satu Långström[3], Mia Westerholm-

Ormio[4], Anu M. Suominen[3], Pia Valle[3], Roosa Sirén[3], Atte Karppinen[5], Päivi Koroknay-Pal[5], Anna

Piippo-Karjalainen[5], Jarno Satopää[5], Nuutti Vartiainen[5], Olli Tynninen[6], Jukka Kanerva[3], Virve

Pentikäinen[1,3], Katja Eloranta[1,7]

Affiliations:

1. Pediatric Research Center, Children's Hospital, Helsinki University Hospital and University of Helsinki, Helsinki, Finland. 2. Division of Genetics and Clinical Pharmacology, Diagnostic Center, Helsinki University Hospital and University of Helsinki, Helsinki, Finland. 3. Division of Hematology-Oncology and Stem Cell Transplantation, Children's Hospital, Helsinki University Hospital and University of Helsinki, Helsinki, Finland. 4. Division of Pediatric Neurology, Children's Hospital, Helsinki University Hospital and University of Helsinki, Helsinki, Finland. 5. Department of Neurosurgery, Helsinki University Hospital and University of Helsinki, Helsinki, Finland. 6. Department of Pathology, Diagnostic Center, Helsinki University Hospital and University of Helsinki, Helsinki, Finland. 7. Institute for Molecular Medicine Finland, Helsinki Institute of Life Science, HiLIFE, University of Helsinki, Helsinki, Finland

Abstract:

Pediatric brain tumors are biologically heterogeneous diseases for which minimally invasive approaches to characterize disease-associated changes and monitor patients are needed. We investigated systemic immune alterations in pediatric brain tumor patients using single-cell RNA sequencing (scRNA-seq) of peripheral blood mononuclear cells (PBMCs), complemented by image-

based phenotypic profiling.

The initial cohort comprised 24 PBMC samples from 9 cancer-free pediatric controls and 10 pediatric brain tumor patients, including patients with diffuse midline gliomas, pediatric-type high-grade gliomas, medulloblastomas, and ependymomas. The cohort also included longitudinal samples collected during or after treatment. scRNA-seq was used to characterize immune cell composition and cell-type-specific transcriptional states. Differential abundance, differential gene expression, and pathway enrichment analyses were performed between patient and control samples. Complementary Blood Cell Painting is being used to assess whether transcriptomic changes are accompanied by alterations in cellular morphology and organelle-related phenotypes.

Analysis of the initial scRNA-seq cohort revealed differences in both immune cell composition and transcriptional profiles between patients and controls. Patient

samples showed reduced proportions ofdendritic cells, $CD14^+$ monocytes, and $CD4^+$ central memory T cells, while activated and XCL1/XCL2-expressing NK cells, effector $CD8^+$ T cells, and cycling T cells tended to be increased. Transcriptional alterations were especially prominent in monocytes, NK cells, and T-cell subsets, with pathway changes related to immune activation, cytotoxicity, oxidative phosphorylation, RNA processing, and protein folding. These findings suggest systemic immune remodeling in pediatric brain tumor patients and support further investigation of PBMC profiling as a minimally invasive approach for disease characterization

and longitudinal monitoring.

5. Embryonic cues shape neuroblastoma cell plasticity

Authors:

Stina Östenberg[1,2,3], Anja Zethraeus[1,2,3], Marina Mazariegos[1,2,3], Sudip Ghosh[3], Jenny Hansson[3] and Sofie Mohlin[1,2,3]

Affiliations:

1. Division of Pediatrics, Department of Clinical Sciences, Lund University, Lund, Sweden. 2. Lund University Cancer Center, Lund University, Lund, Sweden. 3. StemTherapy, Lund Stem Cell Center, Lund University, Lund, Sweden.

Abstract:

Neuroblastoma comprises interconvertible adrenergic (ADRN) and mesenchymal (MES) cell phenotypes that differ in developmental identity and therapy response. Although neuroblastoma arises from the developing sympathetic nervous system of the neural crest, how the embryonic microenvironment influences tumor cell plasticity remains poorly understood. Here, we used a chick embryo model to investigate how neural crest-derived signals regulate neuroblastoma cell identity and

behavior.

Following grafting into the trunk part of the neural tube, both ADRN and MES neuroblastoma cells migrated alongside endogenous neural crest cells. To investigate the underlying signaling interactions, we isolated trunk neural crest cells and generated cell-free conditioned medium to model the embryonic microenvironment. Transcriptomic profiling revealed phenotype-specific responses. ADRN cells showed enrichment of BMP/TGF-β signaling and extracellular matrix (ECM)-associated programs, downregulation of nervous system development programs, and an increased MES signature score, whereas MES cells showed enrichment of TGF-β signaling and proliferation- associated processes. Strikingly, conditioned medium induced loss of adhesion and spheroid formation in ADRN cells. Upon withdrawal of conditioned medium, spheroids returned to adherent growth, demonstrating that this response is reversible and dependent on extracellular cues. Proteomic characterization of the neural crest secretome identified 51 candidate extracellular proteins, highlighting networks associated with ECM organization and growth factor signaling.

Together, our findings demonstrate that the embryonic neural crest microenvironment can dynamically and reversibly reshape neuroblastoma cell phenotype and growth behavior, highlighting developmental extracellular cues as potential regulators of tumor cell plasticity.

6. Proximity labeling identifies a neuronal Alk interactome in the Drosophila CNS

Ezgi Uckun[1], Pengfei Li[1], Jikui Guan[1], Vimala Anthonydhason[1], Kathrin Pfeifer[1] and Ruth Palmer[1]

1Department of Medical Biochemistry and Cell Biology, University of Gothenburg

Anaplastic lymphoma kinase (Alk) is a receptor tyrosine kinase of the insulin receptor family. In addition to its role in cancer, studies have revealed a role for Alk signaling in the central nervous system (CNS) including axon targeting, synapse development, growth and body size regulation, memory formation and learning, circadian rhythm, and longevity. Although the Alk receptor is associated with various processes, downstream components are not well characterized. Our aim was to identify Alk interactome in Drosophila CNS using proximity labeling and characterize components of Alk signaling.

Proximity-dependent biotin identification (BioID) is a powerful method for mapping protein-protein interaction networks. BioID employs mutant forms of BirA protein ligase (BirAR118G, miniTurbo or TurboID) which has lower affinity for reactive biotinoyl-5'-AMP compared to wild-type BirA and thereby prematurely releases reactive biotin resulting in biotinylation of proximal proteins. TurboID is a variant which displays higher catalytic activity enabling efficient biotin labeling in flies. Biotinylated proteins are isolated by pull-down and further identified by LC-MS3.

We described the neuronal "Alk proximitome" by introducing TurboID into the Drosophila Alk locus by CRISPR/Cas9 genome editing. In addition, we revealed the effect of Alk-activation via gain-of-function mutation of the Alk locus (AlkY1355S) in CNS. Here, we confirmed the importance of known Alk signaling components and identified novel interactors of Alk signaling such as Vav guanine nucleotide exchange factor (Vav), C3G guanyl-nucleotide exchange factor (C3G) and the LDL Receptor Related Protein 4 (Lrp4). We further validated the interaction between them by immunoprecipitation as well as genetic interaction assays.

7. FOSL1 degradation enhances ferroptosis sensitivity and suppresses MYCN in neuroblastoma

Authors:

Rodrigo Machado Costa da Cruz[1,2], Tilde Sandvik[1,2], Maria de Santisteban[3], Adriana Mañas Núñez[3], Susanne Fransson[1,2], Ganesh Umapathy[1,2]

Affiliations:

1. Department of Laboratory Medicine, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden. 2. Department of Clinical Genetics and Genomics, Sahlgrenska University Hospital, Region Vastra Gotaland, Gothenburg, Sweden. 3. Spanish National Cancer Research Centre, Madrid, Spain.

Abstract

Aim

We aimed to investigate the role of FOSL1 in adaptive ferroptosis resistance in neuroblastoma (NB) and determine whether FOSL1 degradation enhances the antitumor activity of the GPX4 inhibitor RSL3.

Methods

MYCN-amplified and non-amplified NB cell lines were treated with RSL3, a FOSL1 degrader, or their combination. RSL3-induced transcriptional responses were characterized by RNA sequencing. Cell viability, clonogenic growth, and protein expression were assessed using resazurin assays, colony formation assays, and western blotting, respectively. Drug interactions were evaluated using the Bliss independence model.

Results

RSL3 reduced NB cell viability and clonogenic growth in a cell line-dependent manner. RNA sequencing identified FOSL1 as a prominent RSL3-induced gene in both MYCN-amplified and non-amplified NB cells, suggesting activation of an adaptive stress response following GPX4 inhibition. FOSL1 degradation reduced cell viability and markedly decreased FOSL1, GPX4, and MYCN protein expression. In contrast, MYCN mRNA was not reduced following FOSL1 degradation, suggesting that the effect on MYCN occurs predominantly at the post-transcriptional level. Combining FOSL1 degradation with RSL3 produced synergistic antitumor activity, although the magnitude of synergy varied between cell lines. High FOSL1 expression was associated with significantly poorer overall survival in NB patient cohorts. Furthermore, FOSL1 degradation significantly suppressed tumour growth in the KELLY xenograft model.

Conclusion

Our findings identify FOSL1 as a potential adaptive resistance mechanism to ferroptotic stress in NB and reveal a previously unexplored relationship between FOSL1 and MYCN protein regulation. Targeting FOSL1 enhances sensitivity to

RSL3-induced ferroptosis. These findings provide a rationale for combined FOSL1 degradation and ferroptosis induction as a potential therapeutic strategy for neuroblastoma.

8. Erastin-induced ferroptosis enhances natural killer cell antitumor activity in neuroblastoma

Authors:

Vimala Anthonydhason[1,2], Erna Islamagic[3], Jennie Gaarder[1,2], Tommy Martinsson[1,2], Susanne Fransson[1,2], Fredrik B Thoren[3], **Ganesh Umapathy**[1,2]

Affiliations: 1. Department of Laboratory Medicine, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden. 2. Department of Clinical Genetics and Genomics, Sahlgrenska University Hospital, Region Vastra Gotaland, Gothenburg, Sweden. 3. Tumor Immunology (TIMM) Laboratory at Sahlgrenska Center for Cancer Research, University of Gothenburg, Gothenburg, Sweden

Abstract

Aim

We aimed to investigate whether Erastin-induced ferroptosis enhances natural killer (NK) cell-mediated cytotoxicity in neuroblastoma (NB) and to identify the molecular mechanisms linking ferroptotic stress to increased NK cell recognition.

Methods

MYCN-amplified and non-amplified NB cell lines were treated with the ferroptosis inducer Erastin and subsequently co-cultured with NK cells. Cell viability, ferroptosis, and NK cell-mediated cytotoxicity were assessed using functional assays. Ferroptosis specificity was evaluated using the ferroptosis inhibitor Liproxstatin-1. RNA sequencing was performed following Erastin treatment to characterize transcriptional responses. Expression of stress-response proteins and NK cell-activating ligands was assessed by RT- qPCR, western blotting, and flow cytometry. The clinical relevance of ULBP1 expression was evaluated using NB patient survival datasets.

Results

Erastin induced ferroptotic cell death in NB cells and significantly enhanced their susceptibility to NK cell- mediated cytotoxicity. Inhibition of ferroptosis with Liproxstatin-1 attenuated these effects, supporting a ferroptosis-dependent mechanism. Transcriptomic analysis revealed activation of ferroptosis-, amino acid metabolism-, and cellular stress-associated pathways following Erastin treatment. Importantly, Erastin increased expression of the NK cell-activating ligand ULBP1 through an ATF4-dependent mechanism,

thereby enhancing recognition and cytotoxicity by NK cells. Analysis of NB patient datasets further demonstrated that low ULBP1 expression was associated with poorer overall survival, including in clinically aggressive NB subgroups.

Conclusion

Our findings demonstrate that Erastin-induced ferroptotic stress enhances NK cell-mediated antitumor activity in NB through ATF4-dependent upregulation of

ULBP1. These results reveal a mechanistic link between ferroptosis and NK cell recognition and support ferroptosis induction as a potential strategy to enhance antitumor immunity in NB.

9. The Swedish Childhood Tumor Biobank - A national omics and tissue research resource for pediatric cancers

The Swedish Childhood Tumor Biobank -

A national omics and tissue research resource for pediatric cancers

Authors:

Teresita Díaz de Ståhl[1,2], Gabriela Prochazka[1], Praveen Raj Somarajan[1], Elisa Basmaci[1], Katarzyna Zielinska-Chomej[1], Jenny von Salomé[1], Duohui Cao[1], Arman Ardalan[1], Sandra Wessman [1,2], Christpher Illies[1,2], Gustaf Ljungman[3] and **Johanna Sandgren** [1,2]

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3Department of Women´s and Children's Health, Uppsala University, Uppsala, Sweden

Introduction: In Sweden, around 350 children are diagnosed with cancer annually. With an 85% survival rate, cancer still remains a leading cause of childhood death. Survivors often suffers sequelae due to treatment, underscoring the need for deeper biological insights to improve survival and quality of life. The Swedish Childhood Tumor Biobank (Barntumörbanken, BTB) aims to enhance the understanding of pediatric solid tumors by providing infrastructure resources, biological samples, and molecular/genomic data for research.

Material & Methods: BTB collaborates with the six university hospitals treating pediatric cancer patients nationwide. Fresh frozen tumors, blood samples, CSF, viable tumor cells, digital pathology slides and parental blood are collected. BTB registers, prepares, and stores biobank samples and linked patient information, performing whole genome sequencing, whole transcriptome sequencing, proteomics and methylation array profiling. BTB collaborates with The Science for Life Laboratory for the comprehensive genomic and molecular characterization, and for developing bioinformatic pipelines. Internal variant databases and data portal structures are established for secure data organization, traceability and visualizations.

Results: Over 2800 cases are registered and beyond 66,000 samples collected/prepared. More than 1600 cases have been genomically characterized, with BTB managing and harmonizing the data, including for the Genomic Medicine Sweden Childhood Cancer study. BTB has shared samples and data with more than 20 research projects following formal application processes. Medicolegal and scientific assessment and specific agreements govern sample and data

access. BTB also supports clinical studies with sample logistics, regulatory assistance and data analysis/interpretation.

Conclusions: BTB systematically collects biological specimens, linked patient information and informed consent from over 90% of Swedish pediatric patients with solid tumors, as well as producing and managing high-quality research data. Continuous use of samples and omics data in approved studies will likely improve future clinical care for children with cancer.

10. MYC-Driven Pediatric Brain Tumors Depend on the ATR-CHK1-WEE1 Checkpoint to Tolerate Chemotherapy-Induced Replication Stress

Authors: Anjali Sivakumar[1], Fredrik Swartling[1], Xiaonan Zhang[1], Miao Zhao[1]
Affiliations: 1. Department of Immunology, Genetics and Pathology (IGP), Uppsala University, Uppsala, Sweden.

Abstract:

MYC-amplified pediatric brain tumors, including aggressive subgroups of medulloblastoma and pineoblastoma, are associated with poor outcomes and remain heavily dependent on cytotoxic chemotherapy. However, chemotherapy has limited tumor selectivity and can cause substantial toxicity to the developing brain, underscoring the need for more effective and tumor-selective therapeutic strategies. Whether MYC-driven tumor cells harbor a distinct, therapeutically exploitable vulnerability remains unclear.

We investigated this question using transgenic MYC-driven tumor models and control neural stem cells exposed to a panel of chemotherapeutic agents and DNA damage response (DDR) inhibitors targeting ATR, CHK1/2, WEE1, and PARP. Using a doxycycline-inducible MYC system, we dynamically suppressed and restored MYC expression to assess replication stress, checkpoint activation, and drug sensitivity in vitro. Although MYC-driven tumor cells were responsive to conventional chemotherapy, control neural stem cells were more sensitive, highlighting the limited therapeutic window of cytotoxic treatment and its potential contribution to neurocognitive toxicity. In contrast, MYC-driven tumor cells demonstrated pronounced and selective sensitivity to inhibition of ATR, CHK1/2, and WEE1, whereas PARP inhibition showed substantially less selectivity. Importantly, suppression of MYC reduced sensitivity to ATR, CHK1/2, and WEE1 inhibition, while restoration of MYC re-established this vulnerability, demonstrating a causal dependence on MYC activity.

These findings indicate that MYC-driven pediatric brain tumor cells rely on the ATR–CHK1–WEE1 checkpoint axis to tolerate MYC-associated replication stress. Targeting this dependency may provide a more tumor-selective therapeutic strategy than chemotherapy alone. Studies evaluating the potential for checkpoint inhibition to synergize with chemotherapy are ongoing. Collectively, our results provide a rationale for combining replication-stress checkpoint inhibitors with chemotherapy as a therapeutic approach for MYC-driven pediatric brain tumors.

11. MYCN inhibition rewires fatty acid metabolism and lipid droplet dynamics in neuroblastoma

Lourdes Sainero-Alcolado[1,2], Julia Brown-Casquet[1], Jiansheng Wang[1], Elisa Garde-Lapido[1], Gerard Evan2 and Marie Arsenian-Henriksson[1]

1Department of Microbiology, Tumor and Cell Biology (MTC), Karolinska Institutet, Stockholm, Sweden

2The Francis Crick Institute, 1 Midland Rd, London NW1 1AT, United Kingdom.

Abstract:

Neuroblastoma (NB) is a childhood cancer characterized by therapy resistance and relapse. Although MYCN amplification is an oncogenic driver, the metabolic mechanisms supporting NB progression remain poorly understood. Emerging evidence implicates altered lipid metabolism in NB growth, immune evasion, and therapy resistance by reshaping the tumor microenvironment (TME), yet the role of lipid droplets (LDs) remains unexplored. Here, we investigate how lipid reprogramming, particularly LD biology, drives NB progression, TME remodeling, and therapy response, to identify metabolic vulnerabilities for combination therapies. Proteomic analysis of SK-N-BE(2) NB cells upon MYCN inhibition revealed downregulation of fatty acid oxidation (FAO) enzymes, which was further confirmed by metabolic profiling. Reduced lipid availability prevented LD formation, indicating that impaired FAO drives LD accumulation through excess fatty acids. In addition, inhibition of triglyceride (TG) synthesis reduced LD formation and decreased NB cell proliferation, highlighting a potential vulnerability. Further proteomic analysis revealed upregulation of caveolin-1 (CAV-1), a structural protein of caveolae involved in lipid transport, and lysophosphatidylcholine acyltransferase-1 (LPCAT1), an enzyme in the TG synthesis pathway. We propose that impaired FAO drives toxic fatty acid accumulation, inducing CAV1- and LPCAT1-mediated LD formation to protect NB cells from lipotoxicity. Importantly, CAV1 expression suggests caveolae-dependent lipid trafficking between cells might occur. Conditioned media from MYCN-downregulated NB cells induced LD accumulation in stromal cells, while fluorescent fatty acid tracing demonstrated direct lipid transfer from MYCN-downregulated NB to stromal cells.

Collectively, MYCN downregulation promotes LD accumulation through disrupted FAO and activation of lipid metabolic programs, uncovering potential therapeutic vulnerabilities in neuroblastoma.

12. High-Throughput Drug Screening in Patient-Derived Cancer Stem Cell Models Identifies Promising Candidate Therapies for Pediatric High-Grade Glioma

Ida Sjöberg[1], Teresia Kling[1], Helena Carén[1]

E-mail (presenting author): ida.sjoberg@gu.se

Sahlgrenska Centre for Cancer Research, Dept. of Medical Biochemistry and Cell Biology, Institute of Biomedicine, University of Gothenburg, Gothenburg, Sweden

High-grade gliomas are among the deadliest of all childhood cancers and the leading cause of cancer-related mortality in pediatric patients with a 5-year survival rate of only ~19 %. There are few effective treatment options and clinical translation is hindered by tumor heterogeneity and lack of representative preclinical models. Tumor recurrence is driven by treatment-resistant cancer stem cells (CSCs), highlighting the need to systematically identify therapies that target the CSC-associated survival and invasiveness.

We performed high-throughput screening of established drug libraries with a total of 607 compounds targeting epigenetic, histone-modifying and differentiation mechanisms. The primary screenings included 20 patient-derived stem cell lines, from which four drug candidates were prioritized for further analysis. They were subsequently evaluated using migration assays, apoptosis and cytotoxicity measurements, cell-cycle analysis, and immunocytochemistry to determine effect on stem cell markers, invasiveness and maintenance of stem cell-like properties. The selected compounds consistently impaired migration and disrupted cell-cycle regulation, leading to apoptosis through both caspase-dependent and non-caspase pathways across all tested cell lines. This multi-parametric workflow provides integrated mechanistic and phenotypic evidence to support the selection of candidates for preclinical development.

In summary, CSC-focused high-throughput screening combined with functional validation assays constitutes a clinically relevant strategy to identify and characterize potential therapeutic candidates for pediatric high-grade glioma, with the aim of accelerating translation into in vivo validation, rational combination therapies, and patient-guided treatment approaches.

13. Exploiting microbiome-derived metabolites for targeted therapy in medulloblastoma

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Abstract:

Medulloblastoma (MB) is the most common malignant pediatric brain tumor, and high-risk and relapsed disease remains associated with poor outcomes. Therefore, novel therapeutic strategies targeting tumor-specific vulnerabilities are urgently needed. In our study, we identified a bacteria-derived metabolite with selective cytotoxicity against MB cells while sparing most other cell types, such as neurons, immune and primary cells.

MB cell lines representing primary (D425) and metastatic/relapsed (D458) state were treated with a metabolite fraction isolated from bacterium X (Bx) supernatant. Small molecules were isolated by filtering bacterial supernatants using 3kDa cutoff filters. The bacterial metabolite selectively reduced viability of MB cell lines and demonstrated activity against MB tumoroids. Treatment induced PARP cleavage and alterations in proteins associated with cell-cycle regulation and apoptosis. Proteomics analysis identified increased DKK4 expression and reduced β-catenin levels following metabolite treatment, implicating suppression of WNT signaling in the mechanism of action. Luciferase reporter assays further demonstrated that the metabolite inhibits the noncanonical WNT pathway in MB cells. Biochemical characterization indicates that the active molecule is water-soluble and not a peptide. Our untargeted metabolomics analysis has identified several candidate metabolites that will undergo screening in the above models.

We identified a bacteria-derived, water-soluble and heat-stable metabolic fraction that selectively targets MB cells and inhibits noncanonical WNT signaling. These findings reveal a potentially novel microbiome-derived treatment and provide a basis for further structural identification and therapeutic development.

14. Improving neuroblastoma differentiation therapy using combinatorial single-cell CRISPR screens

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Abstract:

In high-risk neuroblastoma (NB), retinoic acid derivatives are routinely used to induce differentiation of treatment-surviving tumor cells, but fatal relapses remain common and the mechanisms underlying this differentiation remain poorly understood. Pooled CRISPR knockout (KO) screens with single-cell RNA sequencing (scRNA-seq) readouts offer a rich and scalable approach to study genetic dependencies of differentiation, but are typically limited to one KO per cell.

To address this, we are developing a Cas12a-based platform for pooled CRISPR screens enabling combinatorial KOs that is directly compatible with standard scRNA-seq workflows. Using this approach, we aim to identify novel targets that can be therapeutically modulated to achieve robust differentiation in neuroblastoma.

In a proof-of-concept study, we performed pooled KOs of 60 differentiation-related genes in SK-N-BE(2) cells with inducible expression of Cas12a using a dual-guide, single-target library and processed the cells with either 10X Genomics 3' capture or Parse WT 3' scRNA-seq. Both modalities displayed efficient capture of the CRISPR gRNAs with negligible guide recombination, enabling confident assignment of transcriptional profiles to individual perturbations. Long-read sequencing of the scRNA-seq cDNA together with bulk DNA readouts of guide distributions in long-term treatments further confirmed the platform's capacity for functional gene editing.

Next, this platform will be expanded to four-guide libraries, enabling exploration of dual-gene KOs with maintained scRNA-seq compatibility and with potential for combination with various spatial readouts. Overall, this approach holds promise as a scalable strategy to identify targets for stable neuroblastoma differentiation – with the potential for application in many other complex phenotypes.

15. Chemical Proteomic Screening identifies Serine Proteases (SP99 and SP50) downstream of the Anaplastic Lymphoma Kinase receptor tyrosine kinase in Drosophila

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Oncogenic mutations in anaplastic lymphoma kinase (ALK), a receptor tyrosine kinase, drive pediatric neuroblastoma and are associated with poor prognosis. Introducing the activating kinase domain mutation orthologous to ALK-Y1278S into Drosophila melanogaster (AlkY1355S) enhances Alk signaling, evident from increased numbers of Alk-positive neurons and elevated neuropeptides and receptor ligand expression (Sukumar et. al. eLife 2023;12:RP88985; Mendoza-Garcia et. al., Development (2021) 148(23):dev199465). These secreted factors are often regulated extracellularly by poorly characterised serine proteases members of the serine hydrolase (SH) superfamily. Based on the AlkY1355S gain-of-function phenotype, we hypothesized that oncogenic Alk activation may result in deregulated SP activity. Employing activity-based protein profiling (ABPP), a chemical proteomics technique enabling functional interrogation of SH activity in complex biological systems (Kumar et. al., Biochemistry 2021 60 (16), 1312-1324), we identified the brain-specific protease SP99 as significantly activated in the AlkY1355S model. Biochemical characterization revealed SP99 as a trypsin-like protease. Peptidomics analysis identified pertubations of the Alk ligand Jeb and the neuroendocrine neuropeptide Spar in SP99 CRISPR knockout flies. And we also identified SP50, a paralogue of SP99, in a second ABPP screen. These findings were further validated using immunoblotting and immunostaining analyses. Our findings highlight the power of integrated chemical proteomic, peptidomic and genetics approach to identify a novel class of downstream effectors, the serine proteases, in oncogenic AlkY1355S models. These novel Alk effectors play a role in regulation of peptide ligand processing and potentially modulate signaling pathway output in response to Alk activation.

16. Linking cell-state plasticity to enhancer–gene regulatory networks and therapeutic vulnerabilities in neuroblastoma and glioblastoma

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Abstract:

Developmental plasticity contributes to intratumour heterogeneity and therapeutic resistance in neuroblastoma and glioblastoma. Here, we combine interpretable neural-network learning, multi-omics integration and functional experiments to link plastic cell states to regulatory dependencies.

Using scCAMEL, our interpretable neural-network toolkit, we integrate single-cell transcriptomes, chromatin-derived gene activity and spatial profiles across datasets and species. Our expanding resources comprise ~4 million glioma-associated cells from 937 donors across 48 datasets, alongside 367 spatial transcriptomic samples from 179 glioblastoma donors. Neuroblastoma resources include ~614,000 single-cell transcriptomes from 130 donors, ~95,000 chromatin-accessibility profiles from 17 donors and 1.59 million spatial spots/bins from 11 patients.

In our earlier glioma study, projection of 73,495 cells from 100 patients onto normal developmental trajectories identified brain-neural/radial-glial and neural-crest–brain-perivascular lineage-associated programmes, supported by experimental validation in vitro and in vivo [1]. In neuroblastoma, cross-species multi-omics revealed conserved developmental programmes, an intermediate Bridge state associated with high clinical risk and extensive epigenetic priming.

E2F7 overexpression reinforced the Bridge state and promoted tumour growth, whereas HMX1 overexpression promoted differentiation and delayed growth in the tested MYCN-wild-type models [2]. A super-enhancer-driven IRF2BP2 - AP-1 -ALK programme supported neuroblastoma proliferation. CRISPR interference of the IRF2BP2 E3 enhancer reduced IRF2BP2 expression and tumor growth [3].

Together, these studies connect developmental cell-state mapping to regulatory dependencies and candidate therapeutic vulnerabilities. By combining clinically annotated tissues and experimental models with scCAMEL-based analysis, we aim to identify and functionally validate evolutionarily conserved tumour-driving gene regulatory networks and develop state-informed therapeutic strategies.

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scCAMEL: https://sccamel.readthedocs.io/en/latest/

17. Anaplastic lymphoma kinase harnesses the SLC4A7 bicarbonate transporter and de novo nucleotide synthesis in neuroblastoma

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Abstract:

There is growing recognition of how metabolic dependencies influence sensitivity or resistance to neuroblastoma therapies. The role of Anaplastic Lymphoma Kinase (ALK), a clinical target in neuroblastoma, in regulating cellular metabolism is unclear. Using phosphoproteomics and BioID proximity labeling, we identified the sodium bicarbonate transporter SLC4A7 and CAD (Carbamoyl Phosphate Synthetase 2–Aspartate Transcarbamylase–Dihydroorotase), pyrimidine nucleotide metabolism components, as downstream ALK effectors. ALK activation led to phosphorylation of both SLC4A7 and CAD, resulting in increased sodium bicarbonate transport and pyrimidine biosynthesis that was blocked by addition of ALK tyrosine kinase inhibitors (TKIs). Combined targeting of ALK and nucleotide synthesis led to an effective inhibition of neuroblastoma tumor growth in both cell and genetically modified mouse models. ALK-dependent phosphorylation of SLC4A7 and CAD was also detected in EML4-ALK-positive NSCLC cells, suggesting that this metabolic dependency may extend to other ALK-driven malignancies.

18. Molecular mechanisms of ALK-inhibitor resistance in neuroblastoma

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Abstract:

Neuroblastoma, an embryonal tumor arising from the sympathoadrenal neural crest, accounts for 8% of pediatric cancers and 15% of related deaths in children. ALK-activating mutations are associated with poor prognosis, and ALK inhibitors (ALKi) are employed in the clinic,in addition to standard therapies. While neuroblastoma patients on ALK inhibitors generally respond robustly, resistance to ALKi therapy presents a challenging scenario. To investigate this unmet clinical need, we developed ALKi-resistant cell lines to identify molecular mechanisms underlying ALKi resistance. To this end, we developed ALKi-resistant neuroblastoma cells using the IC75 concentration of Neladalkib, a fourth-generation ALK inhibitor. After the characterization of ALKi-resistant neuroblastoma cells, we performed RNA-seq and mass spectrometry to determine dysregulated genes, proteins and phosphoproteins to identify ALKi-resistance mechanisms. Our analyses revealed that ALKi-resistant neuroblastoma cells have increased RAS-MAPK pathway activity. In agreement with our findings,ALKi-resistant cells were sensitive to RAS inhibitors, suggesting that RAS-MAPK pathway inhibition may provide a therapeutic strategy for overcoming ALKi resistance in neuroblastoma. Together, our findings confirm RAS-MAPK signaling activity as a mechanism of ALKi resistance and suggest RAS inhibition as a promising therapeutic strategy in ALKi-resistant neuroblastoma.

19. Hypoxia signaling is elevated downstream of mutant ACVR1 in pediatric high-grade glioma

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Abstract:

Background: Pediatric diffuse midline gliomas (pDMG) are lethal tumors, with approximately 80% harboring a histone H3 lysine-to-methionine mutation that reduces H3K27 trimethylation and establishes a distinct epigenetic landscape. Approximately 25% also carry activating mutations in ACVR1, encoding the BMP receptor ALK2. Although ACVR1mutations activate BMP signaling in pDMG, their contribution to gliomagenesis and downstream pathways remain poorly understood. Evidence from Fibrodysplasia ossificans progressiva (FOP), which also harbors ACVR1 mutations, suggests links between BMP and hypoxia signaling.

Methods: We analyzed clinical gene expression datasets for hypoxia signatures in ACVR1-mutant versus wildtype pediatric gliomas. Hypoxia pathway activity was further assessed in a genetically engineered pDMG mouse model, cultured pediatric and adult glioma cells, and NSC-derived experimental pDMG cultures.

Results: Hypoxia signatures were elevated in ACVR1-mutant pediatric gliomas. In vitro, ACVR1 activation increased hypoxia signaling and HIF-2α expression under low oxygen conditions in pDMG, but not adult glioma cells. ACVR1-mutant murine pDMGs showed increased vascularization and HIF-2α expression. However, genetic and pharmacological targeting of HIF-2α did not suppress ACVR1-mediated hypoxia signaling.

Conclusions: ACVR1 mutations enhance hypoxia signaling in pDMG. Our findings implicate HIF-1α and HIF-2α as potential mediators of the ACVR1-driven transcriptional program and reveal a previously underexplored link between oncogenic BMP and hypoxia signaling in pDMG.

20. Targeting Heparan Sulfate Remodeling as a Therapeutic Strategy in Glioblastoma

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Glioblastoma (GB) is an aggressive brain tumor characterized by extensive extracellular matrix (ECM) remodeling that promotes tumor growth, invasion, and therapy resistance. Heparan sulfate proteoglycans are key regulators of these processes, while heparanase (HPSE), the enzyme responsible for heparan sulfate degradation, is associated with enhanced malignancy and poor patient survival. We investigated the polynuclear platinum compound TriplatinNC as a metalloshielding strategy to disrupt heparan sulfate remodeling and inhibit HPSE-driven tumor progression. TriplatinNC crosses the blood–brain barrier without compromising its integrity and exerts dual activity through DNA binding and heparan sulfate inhibition. In patient-derived primary GB cell lines, TriplatinNC induced potent concentration-dependent growth inhibition, significantly reduced HPSE expression, and promoted prolonged G0/G1 cell-cycle arrest. In a patient-derived GB xenograft mouse model, treatment reduced tumor growth and prolonged overall survival. These findings identify TriplatinNC as a promising therapeutic candidate for glioblastoma and support ECM-targeted approaches as complementary strategies for GB treatment.

21. Targeting OPC transcriptional states enhances the effect of p53 reactivation in diffuse midline glioma

Authors: Elin Vikström[1], Eric Morin[2], Leslie Lupien[3], Noelia Ares Boveda[1], Srijita Banerjee[1], Alper Koc[1], Thale Olsen[1], Tobias Bergström[1], Ananya Roy[1], Cecilia Krona[1], Inês Neves[1], Lene Uhrbom[1], Sven Nelander[1], Keith Ligon[4,5], Géraldine Giraud [1], [2], Wojciech Michno[6,7], Fredrik J. Swartling[1], Pratiti Bandopadhayay[4,5], Veronica Rendo[1]

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Abstract:

Background

Diffuse midline glioma (DMG) is the most lethal pediatric brain tumor, with a median survival of 9–11 months. Approximately half of DMGs retain functional TP53, making MDM2 inhibitors such as navtemadlin an attractive approach for p53 reactivation. However, clinical responses remain limited and resistance is associated with adaptive transcriptional changes. We hypothesized that cell states modulate context-dependent p53 responses promoting therapeutic resistance.

Material and Methods

We profiled patient-derived DMG cell lines treated with navtemadlin using bulk and single-cell RNA sequencing. A genome-wide CRISPRa screen identified regulators of resistance, followed by functional validation in isogenic models. TP53 binding dynamics were analyzed by CUT&RUN. Combination treatments were evaluated in vitro, in a PDX model, and in cortical organoid–tumor assembloids.

Results

Navtemadlin induced selective enrichment of an oligodendrocyte precursor cell (OPC)-like state in DMG models, sustained upon long-term treatment. The CRISPRa screen identified OLIG2 as a key mediator of resistance. OLIG2 overexpression promoted an OPC-like program, reduced apoptosis, and shifted p53 signaling toward cell cycle arrest. CUT&RUN revealed differential p53 chromatin binding dependent on OLIG2 expression. Combining the OLIG2 inhibitor CT-179 with navtemadlin enhanced treatment response by potentiating

apoptosis, inducing selective tumor cell death at nanomolar concentrations in assembloids and increasing survival in vivo in a DMG PDX model.

Conclusion

Lineage-specific transcriptional states critically influence response to p53 reactivation in DMG. OLIG2-sustained enrichment of an OPC-like state drives resistance to MDM2 inhibition by limiting apoptotic activation. Targeting OLIG2 enhances the therapeutic benefits of p53 reactivation and supports combination strategies in DMG.

22. Using non-coding constraint mutations to find novel candidate genes in medulloblastoma: The story of FGR

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Abstract:

FGR belongs to the nonreceptor src-family of tyrosine-kinases. It is normally involved in cell-growth regulation, innate immunity and inflammation. Previous reports identified its importance in lymphomas and leukemia, but it has not yet been linked to brain tumors.

We use evolutionary conservation scores across mammalian genomes to pinpoint functionally important disease-associated mutations in non-coding regulatory regions. These mutations are termed Non-Coding Constraint Mutations (NCCMs). Our approach has enabled the identification of multiple candidate genes and pathways in cancer, including FGR in Medulloblastoma (MB). Being the most common type of primary malignant pediatric brain tumors, MB is molecularly classified into four subgroups with diagnostic and prognostic relevance. Despite great advance in survival, the most

aggressive forms lack efficient treatment. Whole genome sequencing data of 146 MB cases was intersected with evolutionary constraint positions in the human genome to identify genes enriched in NCCMs. We found 114 genes with high levels of NCCMs, among them FGR with six NCCMs in different positions. Introducing one of the NCCMs by CRISPR/Cas9-editing in MB cells caused enhanced sensitivity to Dasatinib, a src-family inhibitor. We next generated FGR-knockout DAOY and MB002 MB cells. In vitro assays revealed reduced cell growth and adhesion in FGR-KO lines compared to controls. Furthermore, in vivo validation in zebrafish showed that FGR-KO cells gave a lower tumor burden than wild-type counterparts.

In summary, FGR emerges as a promising biomarker and therapeutic target in MB, with potential applications in assessing disease severity and guiding patient stratification.

23. Cytoplasmic Relocalization of AGO2 Restores MYCN Gene Silencing in Neuroblastoma

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Abstract:

RNA interference (RNAi) mediated by Argonaute (AGO) proteins is a central mechanism of post-transcriptional gene regulation. While AGO2 is best known for its cytoplasmic role in microRNA-guided silencing, emerging evidence suggests that its subcellular localization can influence RNAi activity and gene regulatory outcomes. Here, we investigate how AGO2 localization affects RNAi-mediated regulation in neuroblastoma, a pediatric cancer frequently driven by amplification of the MYCN oncogene.

We show that AGO2 is aberrantly localized to the nucleus in MYCN-amplified neuroblastoma cell lines, whereas it remains predominantly cytoplasmic in non–MYCN-amplified cells. Retinoic acid (RA)-induced differentiation triggers a redistribution of AGO2 from the nucleus to the cytoplasm without altering total AGO2 protein levels. This relocalization is accompanied by changes in AGO2-associated microRNA profiles and increased AGO2 binding to mRNA targets, as revealed by fPAR-CLIP analysis. Notably, AGO2 occupancy at the MYCN locus increases following RA treatment, coinciding with a significant reduction in MYCN mRNA and protein levels. Consistent with this observation, forced cytoplasmic localization of AGO2 using a nuclear export signal (NES-AGO2) construct reduces MYCN protein levels independently of RA treatment.

Together, our findings demonstrate that subcellular localization of AGO2 represents a critical regulatory axis controlling RNAi activity and MYCN expression in neuroblastoma. Restoring cytoplasmic AGO2 function reinstates RNAi-mediated repression of MYCN, suggesting that spatial regulation of RNAi machinery may provide a potential therapeutic strategy for MYCN-driven cancers.

24. Withdrawn

25. Combined CDK11/TOPK inhibition with radiotherapy and MDM2 inhibition as a novel therapeutic strategy for glioblastoma

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Abstract:

Introduction

Glioblastoma (GBM) remains the most lethal primary brain tumour, with radioresistance, redundant pathway and heterogeneity, contributing to treatment failure. OTS964, a CDK11/TOPK inhibitor, stabilises p53 and has shown anti-tumour activity in several cancer models, but its potential as a radiosensitiser in GBM has not been explored. We evaluated OTS964 alone and in combination with external beam radiotherapy (EBRT) and the MDM2 inhibitor Navtemadlin in two GBM cell lines, U87MG and U343MG, using 3D spheroid, clonogenic assays and subcutaneous xenograft model. As well as flow cytometry and western blotting to elucidate the therapies' mechanisms.

Results

U87MG and U343MG displayed differential sensitivity to the three agents. OTS964 combined with EBRT or Navtemadlin produced synergistic growth inhibition in U87MG spheroids across multiple dose combinations. 75nM OTS964 with 1uM Navtemadlin induced 41% inhibition while 100nM OTS964 with 2Gy and 4 Gy induced 65 % and 80 % respectively. On the other hand, U343MG showed synergy only with lower-dose EBRT combinations, due to its inherent radiosensitivity, and no synergy with Navtemadlin. Both OTS964 and Navtemadlin increased p53 expression, with the strongest induction seen in combination treatments; Navtemadlin additionally upregulated MDM2. U343MG showed a significant increase in apoptosis with OTS964, while U87MG showed a shift toward G0/G1 arrest. In vivo, OTS964 and EBRT monotherapies modestly improved survival, and the combination achieved the highest survival, although statistical synergy was not reached.

Conclusion

These findings suggest that CDK11/TOPK inhibition can enhance radio- and MDM2-inhibitor sensitivity in GBM in a cell-line-dependent manner, supporting further preclinical evaluation of OTS964-based combination strategies.

26. EZH1 inhibition uncovers a targetable lipid metabolic vulnerability in Sonic Hedgehog medulloblastoma

Authors: Jiansheng Wang[1], Mohammad Alzrigat[1], Hannes Juretzka[1], Famke Zutekouw[1], Xavier Ariza[2,3], Laura Herrero[3,4], Lourdes Sainero-Alcolado[1,5], and Marie Arsenian-Henriksson[1],*

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Abstract:

Background

Medulloblastoma (MB), the most common malignant pediatric brain tumor, exhibits a low mutational burden, highlighting the importance of epigenetic modulation. The Enhancer of Zeste Homologs 1 and 2 (EZH1/EZH2) are key histone methyltransferases, whose roles in MB remain unclear.

Methods

Transcriptomic and lipidomic profiling, lipid droplet (LD) staining and lipid deprivation were used to characterize metabolic reprogramming following EZH1/EZH2 inhibition. Lipid-metabolism inhibitors were analyzed alone or in combination with an EZH1/EZH2 inhibitor to assess effects on lipid metabolism and proliferation in vitro and in vivo. Genetic knockdown was employed to determine the contribution of epigenetic modulators to lipid metabolic reprogramming.

Results

EZH2 expression was associated with poor survival in Sonic Hedgehog (SHH) MB, and dual EZH1/EZH2 inhibition using UNC1999 suppressed cell proliferation, upregulated FASN and HMGCR levels as well as LD accumulation, and increased the dependency on lipid availability. Lipidomic analysis revealed increased triglycerides and cholesteryl esters, while synthesis inhibition prevented LD formation and further impaired proliferation. Combining FASN or HMGCR inhibition with UNC1999, exerted additive or synergistic anti-proliferative effects in vitro and reduced tumor mass in a zebrafish xenograft model. EZH1 silencing, but not EZH2 knockdown, induced LD accumulation and increased FASN and

HMGCR expression, highlighting the predominant role for EZH1 in lipid metabolic reprogramming.

Conclusions

EZH1/EZH2 inhibition induces lipid metabolic reprogramming in SHH MB, with EZH1 playing the key regulatory role. Targeting the EZH1-driven lipid metabolic vulnerability may represent a promising therapeutic strategy for SHH MB.

27. Evaluating IRAK4 Inhibition as a Differentiation Strategy in High-Risk Neuroblastoma

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Background

Differentiation therapy with isotretinoin has improved 5-year survival in high-risk neuroblastoma, but its impact on overall survival remains limited. Treatment-related toxicities and cells reverting to undifferentiated states following drug withdrawal further limit its clinical benefits. Pharmacological inhibition of IRAK4, key regulator of NF-κB and MAPK signaling, emerged as a candidate differentiation strategy in a recent drug screen performed in collaboration with AstraZeneca and the Chemical Biology Consortium Sweden.

Aim

We aim to investigate whether IRAK4 inhibition promotes durable neuroblastoma cell differentiation, define underlying mechanisms of action and assess its potential to improve maintenance therapy.

Methods

IRAK4 expression is being investigated using public scRNA-seq data and multiplex IHC of patient tumor samples. IRAK4 inhibitors are being validated across cell lines, spheroids and patient-derived tumoroids, assessing differentiation, proliferation, cell death, alongside transcriptomic and proteomic changes. To model residual disease, cells will undergo chemotherapy before IRAK4 inhibition, followed by combinations with isotretinoin, dinutuximab and DFMO, which represent the main maintenance therapies clinically used. Lead compounds will be evaluated in vivo.

Results

IHC of patient samples and public scRNA-seq datasets demonstrated IRAK4 expression in tumor and stromal cells, while Western Blotting confirmed its presence in eleven neuroblastoma cell lines. Different IRAK4 inhibitors reduced proliferation and induced neurite outgrowth with effects comparable to isotretinoin in multiple cell lines. Preliminary data suggest that combining IRAK4 inhibition and isotretinoin produces durable differentiation and marked growth suppression.

Significance

This work characterizes the role of IRAK4 signaling in neuroblastoma cell-state regulation and assesses its potential in differentiation therapy.

28. Relapse of ALK-mutated high-risk neuroblastoma after lorlatinib discontinuation can be monitored with circulating tumor DNA and successfully treated - report of two cases

Authors: Torben Ek[1], Hartmut Vogt[2,3], Susanne Fransson[4,5], Jennie Gaarder[4,5], Tommy Martinsson[4], Per Kogner[6] and Martin Dalin1 on behalf of the Swedish ALK-group (Presenting author in bold)

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6. Childhood Cancer Research Unit, Women's_and Children's_Health, Karolinska Institutet, Stockholm, Sweden

Abstract:

BACKGROUND

Patients with metastatic relapse of high-risk neuroblastoma harboring the activating ALK p.R1275Q mutation can achieve complete and durable response to lorlatinib monotherapy (PMID: 39177282). Serial ctDNA monitoring is used for early response evaluation and early detection of relapse. However, the optimal therapeutic strategy following subsequent relapse remains undefined, with no established guidelines available.

METHODS & RESULTS

Two patients were monitored for >5 years using frequent retrospective and prospective ctDNA analysis using a targeted neuroblastoma-ALK sequencing panel. Patient 1, with persistent metastatic disease after Rapid COJEC and BuMel, was switched to lorlatinib. After 32 months in lorlatinib-induced CR1, treatment was discontinued, partly because of toxicity. Rising ALK ctDNA levels preceded clinical relapse by 9 months. The patient responded to lorlatinib reintroduction followed by consolidation with dinutuximab beta and TEMIRI, and remains in CR2 with undetectable ctDNA for 28 months after treatment cessation. Patient 2, with metastatic relapse, achieved CR2 on lorlatinib and remained ctDNA-negative for 40 months. Following planned lorlatinib discontinuation, gradually increasing circulating ALK p.R1275Q preceded detection of a bone metastasis. Reintroduction of lorlatinib induced a response, followed by focal radiotherapy. The patient remains in CR3 on lorlatinib >2 years later.

CONCLUSIONS

Patients with advanced neuroblastoma harboring the ALK p.R1275Q mutation can achieve complete and durable responses to lorlatinib monotherapy. Serial ctDNA

monitoring using the neuroblastoma-ALK panel enables sensitive assessment of treatment response and may provide early detection of relapse. Relapse following lorlatinib discontinuation can respond favorably to reintroduction of ALK inhibition, combined with additional therapeutic measures when appropriate.

29. MYCN-Associated Gene Repression During Sympathetic Neuronal Differentiation: Exploring the Chromatin–m^{6}A Connection

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Sustained MYCN expression disrupts neuronal differentiation, yet how its repressive effects emerge during development and intersect with RNA methylation remains incompletely understood. We investigate the relationship between MYCN, chromatin regulation and N^6- methyladenosine (m^{6}A) using a human embryonic stem cell differentiation system progressing through trunk neural crest cells (tNCCs) and sympathoadrenergic precursors (SAPs) to sympathetic neurons. This system enables comparison of MYCN activity across defined developmental stages and examination of failed developmental activation versus acquired gene silencing. A key objective is to determine whether RNA methylation contributes to transcriptional silencing through chromatin regulation, beyond effects on transcript stability, and whether this contribution varies as neural crest cells progress toward a differentiated neuronal state. Chromatin profiling in tNCCs and SAPs is complemented by SAP transcriptomics, tNCC MYCN and METTL3 ChIP-seq, and m^{6}A RNA immunoprecipitation sequencing in MYCN-inducible cells. Preliminary analyses of SAP cells reveal that MYCN induction is associated with reduced expression of neuronal differentiation and axon development programs, accompanied by loss of H3K27ac and gain of H3K27me3 at associated loci. Increased H3K36me3 enrichment is associated with RNA processing and ribosome biogenesis programs. Integration of these datasets is directed toward identifying candidate links between MYCN–METTL3 occupancy, RNA methylation and chromatin-associated repression.

The contribution of METTL3 catalytic activity, and the relation between RNA-modification and chromatin changes, remain to be established. Together, these findings link MYCN induction to coordinated transcriptional and chromatin remodeling in a developmental neuroblastoma model and provide a basis for investigating m^{6}A as an additional axis regulating differentiation-associated gene repression.

30. Modelling moderate MYCN overexpression in the developmental context of Neuroblastoma

Panagiotis **Alkinoos Polychronopoulos***[1], Mingzhi Liu*[2], Kristina Ihrmark Lundberg*[1], Sara Abu Ajamieh[1], Bethel Tesfai Embaie[1], Thale Kristin Olsen[1], Celine Hafkesbrink[1], Stefania Aliverti[1], Per Kogner[1], Conny Tümmler[1], Malin Wickström[1], Ninib Baryawno[1], Oscar Bedoya Reina[1], Margareta Wilhelm[2], John Inge Johnsen #[1]

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The development of the sympathoadrenal lineage, which gives rise to the adrenal medulla and sympathetic ganglia, is a complex process. The disruption of the differentiaton process within this lineage can give rise to neuroblastoma, the most common extracranial solid tumor of childhood. A hallmark of high-risk neuroblastoma is MYCN genomic amplification, however many tumors exhibit high MYCN protein expression without amplification ("MYCN gain"), suggesting that aberrant MYCN expression alone is a critical oncogenic event. Current neuroblastoma models harbor constitutive MYCN-overexpression in committed adrenergic cells and fail to capture the impact of MYCN gain in earlier, multipotent neural crest progenitors.

In this study we employ a human induced pluripotent stem cell (iPSC)-derived model of trunk neural crest cells (tNCC) and cells of the sympathoadrenal lineage to drive moderate MYCN overexpression in this progenitor pool, testing the hypothesis that a more physiologically relevant increase in MYCN expression is sufficient to alter cell fate and initiate early molecular events of tumorigenesis. Following the generation and successful differentiation of a moderate MYCN overexpressing iPSC line, key progenitor cell populations were characterized transcriptomically and stained for relevant markers.

Our preliminary results reveal that moderate MYCN overexpression within the SOX10+ tNCC population induces a distinct molecular profile, providing a unique window into early pathogenic events. Our findings establish a novel human iPSC-derived model to investigate the consequences of moderate MYCN overexpression in trunk neural crest cells and the sympathoadrenal lineage development, in order to understand the molecular mechanisms of MYCN gain-induced cellular transformation in neuroblastoma development.

31. Targeting Developmental Programs in Pediatric Medulloblastoma Tumors

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Abstract:

Medulloblastoma (MB) is an aggressive pediatric brain malignancy arising from cerebellar progenitor populations, accounting for approximately 15% of childhood central nervous system tumors. Despite molecular classification into WNT, SHH, Group 3, and Group 4 subgroups, the mechanisms underlying tumor invasion and metastatic dissemination remain incompletely defined. Recently, we identified bone morphogenetic protein (BMP) signaling as a context-dependent oncogenic pathway in MB. Across patient-derived xenografts, tumor specimens and MB cell models, active BMP signaling was associated with phosphorylated SMAD1/5 and induction of the transcription factor LHX2. Spatial transcriptomics combined with functional studies demonstrated that LHX2 enhances BMP pathway activity by transcriptionally upregulating the BMP type I receptor ACVR1, establishing a feed-forward BMP–LHX2–ACVR1 circuit. This signaling module promotes cancer stem cell-like properties, chemoresistance, and tumor-propagating capacity in vivo, with LHX2-high/BMP-high recurrent Group 4 cells displaying increased metastatic potential. In complementary ongoing studies, integrated transcriptomic analyses of various MB models implicate the evolutionarily conserved ROBO/SLIT guidance pathway as an important regulator of MB cell invasion. Functional studies suggest that sustained ROBO1/3 expression restrains the invasive phenotype, whereas modulation of SLIT ligands alters receptor stability and invasive behavior. Notably, transient SLIT depletion stabilizes ROBO receptors and reduces MB cell invasion in vitro, suggesting that this neuronal guidance program may restrain metastatic spread in specific contexts. Together, these findings reveal complementary developmental signaling networks governing MB stemness and invasion. Defining how oncogenic BMP–LHX2 signaling intersects with ROBO/SLIT pathway may identify biomarkers of aggressive disease and actionable vulnerabilities to suppress tumor metastatic spread.

32. SOX2 orchestrates mitochondrial biogenesis and metabolic remodelling via PGC1α to regulate glioblastoma growth and chemoresistance

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Abstract:

Glioblastomas are heterogeneous tumors characterized by diverse transcriptional subtypes and complex metabolic dependencies. We previously showed that SOX2 facilitates a transition of glioblastoma cells from a mesenchymal to a non-mesenchymal state and promotes tumor cell proliferation. However, the role of SOX2 in regulating mitochondrial metabolic reprogramming remains unclear. Using xenograft and allograft models, cell proliferation assay, immunofluorescence, confocal microscopy, flow cytometry, extracellular flux assay, Western blotting, and RNA sequencing analysis, we demonstrate that SOX2 overexpression enhances tumor growth, increases mitochondrial oxidative phosphorylation, elevates mitochondrial biogenesis and shifts mitochondrial distribution from a perinuclear accumulation to a tubular network. Conversely, SOX2 suppression leads to the opposite effects. Mechanistically, SOX2 drives mitochondrial biogenesis and metabolic reprogramming by upregulating PPARGC1A (encoding PGC1α) and increasing the expression of both fission and fusion proteins. Moreover, SOX2-mediated metabolic reprogramming influences glioblastoma cell sensitivity to temozolomide (TMZ), a standard chemotherapeutic agent. These findings highlight SOX2 as a key regulator of mitochondrial metabolism in glioblastoma, linking its activity to tumor growth and chemotherapy response through mitochondrial structural and functional remodelling. This work offers new insights into glioblastoma biology and suggests potential therapeutic strategies targeting SOX2 and mitochondrial metabolism.

33. Prognostic value of the platelet-to-lymphocyte ratio in pediatric CNS tumors and neuroblastoma

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Abstract:

Pediatric CNS tumors and neuroblastoma are associated with substantial morbidity and mortality, particularly in high-risk or relapsed disease. Survivors often face long-term treatment-related complications, highlighting the need for prognostic biomarkers to guide risk-adapted therapy. Blood-count-based inflammatory biomarkers, such as the platelet-to-lymphocyte ratio (PLR), are readily available from routine laboratory testing and may have prognostic value for survival.

The retrospective data used for this research included pediatric patients (<18 years) diagnosed with CNS tumors (n=108) or neuroblastoma (n=35) in the Helsinki University hospital region between 2004 and 2022. Collected variables include demographics, diagnosis, diagnostic test results (including complete blood counts prior to any treatment), treatment details, disease progression, relapse, and follow-up status on 12/2025.

PLR was calculated by dividing the absolute platelet count by the absolute lymphocyte count. Associations between pre-treatment PLR and survival were assessed using Wilcoxon rank-sum tests, ROC analysis, Cox proportional hazards regression with log-transformed PLR, and Kaplan–Meier analysis with ROC-derived PLR cut-offs, both in the overall cohort and by tumor type. ROC-derived PLR cut-offs identified groups with significantly different overall survival in the whole cohort (log-rank $p < 0.01$), CNS tumors ($p < 0.01$), and neuroblastoma ($p < 0.01$). In the overall cohort, higher log- transformed PLR was associated with poorer overall survival in Cox regression ($p < 0.05$). We suggest that pre-treatment PLR may have potential as a prognostic biomarker, but these analyses are exploratory and require validation in a larger cohort.

34. Uncovering Neuron-Tumor Crosstalk controlling vascular dynamics and cellular plasticity in pediatric brain tumors

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Medulloblastoma (MB) progression is strongly influenced by interactions between tumor cells and the brain microenvironment, including the tumor vasculature and blood–brain barrier (BBB). We previously established a Sonic Hedgehog (SHH)-MB model using patient-derived neuroepithelial stem (NES) cells carrying a germline PTCH1 mutation. Orthotopic transplantation into the mouse cerebellum generates tumors that recapitulate key features of human MB, while serial retransplantation of tumor-derived NES (tNES) cells produces progressively more aggressive tumors, with accelerated growth and occasional leptomeningeal dissemination. Here, we investigated vascular remodeling and BBB integrity across successive tumor generations. Advanced tNES tumors exhibited marked hypervascularization and pronounced morphological abnormalities, accompanied by progressive BBB compromise. This was characterized by reduced astrocytic end-foot coverage and decreased expression of endothelial tight-junction proteins. Comparable vascular alterations were observed in patient-derived xenografts, supporting the translational relevance of the model. Given emerging evidence that neural-tumor interactions can influence tumor progression and the microenvironment, we further investigated neuron-tumor crosstalk as a potential regulator of vascular remodeling. In vitro co-culture experiments demonstrated that direct contact between neurons and tumor cells robustly enhanced angiogenesis. Preliminary RNA-sequencing analyses suggest that this pro-angiogenic response may be linked to the formation of aberrant synaptic contacts between neurons and tumor cells. Collectively, our findings identify progressive vascular remodeling and BBB disruption as features of increasing SHH-MB aggressiveness and suggest a potential neuron- tumor-vascular axis. Elucidating the molecular mechanisms connecting aberrant synaptic interactions with angiogenic signaling may reveal novel mechanisms underlying MB progression and metastatic dissemination.

35. Matched primary-relapse PDHGG patient-derived xenograft models to identify TME shift and therapy resistance profiles

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Background: The prognosis of paediatric-type diffuse high-grade glioma (PDHGG) remains poor despite multimodal therapy. Intratumoral heterogeneity, an immunosuppressive microenvironment, and adaptive therapy resistance contribute to treatment failure and disease progression. Characterisation and molecular evolution from primary tumour to relapse may provide insights into mechanisms underlying treatment resistance and disease progression.

Methods: We performed integrated molecular profiling of matched primary (P) and relapse (R) IDHWT-K27WT PDHGG samples from a child treated with autologous tumour-lysate-pulsed DC vaccinations. Bulk and single-cell RNA sequencing were used to characterise transcriptional changes and tumour cell states between P and R disease. Immune cell states were further assessed using single-cell transcriptomic data, including immunosuppression and T-cell exhaustion scores. To study treatment resistance we have developed brain stem organoids and perform 3D expansion of tumour cells in assembloids where different treatment combinations can be tested.

Results: Single-cell analysis showed that most tumour cells exhibited an oligodendrocyte precursor-like (OPC-like) transcriptional phenotype. Relapse was associated with increased radiation- and therapy-response signatures, alongside increased IGFBP2 expression and loss of CDKN2A expression in OPC-like tumour cells. Transcriptomic analysis also indicated high MYC activity, consistent with an aggressive and treatment-resistant tumour state. Immune profiling revealed increased exhaustion among cytotoxic T cells and FOXP3+

regulatory T cells compared with the independent reference dataset. The brain stem organoids are validated and normal and tumoral cells are differentially being labelled for therapeutic studies.

Conclusion: Integrated bulk, single-cell, and immune profiling identified substantial molecular and microenvironmental changes during PDHGG progression from primary tumour to relapse. These findings support incorporating longitudinal molecular and microenvironmental profiling to better understand relapse biology and inform therapeutic strategies for PDHGG. As a next step, these patient-derived tumour cells will be investigated in brainstem organoid models to functionally explore the identified relapse-associated phenotypes and evaluate potential therapeutic vulnerabilities in a more physiologically relevant context.

36. A Humanized Model of Neuroblastoma Bone Metastases during Therapy

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Abstract

High-risk neuroblastoma (NB) frequently metastasizes to the bone and bone marrow (BM), where tumors are particularly difficult to treat. Most available in vivo models rely on a murine tumor microenvironment (TME), limiting their ability to recapitulate human NB–bone/BM interactions and treatment responses. We aimed to develop and characterize humanized in vivo models of NB bone/BM metastasis to investigate NB interactions with the human TME during tumor progression and treatment and to test novel therapies for metastatic NB.

To achieve that, humanized mini-bones (hOss), generated from human mesenchymal stromal cells, were implanted into immunodeficient mice. MYCN-amplified NB patient-derived xenograft (PDX) cells were subsequently injected either directly into hOss or intravenously via the tail vein. Tumor engraftment, progression, and response to chemotherapy were monitored by in vivo IVIS imaging. Samples were then analysed with spatial transcriptomics and multifluorescent stainings.

NB PDX cells showed enhanced engraftment and invasion in hOss, following both direct and intravenous injections, compared with murine femurs, indicating a more favorable TME within the humanized niche. Chemotherapy treatment of hOss-engrafted NB recapitulated clinical responses, including treatment responsive and resistant tumors, with minimal residual disease detectable within the humanized bone niche following treatment. Initial spatial transcriptomic analyses revealed substantial remodeling of the BM microenvironment upon NB growth and treatment.

Together, these findings establish hOss as a clinically-translatable platform for investigating human NB–bone/BM interactions, treatment response, minimal residual disease, and novel anti-metastatic therapeutic strategies.

37. Targeting the NCAM1/FGFR1 axis as a potential therapeutic vulnerability in Neuroblastoma

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Neuroblastoma (NB) is the most common extracranial solid tumour in infants and is characterised by substantial heterogeneity in tumour biology, treatment response and clinical outcome. Despite intensive multimodal therapy, approximately half of the patients with high-risk disease relapse due to therapy resistance and face poor survival, underscoring the need for new targeted treatment strategies. Recently, our single-cell RNA-sequencing data of cell lines and primary human NB samples revealed that NCAM1 is highly expressed by adrenergic tumor cells, while FGFR1 is expressed by mesenchymal stromal cells. We observed that elevated expression of both targets correlates with poorer patient survival. While NCAM1 and FGFR1 have been individually targeted pharmacologically in adult cancers, their combined role in NB has not been explored. Therefore, we aim to evaluate the therapeutic potential of CD56 (NCAM1)-targeting antibody-drug-conjugate Adcitmer® and the FGFR1 inhibitor erdafitinib.

Therapeutic targeting of the axis was assessed using NB cell lines and multicellular tumour spheroids co-cultures with stromal cells. Pharmacological inhibition impaired tumour cell viability as single agent and in combination with combined treatment showing an additive effect in cell lines and synergy in multicellular tumour spheroids. Moreover, FGFR1 blockade affected multiple downstream pathways, including RAS-MAPK, PI3K-AKT, PLCγ and STAT signaling. In vivo, erdafitinib reduced tumor growth in an IMR32 xenograft model in NMRI-nude mice at doses of 10-15 mg/kg.

Together, these findings identify the NCAM1-FGFR1 axis as a therapeutically actionable target in NB and provides a rationale for further preclinical and clinical evaluation of Adcitmer® and erdafitinib.

38. KD025-mediated ROCK2 inhibition attenuates metastatic progression and hypoxic tumour growth in neuroblastoma

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Abstract:

Neuroblastoma is characterized by a high rate of metastases, frequently present at diagnosis. This underscores the urgent need for therapeutic agents capable of simultaneously targeting primary tumour growth and metastatic spread. The Rho/ROCK-signalling axis has emerged as an attractive novel therapeutic target in neuroblastoma, given its well established roles in key processes that drive metastatic progression, including cytoskeletal remodelling and actin dynamics.

This study investigates the anti-metastatic potential of KD025 (Belumosudil), an FDA and EMA-approved ROCK2-specific inhibitor, in neuroblastoma.

In vitro migration and invasion assays were performed in a panel of neuroblastoma cell lines grown as monolayers and tumour spheroids, using the Incucyte® Live-Cell Analysis System. The effects of KD025 were also assessed under hypoxia in comparison to chemotherapeutics used in standard neuroblastoma treatment protocols.

KD025 inhibited neuroblastoma growth more potently under hypoxic conditions than normoxia, in contrast to standard cytostatic drugs. In monolayer cultures, KD025 reduced neuroblastoma cell migration and blocked cell invasion. Additionally, in the spheroid model, KD025 suppressed 3D tumour cell invasion into a Matrigel matrix in a dose-dependent manner. Collectively, our findings provide a preclinical rationale for investigating the effects of KD025 on neuroblastoma metastasis in vivo.

High-risk neuroblastoma is frequently associated with metastatic disease, and hypoxia is an important microenvironmental driver of metastatic progression. Our findings suggest that inhibition of ROCK2 can target both tumour growth and invasive capacity in neuroblastoma, with enhanced efficacy under hypoxic conditions. Thus, inhibition of Rho/ROCK signalling may offer a promising therapeutic strategy to combat metastatic neuroblastoma.

39. Dual PI3K/AKT and CDK4/6 inhibition reveals selective sensitivity in an SHH medulloblastoma stem cell model

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Abstract:

Medulloblastoma (MB) is a brain tumor for which current treatments cause serious side effects and are not curative for all patients, highlighting the need for more effective and brain-protecting therapies. Recently, we combined phosphoinositide 3-kinase (PI3K) inhibitor (BYL719), fibroblast growth factor receptor (FGFR) inhibitor (JNJ-42756493) and cyclin-dependent kinase (CDK)4/6 inhibitor (PD-0332991) in MB cells, and discovered synergistic effects. In the current study, we investigate the most efficient therapies in a normal/tumorigenic neural stem cell model. A sonic hedgehog (SHH)-MB model, including a Gorlin syndrome patient neuroepithelial stem cell line (NES) and its tumor derivative (tNES), was used to evaluate single and combined treatments of PI3K, AKT, FGFR, and CDK4/6 inhibitors (BYL719, AZD5363, JNJ-42756493, and PD-0332991). Effects on viability, cell confluence and apoptosis were tested on NES and tNES cells cultured in 2D and 3D. We found that 2D tNES cells were generally more sensitive to the inhibitory effects of both single and combination treatments compared to 2D NES cells. In 3D, all single drugs were more effective against tNES than NES, except for JNJ-42756493, which showed the opposite trend. Drug combinations in 3D cultures generally resulted in synergistic or additive effects on cell viability in NES and tNES. This study illustrates that single and combined administrations of PI3K, FGFR, CDK4/6, and AKT inhibitors in a NES/tNES model have dose-dependent and additive/synergistic anti-MB activity impacting tumor growth. Their effects on tNES cells were generally more pronounced than on NES; however, the difference in proliferative capacity between the cells should be considered.

40. HIF2α mediated downregulation of MYCN oncoprotein is associated with subcellular localization of GSK-3(β)

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Neuroblastoma (NB) the most common extarcranial form of solid pediatric tumours having a high variable severity, ranging from spontaneous regression to fatal progression. Hypoxia inducible factor 2 alpha (HIF-2α) is responsible for longterm hypoxia related adaptation in the body. However its role in NB remains inconclusive. MYCN amplification is a strong predictor of high risk (HR) and recurrence in the NB patients. Our previous publication reported an anti-proliferative role of HIF-2α in NB via MYCN downregulation. Hence we aimed to understand the mechanisms through HIF-2α can downregulate MYCN and thereby reduce NB cellular proliferation. Endogenous HIF-2α was stabilised by dose optimised treatment with Dimethyloxalylglycine (DMOG) in various HR-NB cell lines. Treated cells were used for bulk RNA sequencing and Mass Spectrometry (MS) followed by candidate based marker validation through western blot (WB) and immuno histochemistry (IHC). DMOG specifically elevated HIF-2α along with a concomitant reduction of MYCN, which could be restored through proteosomal inhibition. Further exploration of MS data revealed decreased levels of Aurora Kinase A and PLK1, both of which contribute to MYCN stability. Specific MYCN regions, responsible for proteosomal degradation, were also found to be altered both in MS and WB. CHIR99021, a potent GSK-3 inhibitor could restore MYCN level and rescued NB from G1/S cell cycle arrest, implying a role of GSK-3 in MYCN degradation. Further investigation revealed a preferential nuclear localisation of GSK-3β along with HIF-2α. As a consequence, GSK-3β potentially facilitated increased ubiquitination and subsequent degradation of MYCN within nucleus. Our present data has identified a possible mechanism of HIF-2α mediated MYCN downregulation, further implicating a tumor supressive role in NB.

41. The effect of Galectin-3 on the microglia-tumor cell crosstalk in Medulloblastoma

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Galectin-3 (Gal3), a soluble glycan-binding lectin, is overexpressed in Sonic Hedgehog medulloblastoma (SHH-MB), where it promotes tumor growth, invasion, and metastasis. Single cell RNA-sequencing data of MB patients (Riemondy GSE155446) showed that myeloid cells are the main LGALS3 positive cell population in the TME. Previous studies demonstrated that Gal3 drives microglia polarization towards an anti-inflammatory phenotype, thereby facilitating immune evasion and tumor progression. Additionally, prior publications have shown that tumor-associated astrocytes secrete IL-4 to stimulate microglial IGF1 production, which activates downstream signalling promoting MB growth. However, the role of Gal3 within the tumor microenvironment (TME), and specifically myeloid cells, remains poorly understood. To investigate this, we performed immunofluorescent staining for Gal3 and Iba1 on orthotopically transplanted human neuroepithelial stem (NES) cell brain tissue slides. This revealed an enrichment of Gal3-positive microglia within tumor regions, accompanied by a morphological shift from highly ramified to amoeboid states, indicative of microglia activation.

Furthermore, phospho-RTK array analysis in Gal3-deficient NES cells revealed downregulation of IGF1R signalling, which suggests reduced responsiveness of MB cells to microglia-derived IGF1. To further dissect the role of Gal3 in tumor-microglia interactions, we established an in vitro co-culture system combining SHH-MB spheroids with iPSC-derived microglia harbouring inducible LGALS3 knockdowns. This model will allow us to further investigate microglia infiltration, polarisation, overall proliferation, and changes in secretome and transcription levels dependent on Gal3. Collectively, our data identified Gal3 as a potential mediator of microglia-tumor cell crosstalk in MB and presents as a potential novel therapeutic target to disrupt tumor–microenvironment interactions.

42. ALK-driven tumor model development in Drosophila melanogaster

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Abstract:

Drosophila melanogaster is a well-established model organism in cancer research due to its similarity in key signaling pathways with humans. Classical tumor models in flies often rely on activating a cancer-driving gene like Ras together with mutations that disrupt cell polarity, leading to uncontrolled growth in developing tissues.

In our study, we discovered that overexpressing the normal (wild-type) form of the Drosophila ALK gene is sufficient to induce tumor-like overgrowth in epithelial tissues, specifically in the imaginal discs. Interestingly, this phenotype is more severe than the effects seen with activated Ras alone, suggesting ALK activates more aggressive signaling program. Gene expression analysis (RNA-seq) showed that ALK overexpression strongly activates several downstream pathways, including JNK and JAK/STAT signaling.

Through protein interaction and phosphorylation analyses, we identified two key chromatin-associated protein complexes — Spt6 and the FACT complex — as important regulators of ALK-induced tumor signaling. Both are histone chaperones involved in gene regulation and are conserved across species. Our findings point to a previously unrecognized link between ALK signaling and chromatin regulation through histone chaperones. This might inform new strategies for targeting ALK-driven cancers in humans, such as neuroblastoma.

43. m6A-dependent TERRA condensates as a regulatory mechanism in ALT-positiveneuroblastoma

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Abstract

High-risk neuroblastoma (NB) tumors require a telomere maintenance mechanism (TMM) to sustain tumor growth. While most tumors activate telomerase, a subset of high-risk NB maintains long telomeres through the telomerase-independent alternative lengthening of telomeres (ALT) pathway. ALT-positive (ALT+) NB is associated with frequent relapse and poor clinical outcomes, highlighting the need to better understand its underlying biology and identify new therapeutic strategies. ALT+ tumors show increased expression of TERRA, a telomeric repeat-derived noncoding RNA that localizes to telomeres and interacts with proteins involved in ALT. We found that TERRA forms RNA condensates at telomeres in an N6-methyladenosine (m6A)-dependent manner. Inhibition of the m6A writer METTL3, alone or in combination with telomere-damaging agents, synergistically reduced the proliferation of ALT+ NB cells.

Mechanistically, we show that TERRA G-quadruplex (G4) structures cooperate with m6A modification to promote TERRA foci formation. We recapitulated TERRA condensate formation in vitro using the TERRA-binding protein hnRNPA2B1 and further demonstrated the cooperation between G4 structures and m6A. The

intrinsically disordered region of hnRNPA2B1 was essential for TERRA condensate formation both in vivo and in vitro.

Overall, our findings establish a mechanistic framework for TERRA condensate formation in ALT+ NB and identify m6A-dependent TERRA condensation as a potential therapeutic vulnerability.

44. Targeting MDM2 stabilization to enhance p53 reactivation in Diffuse Midline Glioma

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Diffuse Midline Glioma (DMG) is a universally fatal and incurable brain tumor affecting children and young adults. These tumors are driven by lysine 27-to-methionine mutations in histone 3 (H3K27M) that rewire the transcriptional and epigenetic landscape and promote tumor heterogeneity, ultimately hindering the development of novel targeted therapies. Over 40% of DMGs retain wild-type and functional tumor suppressor TP53 which renders them susceptible to therapeutic approaches that reactivate p53, including MDM2 inhibition. In this context, a window of opportunity trial in adult glioma patients showed that MDM2 inhibitor navtemadlin penetrates the blood-brain barrier and exerts a pharmacodynamic effect but achieves limited responses as a single-agent treatment. We suggest its potential use in TP53 wild-type DMG, and devise the need for combination treatments. Here we identify and characterize actionable vulnerabilities that enhance the effects of p53 reactivation in DMG. To do this, we have identified genes whose depletion confers sensitivity to navtemadlin. Among the highest-ranking hits is Ubiquitin Specific Protease 7 (USP7), a deubiquitinase that regulates MDM2 stabilization and degradation. To verify that co-targeting MDM2 and USP7 enhances reactivation of p53, we have characterized a panel of 10 patient-derived DMG cell lines and assessed their response to MDM2 and USP7 inhibition alone and in combination. These models include both TP53 wild-type and mutant models and cover different mutational signatures. By applying different phenotypic assays, we show that combined inhibition synergizes in vitro at nanomolar concentrations and exceeds the anti-tumor response observed with monotherapies. Altogether, our efforts will dissect the effects of this combination treatment to advance targeted therapies in this subset of fatal pediatric brain tumors.

45. Targeting transcriptional states and dependencies of chemotherapy-persistent Atypical Teratoid Rhabdoid Tumors to enhance treatment efficacy

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Abstract:

Atypical Teratoid Rhabdoid Tumors (AT/RTs) are rare and aggressive embryonal brain tumors affecting infants. Despite their low mutational burden, with loss of SMARCB1 as the almost exclusive recurrent event, these tumors are heterogeneous at the epigenetic level and remain poorly understood. Current AT/RT clinical regimens are limited to high-dose multimodal chemotherapy and occasionally radiation, which are highly toxic and only achieve partial responses leading to recurrence in over 50% of patients. Although the responses of AT/RTs to chemotherapy involve profound transcriptional changes, the molecular landscape of AT/RT cells surviving first-line chemotherapy remains unknown. Here, we performed cellular barcoding of a panel of treatment-sensitive patient-derived AT/RT models to track chemo-persistent populations and investigate their clonal dynamics over time. By leveraging single-cell RNA sequencing, we identified the features of cells that persist treatment with five different chemotherapies when evaluated individually. We show that barcodes cluster closely between chemotherapies with the same mechanism of action, suggesting functional redundancies and overlapping mechanisms of action across chemotherapy agents that constitute today's clinical protocols. Moreover, we will conduct a CRISPR Cas12a knock-out screen in AT/RT cell lines to identify which genes, when depleted, enhance the effects of five chemotherapy agents. We will evaluate overlapping and unique screen hits for AT/RT populations treated with each chemotherapy agent and propose novel targeted combination treatments that will be validated in vitro, in vivo, and in patient material. By leveraging state-of-the-art -omics approaches embedded with a translational component, we aim to accelerate target discovery and enhance the treatment efficacy and survival of AT/RT patients.

46. HIF2A creates a cell-state-dependent ferroptosis vulnerability in neuroblastoma

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Abstract:

High-risk neuroblastoma contains hypoxic and immature cell states that resist conventional therapy. Whether hypoxia-inducible factor 2 alpha (HIF2a), a regulator of these states, creates a therapeutically actionable ferroptosis vulnerability remains unknown. We combined inducible HIF2a gain-of-function and EPAS1 knockout models with time-resolved transcriptomics, proteomics, dose-response assays using erastin or RSL3, and rescue with ferrostatin-1 or deferoxamine in LAN1 and SHEP2 neuroblastoma cells. HIF2a induction produced marked, cell-state-dependent metabolic remodeling. In LAN1 cells, early changes in iron and heme pathways preceded late activation of polyunsaturated- fatty-acid lipid-peroxidation programs, while sustained reactive-oxygen-species signaling and reduced NADPH-associated buffering indicated increasing oxidative pressure. SHEP2 cells showed a distinct transcriptional trajectory, demonstrating that HIF2a does not activate a uniform canonical ferroptosis program. Nevertheless, HIF2a induction increased sensitivity to both erastin and RSL3 in functional assays. Erastin-induced loss of viability in HIF2a-expressing LAN1 cells was almost completely rescued by ferrostatin-1 and deferoxamine, supporting lipid-radical- and iron-dependent cell death.

Conversely, EPAS1 knockout altered ferroptosis dose responses in a clone-dependent manner, with pronounced erastin resistance in one knockout clone. These findings identify HIF2a as a context- dependent regulator of ferroptosis susceptibility rather than a simple on-off switch. Integrating HIF2a status with tumor cell state may therefore provide a biomarker-guided strategy to expose treatment- resistant neuroblastoma cells to ferroptosis-inducing therapy and inform rational selection of ferroptosis agents for future combination studies.

47. Exploring long-read whole genome sequencing for pediatric CNS tumors in a clinical setting

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Abstract:

Twenty paired pediatric brain tumor (90×)/normal (30×) samples and four germline samples with known DNA variants were sequenced using Oxford Nanopore Technologies (ONT; PromethION). Five paired samples were additionally sequenced using Pacific Biosciences (PacBio; Revio). Variant calling was performed using standard paired-somatic and single-sample workflows, followed by clinical filtering pipelines. Methylation classification was performed using Sturgeon and ROBIN (13/20 samples), which incorporates Sturgeon, NanoDX, PanNanoDx, and Random Forest classifiers.

We conclude that lrWGS, preferably with ONT, is well suited for clinical pediatric cancer diagnostics, providing a much faster and more comprehensive detection of genetic variants and direct epigenetic classification.

While data output from blood samples was high (mean coverage 29.2x/SMRT cell and 35.0x/ONT cell), tumor data yield was generally lower (21.7x/SMRT cell and 30,7x/ONT cell). All clinically relevant single nucleotide variants (SNV) insertions/deletions (indels), and structural variants (SVs) were detected by both lr-platforms. LrWGS also resolved complex structural rearrangements and variants in challenging genomic regions and identified additional structural variants not detected by srWGS. Sturgeon and at least one classifier within ROBIN correctly classified all tumors (tumor cell content >50%) into types represented in Heidelberg v11b4.

Whole genome sequencing (WGS) of childhood cancer has been implemented at all University Hospitals in Sweden, improving tumor diagnostics and informing treatment. However, short-read (sr) sequencing has limitations in resolving large genomic rearrangements, and methylation arrays used for CNS-diagnostics is a laborious process. We have investigated the potential of using long read (lr)WGS for joint genomic and epigenetic profiling of pediatric brain tumors.

48. Development of Proximity Ligation Assays for Blood-Brain Barrier Assessment in Healthy and Diseased Human Brain Tissue

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Abstract:

The blood-brain barrier (BBB) is a highly specialized vascular interface that maintains central nervous system homeostasis by regulating the exchange of molecules, nutrients, and immune cells between the circulation and the brain. The BBB poses a barrier for therapeutic drug entry into the brain, and BBB dysfunction is implicated in numerous neurological disorders, including neurodegenerative diseases, cerebrovascular disorders, and glioblastoma tumours. However, the molecular mechanisms underlying BBB function and alterations remain poorly understood.

Here, we present the development of a proximity ligation assay (PLA)-based toolbox for spatial characterization of brain and BBB-associated proteins and protein interactions. Unlike transcriptomic approaches that measure gene expression, or conventional in situ proteomics methods that primarily quantify protein abundance, PLA enables spatially resolved analysis of protein interactions, providing a functional interactomics readout directly in intact tissue sections.

A panel of functional interactomic assays targeting BBB structure, signaling, transport, and cellular communication were stained on healthy human cortex and patient-derived glioblastoma tissue samples, enabling visualization and quantification of BBB-associated molecular signatures in healthy and diseased microenvironments. Importantly, the workflow is compatible with formalin-fixed paraffin-embedded (FFPE) tissue, facilitating retrospective studies and supporting integration into translational and clinical research pipelines.

By enabling in situ analysis of functional protein networks, this approach complements transcriptomics and other spatial profiling technologies. The resulting BBB-focused PLA panel establishes a spatial proteomics and interactomics resource for neuroscience and neuro-oncology research and is being developed into standardized products that will be made available to the broader research community.

49. MYC family proteins interact directly with a conserved pocket on TFIIF via the shared MB0 moti

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Abstract:

MYC family oncoproteins are major drivers of tumor initiation and progression, and deregulation of N-MYC is a hallmark of high-risk neuroblastoma. Despite their central role in cancer biology, MYC proteins remain difficult therapeutic targets because their intrinsically disordered nature limits conventional small-molecule drug discovery. One promising strategy is to define and disrupt essential MYC protein–protein interactions that support oncogenic transcription.

Here, we characterize a direct interaction between MYC family proteins and the general transcription factor TFIIF, a core component of the RNA polymerase II transcription machinery. Using an integrated structural biology approach combining biophysical binding assays, NMR spectroscopy, mutational analysis and molecular modelling, we show that c-MYC, L-MYC and N-MYC all interact directly with TFIIF through the conserved MYC box 0 (MB0) region. NMR analysis reveals that this interaction is dynamic and centered on a short, conserved motif within MB0, with additional effects observed in neighboring MYC box regions. From the TFIIF perspective, chemical shift perturbation mapping and supporting mutational data identify a conserved and accessible pocket at the top of the GTF2F2 β-barrel as the principal MYC-interaction surface.

Together, our data define a previously uncharacterized MYC–TFIIF interaction interface that may contribute to MYC-driven transcriptional regulation. Because this interaction is conserved across the MYC family, including N-MYC, it represents a potential platform for developing new therapeutic strategies aimed at MYC-dependent cancers such as neuroblastoma.

50. Establishing a Human Developmental Model of ALT-Positive Neuroblastoma

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Abstract: Neuroblastoma (NB) is a paediatric malignancy arising from the developing sympathetic nervous system, in which trunk neural crest cells (tNCCs) fail to properly differentiate into sympathetic neurons. A subset of high-risk NB maintains telomeres through the alternative lengthening of telomeres (ALT) pathway, which is strongly associated with alterations in the chromatin-remodelling protein ATRX. Despite the clinical relevance of ALT-positive NB, suitable human developmental models remain limited.

The aim of this study was therefore to establish a human developmental model of ALT-positive NB using human embryonic stem cell (hESC)-derived tNCCs with ATRX deficiency and TP53/CDKN2A knockout. Following differentiation of ATRX-deficient hESCs towards the tNCC lineage, we observed ALT-associated phenotypes, including increased telomeric DNA damage and accumulation of TERRA foci. Furthermore, ATRX depletion impaired differentiation towards sympathetic neurons, as demonstrated by reduced expression of neuronal differentiation markers and abnormal neuronal morphology. These findings suggest that ATRX contributes not only to telomere maintenance but also to sympathetic neuronal lineage progression.

Our goal is to further develop this system into a human developmental model of ALT-positive neuroblastoma. This model will enable us to investigate the developmental biology of ALT-positive tumours and, importantly, to understand how early activation of telomere maintenance through the ALT pathway may contribute to the initiation and development of neuroblastoma.

51. BET inhibition induces metabolic rewiring in medulloblastoma cells

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Medulloblastoma (MB) is the most common malignant pediatric brain tumor and comprises four subgroups: wingless (WNT), sonic hedgehog (SHH), Group 3, and Group 4 with Group 3 showing the worst prognosis. Amplification of MYC oncogenes correlates with high-risk disease and represents a therapeutic target. Bromodomain and extraterminal inhibitors (BETis) epigenetically downregulate MYC. We investigated BETis in non-MYC-amplified SHH and MYC-amplified Group 3 MB cell lines.

BETi treatment reduced proliferation in both subgroups. Notably, SHH UW228-3 cells displayed morphological and biochemical changes. In contrast, SHH DAOY cells underwent senescence, evidenced by X-Gal staining and fried egg-like morphology. In MYC-amplified Group 3 D458 cells, bulk RNA sequencing identified thioredoxin-interacting protein (TXNIP), a glucose uptake regulator, as the top upregulated gene following BETi treatment. Increased TXNIP protein levels were confirmed and accompanied by reduced glycolysis. Given reported MYC-mediated TXNIP suppression via miR-17, we investigated this axis: miR-17 inhibition increased TXNIP mRNA levels, whereas BET inhibition reduced miR-17 levels. We next interrogated whether the glucose-responsive MAX-like protein (MLX)–MLX-interacting protein (MLXIP) complex contributed to this metabolic rewiring. Proximity ligation assays (PLA) revealed an increased MLX–MLXIP PLA signal following BET inhibition, suggesting enhanced transcriptional activation at the TXNIP promoter, consistent with TXNIP being an MLX/MLXIP target.

Our results indicate subtype-specific BETi effects in MB, potentially supporting senescence-based therapies for SHH MB and metabolic rewiring for Group 3 MB.

52. Breaking the Break Repair: Comparing Two DNA-PK Inhibitors in Glioblastoma Models

Authors:

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Abstract:

Background/Aim: Pediatric high-grade gliomas, although less common, are highly aggressive, with a reported 2-year survival rate of approximately 12%. Poor outcomes are largely attributed to tumor invasiveness, radioresistance, and high recurrence, partly driven by efficient repair of radiation-induced DNA double-strand breaks through the non-homologous end joining (NHEJ) pathway. DNA-dependent protein kinase (DNA-PK), a central regulator of NHEJ, has emerged as a potential target for enhancing radiotherapy. This study compared the radiosensitizing effects of the selective DNA-PK inhibitor VX-984 and the dual DNA-PK/mTOR inhibitor CC-115 in glioblastoma models.

Methods: Cytotoxicity of both drugs was assessed using an MTT viability assay. Radiosensitization was evaluated using 3D spheroid growth and invasion assays. Cell-cycle distribution, apoptosis, and protein expression were assessed by flow cytometry and Western blot. The radiosensitizing efficacy of VX-984 was further investigated using clonogenic survival assays and validated in a BALB/c nude xenograft model.

Results: Both inhibitors reduced cell viability in a dose-dependent manner. Although both compounds reduced spheroid growth, VX-984 more strongly enhanced radiation-induced growth inhibition, while invasion responses were cell-line-dependent. Despite comparable suppression of DNA-PKcs phosphorylation, VX-984 produced greater radiosensitization, associated with increased p53 expression, greater G2/M accumulation, and enhanced caspase-3/7 activity. VX-984 reduced clonogenic survival dose-dependently, with dose enhancement factors of ~2 and ~3 at 0.5 μM and 2 μM. In vivo, VX-984 combined with irradiation significantly delayed tumor growth and prolonged survival, with no evident systemic or hematological toxicity.

Conclusion: VX-984 demonstrated superior radiosensitizing activity versus CC-115 in glioblastoma models, supporting further investigation as a DNA-PK-targeting radiosensitizer.

53. Binding and Internalization Kinetics of [^{111}In]In-NOTA-Pep1L in IL13Rα2-Targeted Glioblastoma

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Abstract:

Purpose: Interleukin-13 receptor alpha 2 (IL13Rα2) is a tumor-associated receptor that is upregulated in pediatric glioma and represents a promising molecular target for targeted radiopharmaceutical development. Pep-1L, an IL13Rα2-binding peptide, has emerged as a potential agent for selective tumor targeting. This study aimed to characterize [^{111}In]In-NOTA-Pep1L as a targeted radiopharmaceutical in glioblastoma models.

Methods: Pep-1L-NOTA was radiolabeled with Indium-111 via NOTA chelation using radiolysis scavengers and excess methionine to minimize oxidation and radiolysis. Radiochemical yield and purity were assessed using instant thin layer chromatography (iTLC) and reversed-phase high-performance liquid chromatography (HPLC), respectively. Radiochemical stability was evaluated in different chemical environments over time using iTLC. Cellular association and internalization kinetics were assessed in glioblastoma cell lines with varying IL13Rα2 expression. Real-time binding kinetics were investigated using LigandTracer.

Results: [^{111}In]In-NOTA-Pep1L was successfully radiolabeled with a radiochemical yield of 99% and radiochemical purity exceeding 93%. The tracer remained stable in PBS and complete medium, while stability in mouse serum remained above approximately 70% up to 4h, followed by a gradual loss of stability over time. Cellular association was higher in U373 and U251 cells than in U343 cells, consistent with differences in IL13Rα2 expression and supporting IL13Rα2-associated binding. Internalization showed progressive, time-dependent intracellular accumulation over 24h. LigandTracer revealed concentration-dependent binding with micromolar affinity and limited dissociation.

Conclusion: [^{111}In]In-NOTA-Pep1L demonstrated IL13Rα2-associated cellular binding, progressive internalization, and sustained intracellular retention in glioblastoma models. These findings support further preclinical evaluation of Pep-1L as an IL13Rα2-targeted radiopharmaceutical and its further development toward targeted theranostic applications in glioblastoma.

54. Spatial immune profiling reveals lymphocyte confinement to myeloid–mesenchymal niches and heterogeneous therapeutic T-cell opportunity in pediatric ependymoma

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Abstract:

Pediatric posterior fossa group A (PFA) ependymoma is an aggressive, chemo-resistant brain tumor with a poor 10-year overall survival and no therapeutic options at relapse. Although conventionally classified as immune-cold, the spatial organization and therapeutic relevance of its rare immune infiltrates remain poorly characterized. Here, we present a spatially resolved immune analysis of 14 PFA ependymoma sections using published Visium spatial transcriptomics data analyzed through a multi-stage immune-focused computational framework combining reference-guided deconvolution, functional lymphocyte state scoring, ligand–receptor inference, and αβ/γδ T cell immunotherapy opportunity scoring.

Lymphocyte-associated hotspots were identified in 6 of 14 sections and were dominated by an inflammatory and tissue-retention functional state rather than active cytotoxic or exhausted programs. These hotspots were preferentially confined to myeloid- and mesenchymal-rich tumor zones with near-complete exclusion from epithelial regions; ligand–receptor analysis identified four candidate restriction axes (SPP1–CD44, FN1–integrin, collagen VI–integrin, and APP–CD74). Immunotherapy opportunity scoring revealed heterogeneous and spatially compartmentalized αβ and γδ T cell recognition landscapes across sections: αβT ligand availability and immunosuppressive tone both rose in the mesenchymal compartment, while the two components of γδ phosphoantigen-mediated recognition were spatially and transcriptionally dissociated from one another.

Together, these findings reframe PFA ependymoma not as a monolithic immune desert but as a tumor type with spatially compartmentalized, mechanistically tractable immune niches, and provide a generalizable computational strategy for mapping immune architecture and T cell therapy susceptibility in low-infiltrated pediatric CNS tumors.

55. NADPH oxidases modulate glioblastoma cells tumorigenic capacity

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Abstract:

Glioblastoma (GBM) is the most common and aggressive glioma, with a median survival of 15 months. A subpopulation of glioblastoma stem cells (GSCs) drives tumor initiation, invasion, recurrence, and resistance to therapy. Elevated oxidative stress, caused by an imbalance in reactive oxygen species (ROS) production and antioxidant defenses, is a hallmark of GBM. To investigate the role of NADPH oxidases (NOXs) in GBM progression, we analyzed patient datasets and experimental models.

Analysis of TCGA data revealed that high NOX2 and NOX4 expression correlates with poor patient survival, whereas NOX1 correlates with improved survival. In patient samples, NOX1 and NOX4 were enriched in GSCs, while NOX2 was more highly expressed in differentiated tumor cells. In vivo, NOX1 overexpression reduced tumor progression in both sexes. In contrast, NOX2 and NOX4 promoted tumor growth in male mice but reduced tumor burden in females, highlighting sex-dependent effects. In patient-derived GBM cells, NOX2 and NOX4 enhanced proliferation and stemness.

Transcriptomic analyses showed that NOX overexpression increased genes associated with synapse organization and axonogenesis. NOX2 and NOX4 suppressed immune-response pathways, whereas NOX1 induced immune-related gene expression. Metabolomic analyses further revealed that NOX2 increased GABA levels, while NOX1 and NOX4 reduced them. Consistently, pharmacological inhibition of GABA receptors attenuated NOX2-driven proliferation.

Collectively, these findings demonstrate distinct functions of NOX isoforms in GBM. NOX2 and NOX4 promote tumor progression, while NOX1 exerts tumor-suppressive effects. The identification of sex-specific NOX functions and a NOX2-GABA signaling axis highlights novel mechanisms of GBM progression and potential therapeutic targets for personalized treatment.

56. Complementary in vivo models for studying SHH Medulloblastoma initiation, progression, and microglial remodeling

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Background: Medulloblastoma (MB) is the most common malignant pediatric brain tumor, and Sonic Hedgehog (SHH) MB is one of molecular subgroups characterized by aberrant activation of the SHH signaling pathway, driven by genetic alterations such as PTCH1. SHH-MB arise from granule neuron progenitors (GNPs) during early postnatal development, a period when the cerebellar microenvironment undergoes maturation. Microglia, the resident immune cells of the central nervous system (CNS), are highly dynamic during this developmental window and play important roles in tissue remodeling, phagocytosis, and neuronal development. However, how microglial change from tumor initiation to established disease remains poorly understood, highlighting the need for in vivo models that capture diWerent stages of SHH-MB.

Aim: To establish two in vivo models that capture diWerent stages of SHH-MB development and to investigate stage-dependent microglial remodeling during tumor initiation and progression.

Research progress and results: We generated a Atoh1-Cre;Ptch1fl/fl conditional mouse model in which exon 2 of Ptch1 is flanked by loxP sites. Mutant mice developed rapidly progressive disease, with sickness symptoms emerging around 2 weeks after birth and progression to endpoint by 3 weeks. Histological analysis showed cerebellar structural abnormalities during early postnatal development, followed by extensive tumor formation. Established tumors expressed the SHH-MB markers. Preliminary result showed alterations in microglial morphology. In addition, we also established an orthotopic xenograft model using PTCH1- mutant human neural stem cells(NES). Longitudinal bioluminescence imaging was used to monitor tumor burden and stratify mice into early and late stage groups. Microglia displayed distinct morphological changes across tumor stages. Ongoing work focuses on fluorescence- activated nuclei sorting (FANS) using the myeloid-lineage transcription factor PU.1 to enrich myeloid nuclei for single-nucleus RNA sequencing (snRNA-seq).

57. The c-Myc transactivation domain binds Aurora kinase A through multivalent Myc-box interactions and enhances kinase activity

Authors: Vivian Morad[1], Johanna Hultman[1], Dean Derbyshire[1], Simon Ekström[2] Alexandra Ahlner[1] and Maria Sunnerhagen[1]

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Abstract:

Myc is a master gene regulator involved in normal cellular processes, but its overexpression in many human cancers is associated with poor prognosis[1]. Aurora kinase A is essential for cell division and spindle assembly; however, it is overexpressed in several human cancers[2]. Aurora A is tightly regulated by Myc[3] and interacts with N-Myc, protecting it from proteasomal degradation[4 5]. Aurora A and c-Myc are frequently co-amplified and co-expressed in cancer cells[6] raising the question of whether Aurora A also interact directly with c-Myc.

Here, we use complementary biophysical and structural approaches to characterize the interaction between the Aurora A and the c-Myc transactivation domain. We show that Aurora A binds directly to c-Myc through a dynamic and multivalent mechanism involving multiple Myc-box regions. The full c-Myc transactivation domain binds with higher apparent affinity than the shorter MB0–MBI construct, indicating contributions beyond MBI. HDX-MS data map the interaction mainly to the Aurora A N-lobe, including the regulatory β3–αC/αB–αC region, with additional effects in the C-lobe. This binding is independent of Auroras catalytic activity, but c-Myc enhances Aurora A kinase activity in an MB0-dependent manner. These findings clarify the interplay between two oncogenes in tumorigenesis and may guide future strategies to target c-Myc in Aurora A-overexpressing tumors.

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58. RUVBL1 and RUVBL2 are druggable MYC effector regulators in neuroblastoma cells

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Abstract

High-risk neuroblastoma is characterized by MYCN amplification and high MYCN or MYC gene expression. These patients have a poor prognosis, and there is an urgent need for more effective drugs. While strategies to develop inhibitors that directly target the MYC proteins have remained largely unsuccessful, recent preclinical studies have identified ATR, a key protein of the DNA damage response, as a promising alternative therapeutic target. Here, we identified a strong RUVBL[1] and RUVBL2 signature in transcriptomics data derived from different MYCN-driven mouse tumors treated with ATR inhibitors. The RUVBL proteins form a complex with ATPase activity that has broad cellular functions. We demonstrate that pharmacological inhibition of this complex results in a strong reduction of MYC(N) signaling, cell-cycle arrest, DNA damage, and apoptosis. We confirmed the association with MYCN and identified the RUVBL genes as independent prognostic biomarkers in human primary neuroblastoma data.

59. Multi-Omics Analysis Reveals Regulatory Changes in Pediatric High-Grade Glioma Stem Cells

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Abstract:

Pediatric high-grade gliomas (pHGG) are aggressive brain tumors with poor prognosis and glioma stem cells (GSCs) are thought to play central role through epigenetic mechanisms. This aim of this project is to identify differences in chromatin accessibility, gene expression, and protein abundance in patient-derived pHGG-GSCs compared to normal neural stem cells (NSCs), in order to identify transcription factors (TF) that may be driving the malignant state. We performed integrative analysis of ATAC-seq, RNA-seq, and proteomics data from patient-derived pHGG-GSCs and normal NSCs. The analysis included differential accessibility, expression, and abundance analyses, genomic annotation, motif enrichment, and TF footprinting analysis. We found that pHGG-GSCs increased chromatin accessibility, accompanied by up-regulation of genes related to proliferation and down-regulation of genes related to neuronal development. By combining motif accessibility, TF occupancy, gene expression, and proteomics data, we identified candidate TFs in pHGG-GSCs. Candidate TFs are important for maintaining normal neural stem cell identity, showed reduced activity in pHGG-GSCs. Overall, the results suggest that the transition from NSCs to pHGG-GSCs involved a broad epigenetic shift. These findings may help guide future work on diagnosis markers and therapeutic targets in pediatric high-grade gliomas.

60. Targeting Relapse-Associated Cell Populations in Diffuse Midline Glioma Using CAR T Cell Therapy

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Diffuse midline gliomas (DMGs) are highly aggressive pediatric brain tumors characterized by poor prognosis and limited therapeutic options. Tumor localization frequently results in inoperability that restricts treatment to irradiation, which prolongs survival but rarely prevents relapse. Emerging evidence suggests that quiescent, stem- or stress-like states may converge in relapse-competent brain tumor cells capable of evading standard therapies. CAR T therapy has shown a clinically accepted toxicity profile in DMG patients resulting in an accelerated effort to develop successful strategies. A target with potential promise for CAR-design is the surface marker F3, implicated in dormancy and therapeutic resistance co-expressed with SOX9, a marker associated with relapse in pediatric brain tumors. To evaluate our F3-directed CAR T cells we employ advanced preclinical three-dimensional (3D) assembloid models, DMG patient-derive cell lines (PDLCs) co-cultured with brain organoids. Live cell tracking in DMG assembloids demonstrate tumor cell clearance, and further analysis suggests limited impact on normal cells. Higher levels of F3 are associated with a more rapid and stronger CAR T response validated by higher levels of cytokine- and granzyme b release, with subsequent greater DMG PDCL killing efficiency. This study illustrates the use of DMG assembloids as efficient preclinical models for evaluation of CAR T cell efficacy, with the ultimate goal of contributing to the development of effective immunotherapeutic approaches for recurrent DMG.

61. GSPT1-specific protein degradation is effective in preclinical models of chemoresistant MYCN-amplified neuroblastoma

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Background: Neuroblastoma (NB) is a pediatric malignancy arising from the developing sympathetic nervous system, with high-risk (HR) disease associated with dismal prognosis due to frequent treatment failure and resistance. Targeted protein degradation (TPD) enables pharmacological elimination of disease-driving proteins and has recently emerged as a promising approach in cancer therapy. GSPT1, a translational terminal factor, has been identified as a potential dependency in MYCN-driven malignancies, but whether its degradation can be exploited therapeutically in NB has not previously been established.

Methods: GSPT1-directed molecular glues (MGs) were evaluated in PDX-derived NB organoids. In vivo therapeutic efficacy was assessed in chemoresistant MYCN-amplified PDX models and compared to standard-of-care chemotherapy. Transcriptomic and histopathological analyses characterized treatment responses.

Results: GSPT1 expression was increased in MYCN-amplified NB and correlated with adverse clinical outcome. Treatment with GSPT1-directed MGs resulted in selective GSPT1 depletion, reduced cell viability and increased apoptotic cell death at nanomolar concentrations. In vivo, GSPT1 degradation induced sustained tumor regression in a highly chemoresistant model, resulting in significantly prolonged survival compared with chemotherapy. High-dose treatment achieved complete tumor eradication, with no evidence of recurrence during 150 days of follow-up. Histopathological analyses demonstrated morphological differentiation and increased apoptosis, accompanied by reduced MYCN expression.

Conclusions: GSPT1 degradation is a potential therapeutic approach in MYCN-amplified, chemoresistant HR-NB. The pronounced antitumor response observed in vivo supports further investigation of GSPT1-directed MGs, including optimization of dosing, treatment schedules and evaluation of potential off-target effects, toward future clinical translation.

62. Identifying Mechanisms of Resistance to p53 Reactivation in High-Grade Glioma

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Abstract:

High-grade gliomas (HGG) are a group of brain malignancies, affecting both children and adults, with a dismal prognosis. They are characterised by extensive inter- and intratumoural heterogeneity, posing a significant challenge for treatment. In a subset of these tumours, the tumour suppressor TP53 is functionally inactivated despite retaining wild-type status, making them ideal candidates for targeted therapies aimed at restoring p53 activity. Mouse double minute 2 (MDM2) is a key negative regulator of p53 in this pathway, and its inhibitor, Navtemadlin, has demonstrated blood–brain barrier penetration and p53 reactivation in a Phase 0 glioblastoma trial. However, long-term clinical responses have remained unsustained, suggesting the presence of compensatory survival pathways that cause the tumour to relapse. Therefore, we aim to identify genes that play a critical role in these compensatory pathways, using high-throughput CRISPR activation (CRISPRa) screens, long-term drug exposure models, and functional validation of the candidate mediators of treatment resistance to investigate the mechanisms underlying resistance to p53 reactivation. In parallel, we also employ barcoding of the cell lines to enable lineage tracing and identify the subpopulations that give rise to resistance.

We intend to leverage these findings to design combination therapies that specifically target resistant cell populations within the highly heterogeneous landscape of HGG, in order to promote a sustained therapeutic response. Given the limited treatment options currently available for HGG, we hope that such combination treatments will increase efficacy and extend the duration of clinical benefit, ultimately improving survival outcomes for patients.

63. Single cell analysis of neuroblastoma mutations reveals an early selection of cells with specific chromosomal rearrangements in metastasis

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Abstract:

Neuroblastoma (NB) is a highly heterogeneous disease with a broad range of clinical outcomes and prognosis, and an expected low mutational burden. In our study, we aimed to define the genetic and transcriptional changes from cells in human primary tumors to metastasis. To this end, we obtained and sequenced bulk and single cell-RNA from NB tumors, and studied the transcriptional and mutational landscapes in primary tumors and metastasis.

We identified that metastasis and primary tumors presented no significant difference in the estimated tumor burden from bulk- and pseudo-bulk RNA-sequences. Nevertheless, we found a significant higher mutational burden in neoplastic cells from primary tumors compared with those from metastasis. On the contrary, recurrent chromosomal changes in regions associated with poor outcome, were observed with a significantly higher frequency in cells from metastasis than in cells from primary tumors. Paired with these mutations, a high noradrenergic/low mesenchymal-like transcriptional profile was observed in cells of primary tumors, while cells in the metastasis presented a high mesenchymal-like/low noradrenergic profile.

Our results suggest that cells originating metastasis in NB derive from cells ancestral to terminal primary tumors that undergo early selection events, coupled with transcriptional changes leading to enhanced invasion capabilities. We expect that the results of this research contribute to the early diagnosis and treatment of metastasis in high-risk NB patients.

64. Optimizing low-input profiling of RNA modifications in neuroblastoma clinical samples enables detection of MYCN-associated m6A patterns

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Abstract: RNA modifications are an emerging layer of post-transcriptional regulation in cancer, but their profiling in clinical tumor material is often limited by RNA quantity and quality. We aimed to establish a low-input workflow for simultaneous profiling of N6-methyladenosine (m6A) and inosine in neuroblastoma. We optimized an EpiPlex-based workflow using 300 ng total RNA, focusing on RNA input and fragmentation conditions. Optimization was performed in BE(2) neuroblastoma cells, for which independent m6A-RIP data were available as an orthogonal reference. The workflow was subsequently applied to primary neuroblastoma tumors from a German cohort (n=28) and an ongoing Swedish cohort, together with eight neuroblastoma cell lines and four PDX-derived cell models. Adaptation for formalin-fixed paraffin-embedded (FFPE) RNA is also being evaluated. In BE(2) cells, EpiPlex-derived m6A profiles showed highly similar peak distributions, metagene patterns, and enrichment at known m6A sites compared with m6A-RIP data, supporting the reliability of the optimized workflow. Preliminary analysis of primary tumors further indicates that m6A profiles can distinguish MYCN-amplified from non-MYCN-amplified neuroblastomas. These findings demonstrate the feasibility of low-input m6A and inosine profiling in clinically relevant neuroblastoma material. Further optimization for FFPE-derived RNA could broaden the applicability of epitranscriptomic profiling to routinely collected clinical specimens and enable larger retrospective studies.

65. Liquid biopsies for improved diagnostics and monitoring of paediatric CNS tumours: the Swedish collaboration: SMALL-CNS

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Abstract:

Liquid biopsies are a potential clinically important biomarker for cancer diagnostics and monitoring. In CNS tumours, the cerebrospinal fluid (CSF) contains cell free DNA (cfDNA), extracellular vesicles and proteins that derive from the CNS tumour cells and these can be detected in a minimally invasive lumbar puncture. We have set up a team of clinicians specializing in paediatric CNS tumours together with researchers working in translational projects to measure genomics, methylation and proteomics in CSF. We have shown that targeted droplet digital PCR can detect tumour-derived cfDNA even when very low amounts of cfDNA are shed into CSF. If the tumour sheds more cfDNA into the CSF, multi-omic characterisation with methylation analysis and next generation sequencing profiling of point mutations and copy number aberrations can provide important diagnostic information. We now aim to further develop these tools and to add on proteomics and analysis of extracellular vesicles to enhance the diagnostic yield.

66. Astrocyte-expressed FASN as a novel candidate drug target for glioblastoma

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Keywords: Glioblastoma, Microenvironment

Background: Glioblastoma (GBM) is an aggressive cancer requiring deeper understanding and new therapeutic approaches. The tumoral microenvironment guide cancer cell progression. Our previous studies demonstrated that astrocytes (ACs) enhance GBM proliferation, as evidenced by analyses of clinical samples and experimental studies.

Methods: HTS screening (14000 compounds) was performed on co-cultured GBM cells and astrocytes. Tumor growth and self-renewal capacity were assessed via cell proliferation and tumor-sphere formation assays (Luc-GFP system). Human GBM specimens were analyzed using multiplex profiling to detect FASN+GFAP+ astrocytes and Ki67+ proliferative cancer cells. Mitochondrial transfer from ACs to GBM cells was quantified using confocal imaging and FACS. FASN inhibitors potency screening was used to correlate FASN cellular activity and co-culture activity. Non-FASN inhibitory hits were explored by Proteome Integral Solubility Alteration (PISA) Assay for target identification. An orthotopic xenograft mouse model was employed to evaluate the efficacy of FASN inhibition for treating GBM in vivo.

Results: Primary screening identified approximately 130 specific hits. Bioinformatic analysis indicated fatty acid synthase (FASN) as an enriched target. FASN knockdown in astrocytes significantly reduced their support of GBM proliferation and self-renewal. Selected compound from screening showed reduced or no activity in co-cultures with FASN KD astrocytes. Strong correlation was observed between cellular FASN inhibitory potency and growth-inhibitory activity among FASN inhibitors. Free fatty acid supplementation rescued GBM growth in co-cultures with FASN-knockdown astrocytes but not in wild-type astrocyte co-cultures. FASN knockdown reduced mitochondrial transfer from astrocytes to GBM cells, while MIRO knockdown suggested mitochondrial transfer is essential for FASN-mediated AC-supported growth. PISA analyses of hits not targeting FASN also indicated effects on fatty acid metabolism. Preliminary multiplex staining demonstrated positive correlation between FASN-positive astrocytes and Ki67+EGFR+GBM cells in human samples. In vivo, FASN inhibition significantly extended the overall survival (OS) of mice bearing GBM tumors derived from the co-injection of GBM cells and astrocytes.

Conclusion: Screening results, tissue profiling, and knockdown experiments collectively identify astrocyte-derived FASN and fatty acid metabolism as critical

drivers of astrocyte-supported GBM growth. Mechanistic studies further reveal that FASN mediates mitochondrial transfer from astrocytes to GBM cells, a process essential for tumor progression. Consistently, targeting FASN yields promising therapeutic outcomes in suppressing astrocyte-supported GBM growth in both in vitro and in vivo models.

67. Benchmarking methylation-derived CNV calling for CDKN2A/B and integrating methylation subclassification identifies prognostic hemizygous loss in IDH-mutant astrocytomas

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Abstract:

Background: The 2021 WHO classification of IDH-mutant astrocytomas incorporates histological criteria and homozygous CDKN2A/B deletion for tumor grading. With increasing use of DNA methylation profiling in CNS tumor diagnostics, CDKN2A/B copy-number status can be inferred from array-derived CNV data; however, standardized assessment methods are lacking.

Methods: We used genome-wide SNP array data to evaluate the prognostic significance of CDKN2A/B hemizygous deletion in IDH-mutant astrocytomas. We then compared visual and quantitative approaches for assessing CDKN2A/B status from DNA methylation array-derived CNV profiles in two independent cohorts (~400 cases).

Results: CDKN2A/B copy-number status consistently stratified overall survival using both visual and numerical assessments. Visually defined hemizygous deletions were associated with intermediate survival between homozygous deletion and retained CDKN2A/B. Quantitatively, both shallow (−0.2 to −0.4) and deep (−0.4 to −1.1) hemizygous losses were associated with worse survival than retained CDKN2A/B, with deep losses showing poorer outcomes than shallow losses. These findings support a graded increase in risk as copy-number values approach homozygous deletion (<−1.1). We further developed an integrated model combining methylation subclassification with CDKN2A/B status.

Conclusions: CDKN2A/B hemizygous deletion is an independent prognostic factor associated with intermediate risk in IDH-mutant astrocytomas. Numerical

assessment provides a standardized framework for estimating copy-number status, whereas visual assessment provides whole-genome CNV context. Integrating methylation subclassification with CDKN2A/B status may further refine patient risk stratification.

68. Matched primary-relapse DMG patient-derived xenograft models to identify therapy resistance profiles

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A major challenge in cancer care is predicting a patient's risk of relapse after primary treatment. Diffuse Midline Gliomas (DMGs) are intrinsically resistant to standard chemoradiotherapies, and high-throughput genomic profiling of primary and relapse tumors integrated with functional precision medicine are used here to target relapse-initiating cells early. We established pre-clinical experimental models from a matched primary–relapse pair of an individual DMG patient to study tumor evolution, validating tumor plasticity between the primary and

relapse samples and validate phenotypes observed in patient-derived xenografts (PDXs), xenograft-derived cell models (XDCLs) and three-dimensional assembloids. Cross-model evaluation revealed conserved yet distinct transcriptional programs between primary and relapse, with identification of persistent ACVR1 mutation, increased proliferation associated pathways, specifically PI3K-AKT-mTOR axis and reduced MDM4-TP53-signalling in relapse tumor. Paired analysis offered a longitudinal view of tumor evolution, revealing actionable targets, resistance mechanisms, and opportunities for personalized therapy. Phenotypic characterizations across different models through invasion analyses defined a proliferation-driven bulky growth pattern in relapse tumors with high OPC-like signatures. Relapse cultures displayed reduced sensitivity to radiation treatment which highlighted the emergence of resistance in the relapse induced through increased mTOR signaling and reduced TP53 pathway. While certain PI3K/mTOR inhibitors showcased attenuated growth in relapse derived models, incomplete responses showing compensatory signaling pathways. Combination strategies targeting both mTOR and ACVR1 pathways showed greater reduction in cell growth. Our work underlines the importance of modelling disease evolution, showcasing distinct therapeutic vulnerabilities in relapse compared to primary counterparts.

69. Targeting cross-resistance in neuroblastoma: Mechanistic investigation of immune checkpoint blockade

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Patients with high-risk neuroblastoma frequently relapse or fail to respond to intensive multimodal regimens, which can severely affect the long-term health of survivors. This highlights the need for safer and more effective therapeutic strategies. Immunotherapies have demonstrated clear survival benefits but substantial toxicity in selected patients (1), whereas immune checkpoint blockade (ICB) has a favorable safety profile but no objective responses (2). Our work aims to determine whether reduced chemotherapy effectiveness correlates with mechanisms which resonance resistance to immunotherapy. With the usage of a neuroblastoma cell line pair – CHLA-15 established at diagnosis and CHLA-20 established at progressive disease during therapy – we reveal distinct phenotypic state. Immune-relevant molecules, such as PD-L1, HLA-ABC and HLA-DR/DP/DQ expression on cancer cells help portray how the tumor interacts with the immune system and hypothesize responsiveness to immunotherapy. We observe CHLA-20 retainment of antigen presentation machinery and intact interferon signaling, however, expression of HLA-II failed induction. CHLA-15 and CHLA-20 demonstrated distinct immunogenicity when in co-culture, evidenced by a significant difference in cytokine secretion (CHLA-20 with lower or no response) thus attesting cross-resistant to ICB. A whole genome CRISPR/Cas9 screen identified lipid metabolism as a potential target for restoring sensitivity to PD-1 blockade. Future perspectives comprise public RNA-seq datasets, proteomic profiling, flow cytometry, immunoblotting and pharmacological inhibition of selected gene candidates to support the hypothesis.

Candidates will be evaluated in tumor-immune co-culture assays and findings translated into a humanized mouse model to assess the impact of neuroblastoma evasion tactics and drug efficacy.

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70. Withdrawn

71. Comparative m6A epitranscriptomics reveals tumor- specific RNA modification alterations in pediatric glioma stem cells

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Pediatric type diffuse high-grade gliomas (pHGGs) are aggressive and treatment-resistant brain tumors that contribute to poor survival outcomes in children. Although recent studies have advanced our understanding of the molecular mechanisms underlying the disease, its etiology and pathogenesis remain incompletely understood. Recent research highlights the pivotal role of glioma stem cell-like cells (GSCs) in driving tumor progression, therapeutic resistance, and recurrence. Epitranscriptomics is an emerging field that represents an additional regulatory layer in cellular gene expression. N6-methyladenosine (m6A) is a major post-transcriptional RNA modification with emerging roles in various cancers, yet its landscape in GSCs relative to normal neural stem cells (NSCs) remains poorly defined. We profiled the m6A epitranscriptome and matched transcriptome of nine patient-derived pediatric GSC and six fetal NSC models using m6A MeRIP-seq and RNA-seq and complemented the cohort analysis with Oxford Nanopore direct RNA sequencing in representative GSC and NSC samples. Global analyses revealed a reduction in overall m6A enrichment in GSCs compared with NSCs, while retaining the characteristic transcript-wide distribution of m6A. Analysis of peak prevalence across independent GSC and NSC lines identified GSC-enriched, NSC-enriched, and shared m6A peak classes. Native direct RNA sequencing provided site-resolved validation of selected m6A alterations identified across the MeRIP-seq cohort and revealed consistently higher mean transcript-wide m6A levels in the NSC model than in the GSC model, further supporting a global reduction in m6A level in GSCs relative to NSCs. Metagene profiling recapitulated the canonical transcript-wide distribution of m6A, with prominent enrichment at the CDS-3'UTR boundary. Collectively, these findings indicate that pediatric GSCs are characterized by a global reduction in m6A accompanied by selective redistribution of modification across specific transcripts and transcript regions. This analysis highlights candidate RNA modification alterations in pediatric glioma for future functional investigation.

72. MYC-driven pediatric brain tumors share a common photoreceptor identity

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Medulloblastoma (MB), pineoblastoma (PB) and retinoblastoma (RB), three types of malignant neural pediatric brain tumors are anatomically situated in different parts of brain but share a common feature- elevated expression of a photoreceptor 'program'. MYC-amplified MBs and PBs are often histologically indistinguishable. Both grow aggressively, often relapse after standard treatment and have a dismal prognosis. Trilateral RBs are rare but often present with aggressive eye tumors first and later on PBs. It is important to understand how these photoreceptor-positive tumors arise and to know if they could be similarly treated. CRX, a transcription factor critical for retinal and pineal cell development is also a master regulator for MB-Group3 tumor maintenance. To understand if MYC-driven MB, PB and RB can arise from specific photoreceptor-positive progenitors in the developing brain, we performed Crx-lineage tracing with tamoxifen injections. We could confirm that Crx-positive cells exist in retina and in pineal gland progenitors as expected. However, Crx-traced cells also marked granule neurons in the flocculo-nodular lobe of the cerebellum, which is a recently suggested site of MB-Group3 origin arising from the developing rhombic lip. To investigate if the Myc oncogene can generate different types of pediatric brain tumors in Crx-positive cells from retina, pineal gland and cerebellum, XMYCT58A and XMYC was established by crossing Crx-CreERT2 strain with MycT58A strain and Myc stain. Here MycT58A and Myc oncogenes were turned on with tamoxifen injection after birth. Notably, Myc overexpression resulted in tumor development around 2-3 months of age, which is significantly earlier compared to stabilized MycT58A, where tumors developed around 4-6 months. XMYCT58A and XMYC mice developed brain tumors from pineal gland and cerebellum. Histologically, tumors were non-glial (GFAP-, Olig2-), showed neuronal activity (Neurod1+) and stained positive for photoreceptor markers (CRX+, OTX2+) similar to both MB-Group3 and PB-MYC. Our data suggest that MYC-driven Group 3 MB, PB and RB all could originate photoreceptor positive cells, which has implications for future research and the development of novel treatments targeting these devastating childhood malignancies.

73. Liquid biopsies for improved diagnostics and monitoring of paediatric CNS tumours: the Swedish collaboration: SMALL-CNS

Authors:

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Abstract:

Liquid biopsies are a potential clinically important biomarker for cancer diagnostics and monitoring. In CNS tumours, the cerebrospinal fluid (CSF) contains cell free DNA (cfDNA), extracellular vesicles and proteins that derive from the CNS tumour cells and these can be detected in a minimally invasive lumbar puncture. We have set up a team of clinicians specializing in paediatric CNS tumours together with researchers working in translational projects to measure genomics, methylation and proteomics in CSF. We have shown that targeted droplet digital PCR can detect tumour-derived cfDNA even when very low amounts of cfDNA are shed into CSF. If the tumour sheds more cfDNA into the CSF, multi-omic characterisation with methylation analysis and next generation sequencing profiling of point mutations and copy number aberrations can provide important diagnostic information. We now aim to further develop these tools and to add on proteomics and analysis of extracellular vesicles to enhance the diagnostic yield.

74. Diagnostic Evaluation of Long-Read Nanopore Sequencing for Pediatric CNS Tumors

Authors:

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Abstract:

Long-read nanopore sequencing enables methylation-based CNS tumor classification while providing additional genomic information within a single assay. However, its performance across molecular features relevant to pediatric CNS tumor diagnostics and across available methylation classifiers remains incompletely characterized. We evaluated nanopore sequencing for integrated molecular characterization of pediatric CNS tumors and compared with data from DNA methylation arrays and Illumina short-read sequencing used in the clinical diagnostic work-up.

Twenty tumor samples representing three major pediatric CNS tumor groups (gliomas, ependymomas and medulloblastomas) were subjected to long-read nanopore sequencing, which generated robust long-read data across all tumor groups, with a mean genome coverage of approximately 40x. Nanopore-derived copy-number profiles showed good concordance with methylation array-derived profiles (median Pearson r = 0.816).

Nanopore evidence was identified for 74/78 (95%) clinically relevant small variants detected by Illumina sequencing, although 53/78 (68%) were recovered as exact high-confidence nanopore calls. Structural variant profiles showed limited breakpoint overlap and differed in the number, type, and size distribution of detected events between Illumina and Nanopore sequencing.

Nanopore-derived methylation classifications were largely concordant with integrated WHO CNS5 diagnoses. Ependymomas, medulloblastomas, and pilocytic astrocytomas, were concordantly classified across all evaluated classifiers, although not all classifications reached the respective classifier-specific confidence thresholds. In contrast, diffuse gliomas showed greater variability in classification across classifiers. Downsampling indicated that concordance with integrated WHO CNS5 diagnoses remained high at substantially reduced sequencing depths, whereas the proportion of classifications reaching classifier-specific confidence thresholds was more classifier dependent. Long-read nanopore sequencing enables integrated molecular characterization of pediatric CNS tumors within a single assay, although concordance with established methods varied by genomic feature and tumor type. The preservation of methylation classification performance at reduced sequencing depth supports the feasibility of shorter sequencing workflows. Prospective studies are required to establish performance and turnaround time in routine clinical diagnostics.

75. Spatial Mapping of Tumor Cell States Across Primary, Metastatic, and Relapsed Neuroblastoma

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Abstract

Neuroblastoma exhibits extensive genetic and transcriptional heterogeneity, yet how malignant cell states evolve across primary tumors, metastases, and relapse remains poorly understood. In particular, whether mesenchymal-associated transcriptional programs represent stable malignant cell identities or context-dependent adaptations during tumor progression and treatment remains unresolved.

Here, we integrated high-plex spatial transcriptomics with whole-genome sequencing (WGS) to investigate tumor heterogeneity and clonal evolution across 24 neuroblastoma specimens from nine patients, including matched primary, metastatic, and relapsed tumors. Tumor specimens were obtained through the Swedish Childhood Tumor Biobank (Barntumörbanken). Using the Xenium In Situ platform with a 5,000-gene panel supplemented by 100 custom-selected genes, we profiled approximately 340,000 cells following quality control. Non-negative matrix factorization (NMF) identified recurrent transcriptional metaprograms, while inferCNV revealed putative malignant clones whose characteristic copy-number alterations were independently supported by matched WGS data.

Spatial analysis revealed heterogeneous malignant cell states and distinct microenvironmental compositions across tumor sites and disease stages. To characterize adrenergic and mesenchymal-associated transcriptional programs, we applied the recently described 28-gene ADRN and MES signatures developed by Patel et al. (Cancer Cell, 2026) 1. These signatures revealed variable transcriptional states among malignant cells. Interestingly, elevated MES28 scores were observed in pretreated primary tumors but were less apparent in the two relapsed specimens examined. This observation raises the possibility that MES-associated transcriptional features reflect treatment-related or microenvironment-dependent adaptations rather than exclusively defining stable mesenchymal tumor identities.

Together, our findings establish a spatially resolved framework for investigating the relationships between clonal architecture, transcriptional plasticity, and the tumor microenvironment during neuroblastoma progression. Our observations further highlight the importance of distinguishing mesenchymal-associated

transcriptional programs from stable malignant cell identities and suggest that treatment history may influence the interpretation of ADRN and MES signatures in patient tumors.

1. Patel, A. G. et al. Spatial omics resolves adrenergic and mesenchymal cell states in neuroblastoma. Cancer Cell https://doi.org/10.1016/j.ccell.2026.09.008 (2026) doi:10.1016/j.ccell.2026.09.008.

76. Irradiation Induces a Delayed Meningeal Inflammatory Response

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Background

The meninges are membranous layers covering the central nervous system (CNS). They play critical structural and physiological roles in maintaining CNS homeostasis and are implicated in CNS morbidities, including cancer. The meninges represent a niche for primary and metastatic cancers, where radiotherapy and chemotherapy are among the standard-of-care therapies to treat these malignancies. While the effects of these therapies on CNS parenchymal cells have been extensively studied, the effects of radiotherapy and chemotherapy on meninges remain largely unexplored. Here, we investigated the temporal molecular responses of human meningeal fibroblasts to ionizing irradiation and the chemotherapeutic agent methotrexate.

Methods

Human meningeal fibroblasts were subjected to clinically relevant doses of irradiation and methotrexate. We performed transcriptomic profiling of the cellular components and protein analysis of their secretome 24 hour and 1 week following treatment.

Results

We found that irradiation, but not methotrexate, induces significant and dynamic transcriptional alteration in the meningeal fibroblasts. Irradiation acutely activated cell-cycle arrest programs and triggered inflammatory programs during the delayed phase, leading to significantly increased expression and secretion of cytokines and chemokines, including IL1A, LIF, CXCL1, CXCL3, CXCL6, and CXCL8.

Conclusions

Our findings suggest that the leptomeninges undergo a stromal-mediated adaptive response following radiotherapy, potentially creating a cytokine- and chemokine-rich meningeal niche. The inflammatory meninges may contribute to cancer progression or recurrence within this compartment, or even contribute to neurotoxicity observed in cancer survivors who received cranial radiotherapy, where neuroinflammation is a key factor in this process.

77. Modelling Neuroblastoma Dual Loss of NF1 and CDKN2A

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High-risk neuroblastoma frequently metastasises or relapses despite intensive treatment. Tumour cells occupy different developmental and transcriptional states, but how these states are organised within tumours and change during progression remains poorly understood. In particular, we do not know which malignant states and surrounding tissue niches are associated with metastatic disease.

The overall aim of this project is to identify spatially organised tumour-cell programmes associated with neuroblastoma progression and investigate their functional significance. will analyse primary, metastatic, and relapse samples from Barntumörbanken using spatial transcriptomics and existing single-cell reference data. We will then investigate selected findings in a genetically engineered Dbh-Cre; *Nf1/Cdkn2a* model. Preliminary observations indicate that these mice develop spontaneous metastases at multiple sites, including lung, liver, ovary, pancreas, intestine, and lymph nodes.

We hypothesise that progression is associated with specific tumour-cell programmes and spatial niches, and that Nf1/Cdkn2a loss promotes a metastatic phenotype that can be used to investigate some of these programmes experimentally. We will also test whether CDK4/6-dependent signalling contributes to metastatic behaviour in the model.

Specific Aims

- ***Identify tumour-cell states and spatial niches associated with progression in human neuroblastoma. Compare available paired primary and metastatic or relapse samples, and validate selected findings in additional patient material.***
- ***Define cellular and spatial mechanisms underlying metastasis of the Nf1/Cdkn2a model and assess its relationship to human disease. Establish tumour pathology, metastatic incidence and distribution, and the spatial organisation of primary and metastatic tumours. Determine which progression-associated features identified in human samples are represented in the model.***
- ***Test a prioritised mechanism of metastatic behaviour. Select a candidate supported by the human and mouse analyses, with particular attention to CDK4/6-dependent signalling. Test its effects on tumour-cell state, migration or invasion, and metastatic growth using appropriate experimental models.***

Although distinct malignant cell states have been described in neuroblastoma, their spatial organisation in primary and metastatic tumours remains incompletely understood. Analysis of samples from different disease sites and time points will reveal whether progression is associated with changes in tumour-cell identity, the surrounding tissue, or their interaction.

The *Nf1/Cdkn2a* model provides a unique opportunity to study spontaneous dissemination from a sympathoadrenal tumour across multiple organs. Comparing its primary tumours and metastases with human samples will establish which features of progression are shared and provide a basis for mechanistic experiments. The project therefore connects clinically observed spatial programmes with experimental tests of metastatic behaviour.

78. Advancing Precision Medicine in Childhood Cancer: Towards 100% survival with fewer long-term complications

*Barncancerfonden -the Swedish Childhood Cancer Fund**

Background

Precision medicine is changing how childhood cancer is understood, diagnosed and treated. The Swedish Childhood Cancer Fund supports investigator-driven research across the field, while also making strategic investments in research and healthcare infrastructure that can accelerate progress by connecting molecular discoveries, advanced diagnostics, clinical research and implementation in healthcare.

Aim

Through research grants, infrastructure, education, healthcare support, patient and family support, and information and advocacy, the Swedish Childhood Cancer Fund aims to enable more precise treatment, lower toxicity, fewer long-term complications and better outcomes for children and adolescents with cancer.

From research to implementation

One example is Genomic Medicine Sweden (GMS) Childhood Cancer. In 2021, the first pediatric cancer patient in Sweden received precision diagnostics through this research project, largely funded by the Swedish Childhood Cancer Fund. The project has since been fully implemented in healthcare, and every child with cancer in Sweden is now offered whole genome sequencing at diagnosis. Detailed molecular information about each child's cancer can help identify relevant treatment options and provides new opportunities to advance childhood cancer care and improve outcomes.

Conclusions

Continued progress in precision medicine requires more than advanced diagnostics. It depends on collaboration, data sharing, patient and family involvement, and healthcare structures that can translate new knowledge into care. The Swedish Childhood Cancer Fund works to strengthen these connections and supports initiatives that help bring precision medicine closer to every child with cancer.

Read more at www.barncancerfonden.se

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79. Mapping Neuroblastoma Development, Plasticity, and Metastasis in an ALK/NF1 Mouse Model

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Abstract:

Neuroblastoma exhibits substantial heterogeneity across anatomical sites and cellular states. To address its early development and spontaneous metastatic progression, we built a mouse model combining germline Alk F1178S mutation, corresponding to human ALK F1174S, with Dbh-driven Nf1 deletion.

We assessed tumor distribution and disease-specific survival across genotypes and characterized neonatal lesions, primary tumors and metastases using histopathology and immunofluorescence. Whole-genome sequencing examined copy-number variations in double mutant primary tumors. 10x Visium HD spatial transcriptomics resolved cellular organization in a primary tumor and liver metastasis.

Double mutant mice spontaneously developed adrenal and abdominal tumors, including histologically confirmed neuroblastoma with metastases. At postnatal day 1 (P1), abdominal white mass already contained ganglioneuroblastoma and fuse celiac and superior mesenteric ganglia and the organ of Zuckerkandl. Most sequenced tumors lacked Mycn copy-number gain, while recurrent gains involved Alk and signaling-associated genes including Sos1 and Map2k2. Spatial transcriptomics analyses identified heterogeneous tumor populations, including cycling, neuroblast-like and chromaffin-like cells. P1 whole-mount analyses will compare ALK, RET, PHOX2B and MKI67 patterns across WT, ALK-mutant, NF1-KO and double mutant mice in adrenal and ganglion-Zuckerkandl tissues.

This model enables investigation of early neuroblastic lesions, tumor heterogeneity and spontaneous metastasis, including disease without Mycn amplification. The findings motivate testing whether mutant ALK and NF1 loss cooperate through RAS–MAPK signaling and whether additional copy-number alterations contribute to tumor development. Sustained pathway activation and the relationship between neonatal lesions and subsequent tumors remain to be established.

80. Investigating the role of *MYCN* and *PPM1D* in neural crest cells

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Abstract:
The most common genetic alterations in high-risk neuroblastoma include *MYCN* amplification and segmental gain of chromosome 17q. *PPM1D* located on 17q22.3 encodes the nuclear serine/threonine phosphatase WIP1, a key regulator of DNA damage response and cell cycle progression. Here we have investigated how aberrant expression of these two genes affect neural crest and sympathoadrenal cells in transgenic zebrafish models, and if they contribute to neuroblastoma development.

Transgenic zebrafish were generated using constructs expressing *MYCN* or *PPM1D* together with fluorescent reporters, controlled by the sox10 or dbh promoter. Constructs were injected at the one-cell stage, and the embryos were selected based on fluorescence at 5 days post fertilization (dpf). Live tumor screening in a fluorescence microscope is currently performed every 2 weeks. Whole fish and tumor tissue will be collected for bulk- or single-cell RNA sequencing as well as IHC.

Overexpression of *MYCN* in sox10 cells at 24 and 48 hours post fertilization (hpf) led to intestinal obstruction and severe inflammation in the lower GI-tract in 40% of the fish, which was not observed in controls. These findings suggest an altered development of the neural crest-derived enteric nervous system. Long-term experiments with overexpression at an earlier developmental stage are ongoing. Current studies to assess the role of *MYCN* and *PPM1D* in sympathoadrenal development and neuroblastoma initiation are ongoing.

Our findings suggest that the developmental timing of *MYCN* overexpression is critical for determining its biological effects in neural crest-derived cell populations.