Drug class-specific MAPK sensitivity scores predict sensitivity to MAPK inhibitors in pediatric low-grade gliomas

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Pediatric low-grade gliomas (pLGG) are driven by alterations in the MAPK pathway. Clinical trials have shown the efficacy of MAPK inhibitor (MAPKi) treatment in pLGG. Responses to MAPKi, however, vary even in tumors sharing identical driving MAPK alterations. Additional predictive markers are needed to identify tumors that will be sensitive to MAPK inhibition. We generated a MAPKi sensitivity gene score (MSS) for each MAPKi class (types I and II BRAFi, MEKi, catalytic and dual ERKi), based on MAPK-related genes differentially regulated between MAPKi sensitive and nonsensitive cell lines from the Genomics of Drug Sensitivity in Cancer (GDSC) dataset. Single sample gene set enrichment analysis was used to measure and validate the MSS in the GDSC dataset and an independent patient-derived xenograft (PDX) dataset (XevaDB). The validated MSS were tested in a pLGG-specific background, using both published (Open Pediatric Brain Tumor Atlas) as well as newly generated gene expression and single-cell RNA sequencing data from primary pLGGs and cell lines. The MSS discriminated MAPKi sensitive and non-sensitive cells in the GDSC dataset, and significantly correlated with response to MAPKi in the PDX dataset. The MSS discerned gliomas with varying MAPK alterations from those without MAPK alterations and showed higher scores in pLGG compared to other pediatric CNS tumors and normal brain tissue. Predicted sensitivity to MAPKi was heterogeneous within pLGGs with a common MAPK alteration, in accordance with the observation in MAPKi clinical trials. In an exploratory analysis, a strong positive correlation between the MSS and the predicted immune cell infiltration rate, determined by the ESTIMATE signature, was observed. Our analyses identify novel signatures predicting response to MAPKi in a target class-specific manner with a strong potential to predict response to MAPKi treatment. In addition, our data support a role of immune cell infiltration in the response to MAPKi in pLGG, warranting further validation.
The HIT-LOGGIC Register (Low Grade Glioma in Children and Adolescents) in Germany: added clinical value for pediatric low-grade glioma patients

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Background: The national registry HIT-LOGGIC Register aims at ensuring high quality of clinical and molecular data for all children and adolescents suffering from a low-grade glioma (LGG) and at providing clinical, biological and molecular data for further analysis. The partners within the referral network review all diagnostic steps and therapeutic approaches. As the biology of LGG are still poorly understood therapeutic decisions in patients with non-resectable tumors are often difficult. The weekly tumor board enables counselling of treating pediatric oncologists.

Methods: In Germany patients aged 0–21 years with a radiologic or histologic suspicion of LGG are registered through 59 centers delivering pediatric oncology care. Clinical data are gathered by paper/pencil by fax. Images are mainly uploaded to the mdpe server and centrally reviewed. Tumor tissue and if necessary cerebrospinal fluid undergo central integrated histopathological review including immunohistochemistry, BSLk DNA methylation analysis, gene panel sequencing and if possible, RNA sequencing.

Results: The registry initiated its work in 2018. Since 2020 around 380 new patients are registered per year (increase of 26% of estimated cases). The numbers of all referral services have increased accordingly. The implementation of a national weekly tumor board through video conference including all members of the referral network and the local treating team has been well accepted with an average of 2–3 cases discussed each week.

Conclusion: The registry with its weekly virtual video conferencing promotes an interdisciplinary approach ensuring high quality management of pediatric LGG within Germany. It allows minimization of neurotoxicity due to therapy, which is crucial in patients that per se are suffering from a chronic nonfatal disease and are already often compromised by deficits due to the disease itself.

LOGGIC (Low Grade Glioma in Children) Core BioClinical Data Bank: establishment and added clinical value of an international molecular diagnostic registry for pediatric low-grade glioma patients

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Background: The international multicenter registry LOGGIC Core BioClinical Data Bank aims to enhance the understanding of tumor biology in pediatric low-grade glioma (pLGG) and provide high-quality clinical and molecular data. In addition to routine histopathological and molecular analyses, LOGGIC Core determines the driver alteration as precisely as possible to support treatment decisions and participation in interventional trials. Hence, the question arises whether comprehensive implementation of RNA sequencing using fresh frozen (FrFr) tumor tissue to identify underlying gene fusions improves diagnostic accuracy and provides a clinical benefit. For that aim, an international molecular and clinical registry including the logistical and analytical pipeline was established.

Methods: All patients aged 0–21 years, who were enrolled in Germany between April 2019 and February 2021 and for whom FrFr tissue was available, were analyzed. This included central histopathological reference evaluation, immunohistochemistry, BSLk DNA methylation analysis, gene panel sequencing and RNA sequencing using FrFr tissue.

Results: FrFr tissue was available in 178/379 included cases. RNA sequencing was performed on 125 samples. In this prospective population-based cohort we confirmed KIAA1549:BRAF fusion (57%), BRAFV600E mutation (10%) and FGFR1 changes (11%) as the most frequent alterations. Of the cases 13% presented with rare gene fusions (e.g. TPM3:NTRK1, EWSR1:VGL1, G0PC:ROS1, SH3PXD2A:HTRA1 and PDGFB:LRP1). In 22% of cases, RNA sequencing detected an actionable target not identified by conventional methods. FGFR1 internal tandem duplications (n = 6) were only detected by RNA sequencing using fresh frozen tissue, which led to a change in analysis protocols for future cases.

Conclusion: The addition of RNA sequencing reveals clinically relevant alterations including rare gene fusions. By demonstrating improvement of diagnostic accuracy and making precision oncology studies (MEKi/RAFi/ERKi/NTRKi/FGFRi/ROSi) more accessible, the added value for pLGG patients becomes apparent. We propose to include RNA sequencing as part of routine diagnostic procedures for all pLGG patients, especially in tumors where no common MAPK alteration was identified.
Proteogenomics reveals two distinct biological pilocytic astrocytoma subgroups

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Pilocytic astrocytoma (PA) is the most common pediatric brain tumor and is driven by aberrant MAPK signaling. While 5-year overall survival rates exceed 95%, tumor recurrence constitutes a major clinical challenge in incompletely resected tumors despite chemotherapy or radiation-based therapy. Therefore, we used proteogenomics to discern the biological heterogeneity of PA to improve classification of this tumor entity.

Our proteogenomics approach integrates RNA sequencing and LC/MS-based proteomic profiling data from a cohort of 58 confirmed primary PA samples. An integrative genomics approach was conducted to discern the biological heterogeneity of PA and to identify aberrant pathway activation in these biological groups. Lastly, we utilized immunofluorescence to observe the immune landscape of the PA groups.

Pilocytic astrocytomas segregate into two groups that can be validated in two nonoverlapping cohorts. Importantly, younger patients are significantly associated with group 1. Pathway analyses revealed that gene sets involved in ion exchange and cellular respiration were enriched in group 2, whereas immune response signatures were associated with group 1. Despite there being an overall higher immune score for group 1, Tregs were significantly increased in group 2. Due to the important protumoral and immunosuppressive function that Tregs play in the tumor microenvironment (TME), we confirmed a difference in Treg populations between PA groups using immunofluorescence. Specifically, group 2 was enriched in infiltrating CD4+FOXP3+ Tregs compared to group 1, suggesting an immunosuppressive TME. Interestingly, despite the higher immune score and decreased levels of Tregs in group 1, this group was characterized by decreased progression-free survival suggesting the importance of assessing multiple factors and a combination of immune subsets when considering the utility of prognostic and diagnostic markers.

In summary, our proteogenomic approach revealed important biological heterogeneity within the TME of PA suggesting that differences in immune signatures could acquire importance as prognostic and diagnostic markers.

Novel molecular guided therapies for pediatric low-grade glioma (pLGG)

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Background: Pediatric low-grade gliomas (pLGG) are characterized by activating alterations in the MAPK signal transduction pathway defining this entity as a single pathway disease. Most frequent alterations comprise fusions and mutations in BRAF, followed by FGFR, NTRK and other alterations. Clinical trials have tested molecular therapies targeting these alterations in relapsed pLGG. As a consequence, targeted therapies are now entering first-line therapy in pLGG.

Design: Summary of results of completed and interim data of currently ongoing phases I and II targeted therapy trials in pLGG including market authorization studies. Provide outlook for planned phases I–III trials.

Results: Molecular therapies targeting BRAF and MEK demonstrate promising activity with an overall good safety profile in pLGG. First randomized trials in newly diagnosed pLGG harboring BRAFV600E mutations demonstrate superior efficacy and better safety of BRAF-MEK inhibitors combination treatment compared with standard of care chemotherapy.

Conclusion: Several targeted therapies for treatment of relapsed and newly diagnosed pLGG are expected to receive market authorization in the upcoming few years and thus will be available for all pediatric oncology centers significantly expanding the armamentarium of treatment options for all patients with pLGG. Therefore, molecular diagnostics including target definition is essential for all patients with pLGG at diagnosis. In the GPOH and European countries, this is made available to all pediatric oncology centers through the LOGGIC program. The adverse event profile of targeted therapies requires specific management, in particular treatment of skin toxicities. Open questions requiring further translational and clinical research include duration of treatment, rebound growth following drug holiday, biomarkers for response prediction and development of novel rationale combination therapies. This is currently being addressed by the EVEREST and EPILOGIE international consortia.
Strahlentherapie

Etablierung einer paneuropäischen Plattform zur Untersuchung von Gesundheitsfolgen medizinischer Strahlung für Krebspatienten im Kindes- und Jugendalter – Das EU-Projekt HARMONIC

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Purpose: The etiology of most sporadic pediatric first primary neoplasms (FPNs) and proneness to therapy-associated second primary neoplasms (SPNs) in childhood cancer survivors is unknown. As innate alterations in the DNA damage response predispose to sporadic tumors and carcinogenic effects of genotoxic cancer therapies, we investigated cytogenetic lesions in long-term survivors of a pediatric FPN without or with a subsequent SPN after ionizing radiation and replication stress.

Design and methods: Primary skin fibroblasts were established from 23 long-term survivors of a pediatric FPN, 22 patients with a subsequent SPN, and 22 controls with no neoplasm (NN) in the nested case-control study KiKine (positive vote of the GPOH committee „Long-term consequences“ 07/2015). Cells were exposed to X-rays or aphidicolin-induced replication stress and DNA damage was assessed as chromosome aberrations or micronuclei.

Results: The average level of basal cytogenetic damage was comparable between NN, FPN, and SPN donors. Two donors with SPN had striking spontaneous chromosomal instability occurring at high rates of numerical and structural aberrations or nonclonal and clonal translocations. After X-ray exposure, we observed no significant difference in the average yields of radiation-induced chromosome aberrations between NN, FPN, and SPN donors. In contrast, after replication stress, the yield of micronuclei was significantly elevated in SPN donors compared to FPN and NN donors.

Conclusion: Our results suggest an increased susceptibility to replication stress caused by systemic chemotherapy or low peripheral doses during radiotherapy of an FPN in normal tissues in a subset of former childhood cancer patients, elevating their risk for SPNs. Confirmation in a larger cohort and elucidation of the underlying molecular mechanisms are highly warranted and have the potential to establish predictive biomarkers and functional assays for clinical surveillance, prevention and intervention strategies for patients at high risk of SPNs.

Abstracts

Replikation stress but not ionizing radiation drives genomic instability in fibroblasts of childhood cancer survivors with second primary neoplasms

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Postoperative Protonentherapie bei pädiatrischen Patienten mit diffusen gliogeneuralen Tumoren mit oligodendrogliomähnlichen Features und nukleären Clustern (DGONC)

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Fragestellung: Durch die Entwicklungen in der molekularen Charakterisierung von kindlichen Hirntumoren, insbesondere durch die Anwendung der DNA-Methylierung-basierten Klassifikation wurden in den vergangenen Jahren zahlreiche neue seltene Hirntumorentitäten identifiziert. Durch die Seltenheit dieser molekularen Tumorentitäten ist die Datenlage hinsichtlich der angewandten Therapiekonzepte bzw. des Therapieansprechens begrenzt. In der vorliegenden Arbeit wird das Therapieansprechen bei pädiatrischen Patienten mit DGONC nach postoperativer Protonentherapie exploriert, einhergehend mit umfassender molekularer Aufarbeitung.

Studiendesign: Alleiniges Einschlusskriterium war die Eingruppierung der Patienten in die Methylierungsklasse der DGONC mit einem kalibrierten Score >0,9 im DNA-Methylierung-Classifier. Klinische Patientencharakteristika, Überlebensdaten sowie die Toxizität der Behandlung wurden retrospektiv erhoben. Die Behandlungsschritte wurden mit den präoperativen sowie posttherapeutischen MRT-Bildgebung sowie immunhistochemischen Färbungen und Panel-Sequenzierungen wurden durchgeführt und analysiert.

Ergebnisse: Zwei Patienten (DGONC-1/DGONC-2) wurden mit einem kalibrierten Score >0,9 der Methylierungsklasse DGONC zugeteilt. Nach vollständiger Resektion wurde DGONC-1 initial als „primärender neuroektodermaler Tumor WHO Grad 4“ diagnostiziert und postoperativ SpaceXionentherapie mit einer kumulativen Gesamtdosis von 52,4 Gy(RBE) und einer Temozolomid-Dauertherapie von 1,8 Gy(RBE). DGONC-2 erhielt eine initiale Protonentherapie auf die Tumorregion mit 59,4 Gy(RBE) in 1,8 Gy(RBE) für 6 Wochen. Der Klinikausschuss der Patienten in die Methylierungsklasse der DGONC mit einem kalibrierten Score >0,9 im DNA-Methylierung-Classifier. Klinische Patientencharakteristika, Überlebensdaten sowie die Toxizität der Behandlung wurden retrospektiv erhoben. Die Behandlungsschritte wurden mit den präoperativen sowie posttherapeutischen MRT-Bildgebung sowie immunhistochemischen Färbungen und Panel-Sequenzierungen wurden durchgeführt und analysiert.

Conclusions: Der PSA-Träger sollte als die klinisch relevanten Untersuchungen des PSA-Wertes von Interesse sein. Die klinische Wirksamkeit der PSA-Behandlung kann jedoch nur durch prospektive Studien bewiesen werden. Eine zukünftige Studie sollte die PSA-Werte von Patienten mit PSA-positiven Tumoren im Vergleich zu einem Kontrollpopulation beinhalten, um die Effektivität der PSA-Behandlung zu bewerten.

Moderne Bestrahlungstechniken ermöglichen die Rebestrahlung von Hirntumormassen bei pädiatrischen Patienten – eine monozentrische Analyse

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Fragenstellung: Die moderne Bestrahlungstechnologie ermöglicht die Rebestrahlung von Hirntumormassen bei pädiatrischen Patienten. Die klinische Wirksamkeit und Effektivität der Rebestrahlung können jedoch nur durch prospektive Studien bewiesen werden. Eine zukünftige Studie sollte die PSA-Werte von Patienten mit PSA-positiven Tumoren im Vergleich zu einer Kontrollpopulation beinhalten, um die Effektivität der PSA-Behandlung zu bewerten.
Studiendesign: Diese retrospektive Beobachtungsstudie schloss 22 Kindern mit strahlentherapeutischer Behandlung eines Hirnstammmglioms ein. Effektivität und Toxizität wurden mit Fokus auf neurologische Symptome und die Rolle der Rebestrahlung analysiert.

Ergebnisse: Die Behandlung bestand aus einer Bestrahlung des Hirnstamms mit 51,4–60 Gy (Median: 54 Gy) in Kombination v. a. mit Temozolomid gemäß den HIT-Studien-Protokollen für hochmaligne Gliome. Alle behandelten Kindern zeigten eine signifikante Verbesserung der neurologischen Symptome und die Rolle der Rebestrahlung analysiert. Es traten keine höhergradigen (Grad 3 oder höhere) Toxizitäten auf. Drei Patienten erhielten eine Rebestrahlung mit 19,8 Gy, die nach dem Progress ein Gesamtüberleben von 5 bis 7 Monaten zeigten. Der kombinierte Einsatz zweier Bestrahlungsansätze resultierte in maximalen Hirnstammbelastungen von 75–78 Gy und mittleren Dosen von 69–74 Gy. Das mediane Gesamtüberleben betrug 14 Monate.

Schlussfolgerung: Die Prognose pädiatrischer Patient*innen mit Hirnstammmgliomen bleibt limitiert. Eine Rebestrahlung stellt nach den hier vorgestellten vorläufigen Daten eine Behandlungsoption des Rezidivs dar und erscheint machbar.

Stammzelltransplantation: neue Entwicklungen

Strategien zum Remissionserhalt nach allogener Stammzelltransplantation bei Hochrisikoleukämien im Kindesalter

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Fragenstellung: Der Therapieerfolg der allogenen Stammzelltransplantation (Allo-SZT) zur Behandlung hämatologischer Malignome im Kindesalter ist weiterhin durch das Rezidiv der jeweiligen Grunderkrankung limitiert. Präemptiv anwendbare, klassische Therapiemöglichkeiten wie die Reduktion der Immunsuppression oder Donorlymphozyteninfusionen (DLI) bergen das Risiko einer schwer verlaufenden Graft-versus-Host-Erkrankung (GvHD). Reinduktionschemotherapien, gefolgt von einer weiteren Transplantation, sind zudem mit hohen Morbiditäts- und Mortalitätsraten assoziiert.

Studien(design): Neue Immuntherapien wie Antikörper-Wirkstoff-Konjugate, bispezifische „T-Zell-Engager“ und chimäre Antigenrezeptor-T-Zellen (CAR-T-Zellen) haben in den letzten Jahren v. a. die Therapielandschaft der CD19-positiven ALL revolutioniert und sind nicht Gegenstand dieser Übersicht. „Molecularly targeted agents“ als selektive Inhibitoren von BCR/ABL, FLT3/ITD, IDH1 und IDH2 etc. sowie epigenetische Wirkstoffe wie Azacitidin und Decitabin gewinnen derzeit als Rezidiv-, „Bridging“- oder Erhaltungstherapie in der Behandlung der AML zunehmend an Bedeutung. Auch zellbasierte Immuntherapieansätze, u. a. kombiniert mit zuvor genannten Therapieoptionen, stellen vielversprechende Therapiemöglichkeiten zur Rezidivbehandlung nach Allo-SZT dar.

Ergebnisse: Kombinationsbehandlung von hypomethylierenden Substanzen (HMA) und DLI konnten in einem Drittel erwachsener AML/MDS-Patienten mit Rezidiv ein erneutes Therapieansprechen bewirken und zeigten als Erhaltungstherapie ein krankheitsfreies 2-Jahres-Überleben bis zu 65 %. Sorafenib als Erhaltungstherapie reduzierte das Rezidivrisiko von transplantierten Patienten mit einer FLT3/ITD-mutierten AML im Vergleich zur Kontrollgruppe signifikant. Kombinationen mit DLI bewirkten in bis zu 75 % der präemptiv behandelten Patienten ein zytogenetisches Ansprechen. Bcl-2 Inhibitoren in Kombination mit DLI erzielten ebenfalls in bis zu 50 % der AML-Patienten therapeutische Effekte. Auch Histone-Deacetylase(HDAC)-und IDH1-IDH2-Inhibitoren zeigten potenzielle Behandlungserfolge. Modifizierte DLI bewirkten ebenfalls konsolidierende Immunantworten in frühen klinischen Studien. So zeigten frühzeitige Interventionen mit IL-15-aktivierten zytokininduzierten Killer-Zellen (CIK-FFM) in einem pädiatrischen Patientenkollektiv mit molekularem Rezidiv einer AML Gesamtüberlebensraten bis zu 90 %.

Schlussfolgerung: Kombinationstherapien aus HMA, FLT3- oder Bcl-2-Inhibitoren und konventionellen DLI auf der einen Seite sowie eine Monotherapie mit modifizierten, zellbasierten Immuntherapieansätzen auf der anderen Seite besitzen ein vielversprechendes antileukämisches Potenzial bei minimierten GvHD-Risiko und guter Verträglichkeit.
Incidence of subsequent malignancies after total body irradiation-based allogeneic HSCT in children with ALL—Long-term follow-up from the prospective ALL-SCT 2003 trial

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Total body irradiation (TBI)-based conditioning is associated with superior leukemia-free survival in children with ALL undergoing HSCT. However, the risk for subsequent malignant neoplasms (SMN) remains a significant concern. We analyzed 705 pediatric patients enrolled in the prospective ALL-SCT-BFM 2003 trial and its subsequent registry. Patients >2 years received conditioning with TBI 12 Gy/etoposide (n = 558) and children ≤2 years of age with contraindications for TBI received busulfan/cyclophosphamide/etoposide (n = 110). The 5-year and 10-year cumulative incidence of SMN was 0.02 ± 0.01 and 0.13 ± 0.03, respectively. In total, 39 SMN (34 solid tumors, 5 MDS/AML) were diagnosed in 33 patients at a median age of 5.8 years (1.7–13.4 years), exclusively in the TBI group. Of 33 affected patients, 21 (64%) were alive at a median follow-up of 5.1 years (0.0–9.9 years) after diagnosis of the first SMN. In univariate analysis, neither age at HSCT, donor type, acute GVHD, chronic GVHD, nor CMV constituted a significant risk factor for SMN. The only significant risk factor was TBI versus non-TBI-based conditioning. This analysis confirms and quantifies the increased risk of SMN in children with ALL after conditioning with TBI. Future strategies to avoid TBI will need careful tailoring within prospective, controlled studies to prevent unfavorable outcomes.

Spenderauswahl für die allogene Stammzelltransplantation: aktualisierte Empfehlungen der deutschen Fachgesellschaften

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Hintergrund: Die allogene hämatopoetische Stammzelltransplantation wird jährlich deutschlandweit bei etwa 3000 Patienten mit malignen und nichtmalignen Blut- und Systemerkrankungen durchgeführt. Genetische Unterschiede zwischen Patient und Spender, insbesondere für humane Leukozytenantigene (HLA), vermitteln den mit der allogenen Stammzelltransplantation verbundenen immuntherapeutischen Effekt, aber auch die kollaterale Schädigung gesunder Gewebe („graft-versus-host disease“). Somit kommt der Spenderauswahl eine integrale Rolle zu. Ziel dieses Konsensusprojekts war es, hierfür Empfehlungen für ein standardisiertes Vorgehen bei der Spenderauswahl zu erarbeiten. Methoden: Expertenkonsens auf der Basis einer Recherche der relevanten Fachliteratur durch ein interdisziplinäres Fachgremium. Ergebnisse: Ist kein HLA-identischer Geschwisterspender vorhanden, sollte nach HLA-kompatiblen Fremdspendern gesucht werden. Der wichtigste Faktor bei der Fremdspenderauswahl ist die immunogenetische Kompatibilität an jeweils beiden HLA-A, HLA-B, HLA-C, HLA-DR, HLA-DQ-Genorten (10/10). Findet sich ein 10/10-kompatibler Fremdspender, können alternativ haploidalidentische Familienspender oder 9/10-Fremdspender, Letztere bevorzugt mit HLA-DQ-Differenz, zum Einsatz kommen. Schlussfolgerung: Aufgrund verfeinerter HLA-Typisierung-Techniken und vergrößertem Spenderpool stehen heute für die meisten Patienten mehrere Stammzellspender zur Verfügung. Den daraus resultierenden Herausforderungen für die Spenderauswahl sollte in einem interdisziplinären Ansatz gemäß den hier erarbeiteten Empfehlungen begegnet werden, um das bestmögliche Therapieergebnis zu erzielen.
Knochenmarkversagen

Gain of function RPA1 germline mutation causes dyskeratosis congenita

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The female patient developed pancytopenia and a hypocellular bone marrow at the age of 10 years. Concomitant mucocutaneous findings suggested the presence of dyskeratosis congenita (DC), a bone marrow failure syndrome caused by mutations in telomere-associated genes. Indeed, telomere lengths were < 1st percentile confirming the presence of a telomeraseopathy. A mutation identified by next-generation sequencing in telomerase-related genes could not be identified. Blood counts remained stable, and at the age of 28 years, whole exome sequencing revealed a mutation in replication protein A (RPA1) (c.718G>A, p.E240K). RPA1 is a single stranded DNA binding protein essential for DNA replication, damage repair and telomere maintenance. Functional assays confirmed premature telomere shortening in cells harboring the RPA1 mutation (Sharma et al., Blood, 2021).

Surprisingly, the mutation was found at 50% variant allelic frequency (VAF) in the patient’s fibroblasts, but only at 27% in blood cells. The low VAF could be explained by uniparental disomy (UPD) on chromosome 17p. In addition, a somatic RPA1 p.K579X mutation in cis was identified at 10% VAF in blood cells. We show that both clones expanded over time indicating that they represented somatic rescue events by inactivating the germline gain-of-function RPA1 mutation. In line with this, the patient showed an atypical clinical course with stable blood counts over many years.

We discuss the implications of the gain-of-function RPA1 germline mutation and the somatic rescue events with respect to hematological and non-hematological surveillance. In addition, we present four other patients with varying phenotypic telomeropathies and mutations in the DNA-binding domain A of RPA1. RPA1 mutations should be considered in patients with shortened telomeres and/or the clinical picture of DC. Screening of larger DC patient cohorts will reveal the phenotypic spectrum, genotype-phenotype correlations and risk of neoplasia in the RPA1 syndrome.

A zebrafish model for severe congenital neutropenia (CN) with JAGN1 deficiency

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Introduction: Severe congenital neutropenia (CN) is a bone marrow failure syndrome characterized by a neutrophil maturation defect. CN patients develop life-threatening infections from birth onwards and in the long term they may develop MDS or AML. Autosomal recessive mutations in the Jagunal homolog 1 (JAGN1) gene were described in a group of CN patients. Thus far, no reliable in vivo model of JAGN1-CN has been estab-
Maligne endokrine Tumoren: Neues aus der Studiengruppe

Therapiedauer und Erreichen des Zielspiegels sind die wichtigsten Faktoren für eine effektive Mitotane-Therapie bei Kindern und Jugendlichen mit adrenokortikalen Karzinomen

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Fragestellung: Eine adjuvante Therapie mit Mitotane und Polychemotherapie ist für Patienten mit adrenokortikalen Karzinosaurnen (ACC) empfohlen. Wichtige Fragen zur richtigen Indikation und Dosierung sowie zur notwendigen Therapiedauer sind jedoch bisher ungeklärt.

Studiendesign: Die prospektiv erhobenen Daten des MET-Registers wurden retrospektiv analysiert. Eingeschlossen wurden alle Kinder und Jugendlichen mit ACC, die Mitotane als Erst- oder Zweitlinientherapie erhalten haben.

Ergebnisse: Es wurden 43 Patienten (medianes Alter 7,5 Jahre, Bereich: 0,2 bis 17,8 Jahre; 266 Mädchen) identifiziert. 74,3% der Patienten hatten Lymphknoten- und 24,5% Fernmetastasen. Die mediane Beobachtungszeit betrug 4,1 Jahre (Bereich: 0 bis 20,6 Jahre). Das Gesamt- (OS) und erreichbare Ereignisfreies Überleben (EFS) lag bei 98,9% bzw. 78,1%. Lymphknoten- und Fernmetastasen (p < 0,001), postoperativ nachweisbares Thyreoglobulin (p = 0,006), inkomplette Resektion (p < 0,001) und Lymphgefäßeinbruch (p < 0,001), eine Infiltration des umliegenden Gewebes (p < 0,001) und Lymphknoten- und Fernmetastasen (p < 0,001), postoperativ nachweisbares Thyreoglobulin (p = 0,006), inkomplette Resektion (p = 0,002), Notwendigkeit mehrerer Operationen zum Erreichen einer kompletten Resektion (p = 0,042), ein Kapsel- (p < 0,001) und Lymphgefäßeinbruch (p = 0,005), eine Infiltration des umliegenden Gewebes (p = 0,001), Multifokale Tumoren (p < 0,001, mittlere und Hochrisikogruppe gemäß ATA (p < 0,001) und ein Alter <10 Jahren (p < 0,001) waren Risikofaktoren für das EFS.

Schlussfolgerung: Ein Alter <10 Jahre, ATA-Hochrisiko-Gruppe und ein schlechtes Therapieansprechen als signifikant ungünstige Faktoren für das EFS.

Alter, ATA-Risikogruppe und Therapieansprechen sind entscheidende prognostische Faktoren bei Kindern und Jugendlichen mit differenzierten Schilddrüsenkarzinomen

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Fragestellung: Die Inzidenz differenziierter Schilddrüsenkarzinome (DTC) im Kindes- und Jugendalter steigt in den letzten Jahrzehnten weltweit. DTC werden oft erst in fortgeschrittenen Stadien diagnostiziert, die Prognose bleibt aber ausserzelt.

Wir haben die pädiatrische Klassifikationssystem der American Thyroid Association (ATA) und das bei Erwachsenen validierte „Response-to-therapy“- Strategisierungssystem angewendet, mit der Frage, ob sich daraus Implikationen für eine Therapieanpassung und die Nachsorge ergeben.

Studiendesign: Die prospektiv erhobenen Daten des MET-Registers wurden retrospektiv ausgewertet. Eingeschlossen wurden alle Kinder und Jugendlichen mit DTC, die von 1997 bis Oktober 2019 registriert wurden.

Ergebnisse: Es wurden 354 Patienten (medianes Alter 13,7 Jahre, Bereich: 3,6 bis 17,9 Jahre; 266 Mädchen) identifiziert. 74,3% der Patienten hatten Lymphknoten- und 24,5% Fernmetastasen. Die mediane Beobachtungszeit betrug 4,1 Jahre (Bereich: 0 bis 20,6 Jahre). Das Gesamt- (OS) und erreichtes Ereignisfreies Überleben (EFS) überleben (10 Jahre) lag bei 98,9% bzw. 78,1%. Lymphknoten- und Fernmetastasen (p < 0,001), mittlere und Hochrisikogruppe gemäß ATA (p < 0,001) und ein Alter <10 Jahren (p < 0,001) waren Risikofaktoren für das EFS.

Schlussfolgerung: Ein Alter <10 Jahre, ATA-Hochrisiko-Gruppe und ein schlechtes Therapieansprechen sind signifikant ungünstige Faktoren für das EFS.

Pseudohypoxische Phäochromozytome und Paragangliome dominieren im Kindes- und Jugendalter

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Fragestellung: Phäochromozytome und Paragangliome (PPGL) sind selten neuroendokrine Tumoren, die bei bis zu 80% der Kinder und Jugendlichen mit Tumordispositionssyndromen assoziiert sind. PPGL werden in molekular definierte Subgruppen geteilt: 1) pseudohypoxisch, 2) Kina-
Abstracts

Se-Signalweg und 3) Wnt-aktiviert. Die molekularen Charakteristika dieser Untergruppen eröffnen bei Erwachsenen neue Perspektiven für die Diagnostik und Therapie, sind bei Kindern und Jugendlichen bisher aber nur unzureichend untersucht.

**Studiendesign:** Die prospektiv erhobenen Daten des MET-Registers wurden retrospektiv ausgewertet. Eingeschlossen wurden alle Kinder und Jugendlichen mit PPGL von 1997 bis Dezember 2019.

**Ergebnisse:** Es wurden 88 Patienten (medianes Alter 12,6 Jahre, Bereich: 4,0 bis 18,7 Jahre; 59 Jungen) identifiziert. Phäochromozytome traten bei 56 % der Patienten auf, Paragangliome bei 35 % und synchrone Tumoren bei 9,1 %. Lymphphoknetometastasen lagen bei 5,7 % der Patienten vor. Fernmetastasen bei 10 %. Die mediane Beobachtungszeit lag bei 4,2 Jahren (Bereich: 0 bis 17,1 Jahre). Das Gesamt- (OS) und erkrankungsfreie (DFS) Überleben (5 Jahre) war 98,6 % bzw. 54,0 %. Lokalrezidive, Metastasen und metachrone PPGL traten bei 11 %, 4,5 % bzw. 15 % der Patienten auf. Pathogene Keimbahnvarianten wurden bei 83 % der Patienten identifiziert (VHL 51 %, SDHB 21 %, SDHD 7,8 %, RET und NFI jeweils ein Patient). Ein Patient wurde mit Pacak-Zhuang Syndrom diagnostiziert. 96 % der PPGL waren vom pseudohypoxischen Typ (34 % TCA-Zyklus-assoziiert, 66 % VHL/EPAS-assoziiert). In der multivarianten Analyse war das Ausmaß der Tumorresektion signifikant prognostischer Faktor für das DFS. Mittels eines „cluster approach“ konnte auch für Kinder und Jugendliche mit PPGL – basierend auf der molekularen Subgruppe, der anatomicen Lokalisation und dem Rezidivstatus – ein Risikoscore kalkuliert werden. Bei den Patienten der Hochrisikogruppe traten die meisten Ereignisse auf (metachrone PPGL, Metastasen).

**Schlussfolgerung:** Die meisten PPGL des Kindes- und Jugendalters gehörten zur pseudohypoxischen Subgruppe, die mit einem hohen Risiko für metachrone PPGL und Metastasen assoziiert ist. Umfangreiche molekulare Analysen von PPGL bei Kindern und Jugendlichen ermöglichen eine neue Wege der personalisierte Diagnostik, Therapie und Surveillance.

Der Stellenwert von Strahlentherapie bei pädiatrischen adrenokortikalen Tumoren – systematisches Review und Auswertung aus der GPOH-MET-Datenbank

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**Hintergrund:** Pädiatrische adrenokortikale Karzinome (ACC) sind seltene Tumoren mit einer schlechten Prognose im fortgeschrittenen Stadium. Im Vergleich zu adenalen ACC ist die Lokalrezidivrate deutlich erhöht (81 % vs. 50–60 %), sodass eine Optimierung der Lokaltherapie einen möglichen Ansatzpunkt zur Verbesserung der Prognose darstellt.

**Studiendesign:** Es wurden ein systematisches Literaturreview sowie eine Analyse der MET-Register-Daten durchgeführt, um die aktuelle Datenlage zur Bestrahlung darzustellen.

**Ergebnisse:** Insgesamt fanden sich 76 Patienten in der Literatur, 11 Patienten (davon 7 bereits publizierte) im MET-Register sowie 6 weitere, eigene Patienten, die eine Strahlentherapie (RT) erhielten. Die mediane Alter der in der Literatur berichteten Patienten bei Erstdiagnose betrug 11,1 Jahre (1,1 bis 17,1 Jahre). Daten zum TP53-Status lagen nicht vor. In 78 % der Fälle erfolgte die RT in kurativer Intention in der Ersttherapie und beinhaltete das Tumorbett (76 %). Darüber hinaus wurden auch Metastasen- und palliative RT beschrieben. Indikationen für die RT waren Inoperabilität, inkomplette Resektion und/oder eine Verbesserung der lokalen Kontrolle im Rezidiv. Die verabreichten Strahlendosen lagen zwischen 15–62 Gy (Median 50 Gy). Bei 33 % (16/48) der bestrahlten Patienten trat bei einem medianen Follow-up von 7 Jahren kein Lokalrezidiv im Bestrahungsgebiet auf. Bei 3 von 9 Patienten mit palliativer RT verbesserte die RT die Schmerzkontrolle.

**Zusammenfassung:** Bisher liegen zu wenige Daten vor, um generelle Empfehlungen zur RT bei pädiatrischen ACC zu geben, jedoch profitieren einzelne Patienten in High-Risk-Situationen möglicherweise von einer RT. Daher sollte diese individuell diskutiert werden. Es sind internationale Kollaborationen notwendig, um zukünftig Evidenz zu diesem Thema zu schaffen.

In einem gemeinsamen Projekt werden wir zunächst retrospektiv weitere Daten zur Strahlentherapie bei MET-Patienten erheben.

Analysis of Bulk- and Single-cell-RNA-Seq and ATAC-Seq-Daten von adrenokortikalen Tumoren zur Erstellung eines molekularen Profils

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Das adrenokortikale Karzinom (ACC) ist ein maligner Tumor, der sich aus Zellen der Nebennierenrinde entwickelt. Ein Teil dieser Tumoren ist hormonaktiv und wird aufgrund eines entsprechenden klinischen Symptomatik in der Regel frühzeitig erkannt. Trotzdem gibt es für fortgeschrittene Stadien – abgesehen von einer Therapie mit Mitotane ggf. in Kombination mit einer Polychemotherapie – keine erfolgversprechenden Therapieansätze. Rezidive sind auch bei vollständig resezierten Tumoren möglich. Die wichtigsten Prädiktoren für ein solches sind eine fortgeschrittene Krankheitsstadium, große Tumormassen, unvollständige Resektion, Kortisolproduktion und bestimmte genetische Veränderungen sowie eine hohe Proliferationsrate. Molekulare Determinanten, die ein Rezidiv vorhersagen können, sind häufig unklar.

Wir stellen die Hypothese auf, dass ein molekulares Profiling von adrenokortikalen Tumoren (ACT) dazu beitragen kann, Therapieziele aufzudecken und eine molekulare basierte Risikostatifizierung einzuführen. Wir analysierten die Methylierungswerte von 21 Tumorproben von Patienten mit ACT und führten eine RNA-sequenzierung durch. Des Weiteren führten wir Single-cell-RNA-Seq der Tumoren mithilfe des 10x-Genomics-Systems durch, um eine mögliche intratumorale, molekulare Heterogenität aufzudecken. So konnten sich neue Angriffspunkte für adjuvante Therapien ergeben, und es können möglicherweise klinisch relevante Subgruppen etabliert werden, die Aufschluss über die Prognose und die Rezidivwahrscheinlichkeit geben. Außerdem werden wir die Auswirkungen von Tumordispositionssyndromen und TP53-Varianz auf das molekulare Profil der Tumoren untersuchen.

Das Erstellen eines umfassenden molekularen Profiling soll Wissenslücken in der Tumorökologie und Tumorökologie schließen, die Prognosemöglichkeiten verbessern und Anstöße zur Entwicklung neuer Therapien liefern.
Distinct relapse pattern across ependymoma types and subtypes

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Background/significance: The 2021 WHO classification for brain tumors confirms that ependymoma is not a uniform disease, but is divided into subtypes with biological and clinical heterogeneity. Some have an excellent prognosis, while others display frequent relapses, with attendant morbidity and mortality. However, the pattern of when and where different types of ependymoma relapse is largely unknown.

Methods: We assembled a cohort of 505 intracranial ependymomas from pediatric (78%) and adult (22%) series and correlated molecular information on DNA methylation data and copy number aberrations with clinical information.

Results: The 5-year progression-free survival (PFS) was 51.8 ± 2.5% (median follow-up for 388 survivors: 6.9 years). Metastatic spread at initial diagnosis (n = 15) was not a negative prognostic marker (5-year PFS: 58.7 ± 14.3%, M0: 50.4 ± 3.0%; p = 0.5). First relapses were in the initial tumor bed (local) in 74.5%, 20% were metastatic only, and 5.3% were combined local and metastatic. Relapses of PF-EPN-B occurred later after initial diagnosis than of PF-EPN-A, ST-EPN-YAP1, or ST-EPN-ZFTA (median time to relapse: 4 vs. 1.9/1.9/0.25 years, respectively; p < 0.05) and in cases of metastatic recurrence (9/28 of PF-EPN-B relapsed cases), the relapse was more often located in the spine than for PF-EPN-A or ST-EPN-ZFTA (77.8% vs. 40.5%/11.1%; p < 0.05). No distant relapses were observed in ST-EPN-YAP1 or PF-SE. Within PF-EPN-A relapses, chromosome 6q loss was associated with local relapse, while chromosome 1q gain was associated with distant relapses (p < 0.05 and p = 0.05, respectively). Finally, post-relapse survival (PRS) was poor for PF-EPN-A and ST-EPN-ZFTA (5-year PRS: 43.9 ± 4.6 and 45.0 ± 9.9%, respectively), whereas PF-EPN-B and PF-SE displayed a 5-year PRS of 88.2 ± 8.0 and 90.0 ± 9.5%, respectively (p < 0.05). Remarkably, 10-year PRS for PF-EPN-B dropped to 49.6 ± 18.3%.

Conclusion: Relapse patterns of ependymoma are very heterogeneous. Treatment strategies and surveillance should be planned by incorporating this subtype-related relapse information. Spinal MRI follow-up should be considered as standard procedure for PF-EPN-B as part of long-term surveillance.

DNA methylation profiling improves risk prediction in patients with early childhood sonic hedgehog-activated medulloblastoma with desmoplastic/nodular or extensively nodular histology treated with radiation-avoiding chemotherapy

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Introduction: Clinical and molecular risk factors and DNA methylation patterns in sonic hedgehog (SHH)-activated early childhood desmoplastic/nodular medulloblastoma (DMB) or medulloblastoma with extensive nodularity (MBEN) were evaluated to improve identification of patients at risk for relapse.

Methods: We analyzed 144 patients with DMB (n = 99) or MBEN (n = 45) aged <5 years, diagnosed between 1992 and 2020 and treated with radiation-sparing approaches; 132 received intraventricular methotrexate. DNA methylation profiles of 78 tumors were available.

Results: DMB had less favorable 5-year progression-free and overall survival than MBEN (5-year PFS, 71% [DMB] vs. 93% [MBEN], P = 0.004; 5-year OS, 90% [DMB] vs. 100% [MBEN], P = 0.024). Patients aged >3 years had less favorable 5-year PFS (47% [>3 years] vs. 85% [<1 year] vs. 84% [1–3 years]; P < 0.001). Neither metastatic nor residual disease influenced survival. DNA methylation profiles were reclassified according to the 2021 WHO classification into SHH-1 (n = 39), SHH-2 (n = 38), and SHH-3 (n = 1).

Using hierarchical clustering, SHH-2 was subdivided into two subgroups: SHH-2a (n = 19) and SHH-2b (n = 19). The SHH-2b patients were older (P < 0.001), predominantly displayed DMB histology (P < 0.001), and tu...
mors were more often located in the cerebellar hemispheres (P < 0.022). Cytogenetically, chromosome 2 gains were more frequent in SHH-1, chromosome 9q losses in SHH-2b, while few chromosomal alterations were observed in SHH-2a. SHH-2b had more unfavorable 5-year PFS (58% [SHH-2b] vs. 83% [SHH-1] vs. 95% [SHH-2a]; P = 0.013). In multivariable Cox regression for PFS, DNA methylation subgroups were selected as the strongest adverse prognostic factor. Subclassification of SHH-2 with key clinical and cytogenetic characteristics was confirmed using two independent validation cohorts (total n = 188). In gene mutation analysis, SHH-2a was enriched for SMO mutations.

Conclusion: We identify a subdivision of SHH-2, with SHH-2a representing a very low-risk group, and SHH-2b comprising older patients with an increased risk of relapse when treated with radiation-sparring chemotherapy. These data suggest further heterogeneity within early childhood SHH-DMB/MBEN.

Erste Erfahrungen mit Rivaroxaban bei der Therapie und Sekundärprophylaxe von thrombembolischen Ereignissen bei Kindern und Jugendlichen

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Fragstellung: Seit 2021 ist Rivaroxaban für die Therapie und Sekundärprophylaxe von Venenthrombosen und Lungenembolien bei Kindern und Jugendlichen zugelassen. Die Primärprophylaxe ist derzeit noch außerhalb der Zulassung. Da die Zulassungsstudien ein enorm breites Spektrum unterschiedlicher Thromboselokalisierungen und dies bei verschieden alternativen Altersgruppen umfasst, ist die sorgfältige Dokumentation der ersten klinischen Erfahrungen von Bedeutung.

Kasuistiken: Wir berichten über 3 Säuglinge, 3 Schulkinder und 17 Jugendliche, die wegen thrombembolischer Ereignisse unterschiedlicher Lokalisation (8-mal ZNS, einmal Aorta, einmal Vorhofflimmern, 4-mal Beinvenenthrombose, 4-mal Subklavia/Armvenen, 3-mal primäre Lungenembolie, einmal Primärprophylaxe bei Protein-C-Mangel, einmal ausgehendes Katheterthrombus) mit Rivaroxaban behandelt wurden, davon 3 mit einer onkologischen Grunderkrankung. Wir berichten zusätzlich über eine Intoxikation in suizidaler Absicht.

Ergebnisse: Die Medikation wurde in 21/23 Fällen gut vertragen. Bei einem 7-jährigen Jungen mit katheterassoziierter Thrombose wurde die Medikation wegen Wesensveränderung und psychischer Auffälligkeiten beendet. Bei einem weiteren 17-jährigen Patienten mit Aortenklappenstenose wurde bei vergleichbarer Symptomatik die Dosis reduziert, die Wesensveränderung war danach rückläufig. Bei 2 von 3 Säuglingen wurde nach Spiegelbestimmung die Dosis über das empfohlene Niveau erhöht. Bei einem Jugendlichen mit ALL wurde bei unerwartet erhöhten Spiegeln die Dosis reduziert. Ein Jugendlicher unterannahm einen Suizidversuch mit Rivaroxaban; ohne spezifische Maßnahmen konnte der Verlauf abgewartet werden.

Schlussfolgerung: Rivaroxaban ist eine sichere, peroral verabreichbare, zugelassene Alternative zur bisher praktizierten Therapie und Prophylaxe mit NM-Heparinen. Aus unserer Sicht sollten die Anwendungen in nahe Zukunft bei Kindern vor der Pubertät und bei allen Patient/Innen mit onkologischen Grunderkrankungen mit Spiegelkontrollen und einer Dokumentation der Effektivität (Rekalanalisierung) begleitet werden.

DNT phenotyping and biomarkers predict complex FAS gene or FAS pathway alterations in ALPS-U patients

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Keywords: ALPS-FAS, ALPS-U, DNT, FAS deletion, FAS promoter, FADD Background and aims: Elevated double-negative T cells (DNT) and serum biomarkers have high diagnostic value for identifying FAS mutant patients with autoimmune lymphoproliferative syndrome (ALPS). However, in some patients with clinical features and biomarkers consistent with ALPS, germline or somatic FAS mutations cannot be found with standard Sanger sequencing (ALPS-U). We hypothesized that complex genetic alterations in the FAS gene escaping standard sequencing could explain these cases.

Methods: Our analyses were guided by FAS expression analysis on CD57+ DNT and complemented by FAS cDNA analysis, FAS whole gene sequencing, FAS copy number variation (CNV) analysis as well as WES and targeted FADD sequencing.

Results: Absence of FAS expression can predict somatic loss of heterozygosity (sLOH), which was observed in 31/35 ALPS-FAS patients with extracellular or transmembrane mutations but only in 7/31 patients with intracellular mutations. >Out of 100 patients with elevated DNT and biomarkers 16 did not show FAS mutations upon standard sequencing including DNA from sorted DNT. Of these patients eight lacked FAS expression on CD57+ DNT compatible with heterozygous loss of expression FAS mutations plus acquired somatic second hit in the FAS gene, enriched in DNT. Indeed, seven patients carried deep intronic mutations or large deletions
in the FAS gene combined with sLOH detectable in DNT, three patients had reduced FAS expression, two of which harbored mutations in the FAS promoter, reducing promoter-driven luciferase activity in reporter assays and three unrelated patients with normal FAS expression on DNT carried FADD mutations.

**Conclusion:** A combination of serum biomarkers and DNT phenotyping is superior to conventional FAS sequencing in diagnosing ALPS-FAS. Most clearly defined ALPS-U patients carry FAS pathway mutations but require extended genetic analysis.

Wilms tumor with underlying cancer predisposition syndromes—New insights by placing clinical features in a radiological and molecular genetic context

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**Background:** About 10% of nephroblastoma (WT) patients are diagnosed with cancer predisposition syndrome (CPS) with causative germline genetic or epigenetic variants. Knowledge on CPS is essential for diagnosis and treatment.

**Study design:** This is a retrospective analysis of 2927 consecutive patients with nephroblastoma registered between 1989 and 2017 in the SIOP/GPOH studies. Radiologic aspects and the development of synchronous bilateral WT (BWT) with known predisposition syndromes are included.

**Results:** Beckwith-Wiedemann spectrum (BWS, N = 32, 1.1%), isolated hemihypertrophy (IHH, N = 29, 1.0%), Denys-Drash syndrome (DDS, N = 24, 0.8%) and WAGR syndrome (N = 20, 0.7%) were diagnosed most frequently. Compared to others, these patients were younger at diagnosis (median age 24.5 months vs. 39.0 months), had smaller tumors (349.4 mL vs. 487.5 mL), less often metastasis (8.2% vs. 18%), but more often nephroblastomatosis (12.9% vs. 1.9%) and bilateral tumors (25.7% vs. 8.0%). Nephroblastoma with IHH was associated with blastoma WT and DDS with stromal subtype. Bilateral WTs were common in WAGR (30%), DDS (29%) and BWS (31%). Germline genetics and epigenetic predisposition to BWT development (WT1, WT2) (alterations in 11p15.5 region through ICR1 gain of methylation, paternal uniparental disomy and postzygotic somatic mosaicism), TRIM28, REST are presented. Molecular mechanisms that result in BWT are often also present in multifocal WT in one kidney. Chemotherapy-induced reduction in tumor volume was poor in DDS vs. 487.5 mL), less often metastasis (8.2% vs. 18%), but more often nephroblastomatosis (12.9% vs. 1.9%) and bilateral tumors (25.7% vs. 8.0%).

**Conclusion:** CPS should be considered in nephroblastoma with specific clinical features resulting in a specific surveillance program and referral to a geneticist. Despite mostly favorable clinical characteristics at diagnosis, CPS patients have a relevant risk of local, metachronous relapse requiring adapted treatment concepts. International collaboration is needed in this patient group.

Keimbahnssequenzierung zeigt eine hohe Rate an Krebsprädisposition bei Kindern mit myelodysplastischem Syndrom und identifiziert POT1 als neues Prädispositionsigen für akute myeloische Leukämien im Kindesalter

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**Fragenstellung:** Die Thematisierung der genetischen Prädisposition bei pädiatrischen Krebskrankungen gewinnt vermehrt an Interesse und Aufmerksamkeit. Bis zu 15% der Fälle sind heutzutage nachweislich auf die novo oder vererbte Krebsprädispositionen zurückzuführen (Byrjalsen et al. 2020). Um Therapien individuell auf die Genetik des betroffenen Kindes anzupassen, ist eine lückenlose Identifizierung von Kindern mit einer Krebsprädisposition unabdingbar.

**Studiendesign:** Korrelation der Checkliste „Krebskrankung im Kindesalter: Genetische Beratung indiziert?“ (DKG und GPOH) mit genomischen Sequenzanalysen bei einer unselektierten Kohorte von 139 Kindern mit einer Krebskrankung und deren Eltern.

**Ergebnisse:** Die Auswertung der Studienkohorte anhand der vordefinierten Checkliste zeigt eine hohe Indikation für Krebsprädispositionen. Insgesamt wurde bei 36% (n = 50) der Kinder eine genetische Beratung indiziert, was hauptsächlich durch die beiden Kriterien „Tumordiagnose“ und „phänotypische Auffälligkeiten“ zustande kam. Exomanalysen bestätigten das Vorliegen einer bekannten Krebsprädisposition in 15 Kindern (11%), von denen wiederum 4 klinisch unauffällig (Checkliste negativ) waren. Interessanterweise gab es eine hohe Korrelation zwischen indizierter genetischer Beratung und gefundener Prädisposition bei Kindern mit myelodysplastischem Syndrom (MDS). Hierbei zeigten 50% aller untersuchten Patient*innen (5 aus 10) eine krankheitsrelevante Keimbahnmutation.

Darüber hinaus identifizierten unsere Analysen eine neuartige Stop-Gain-Variante (p.Q199*) im Shelterin-Komplex-Gen POT1, welche bei einem Kind mit akuter myeloischer Leukämie (AML) gefunden wurde. Diesbezüglich konnten wir zeigen, dass Überschneidung der POT1-Variante im Vergleich zur Wildtypkontrolle zu erhöhten DNA-Schäden und chromosomalen Instabilitäten in Zellen führt. Protein- und mRNA-Expressionanalysen in primären Patientenzellen bestätigten, dass die Variante physiologisch ein nichtfunktionalles POT1-Allel im Patienten erzeugt. Nachfolgende Telomerlängenmessungen in primären Patientenzellen sowie POT1-Knockdown-AML-Zellen zeigten eine Verlängerung der Telome- re als hauptsächlichen zugrunde liegenden funktionellen Effekt.

**Schlussfolgerung:** Unsere Ergebnisse unterstreichen die Wichtigkeit der Kombination von klinischen Checklisten und Keimbahnssequenzierung zum Auffinden genetischer Prädispositionen bei Kindern mit einer Krebskrankung, speziell bei Patient*innen mit MDS. Darüber hinaus zeigen wir einen Zusammenhang zwischen POT1 p.Q199* und Telomerlängenveränderung und bestätigten POT1-Keimbahnderegulation als Prädisposition für AML im Kindesalter.
Two rare sides of one rare disease: Ewing sarcoma with and without secondary malignancies—Epidemiological and clinical analysis of an international trial registry

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Background and aims: Intensive multimodal treatment of Ewing sarcoma (EwS) improves survival at the expense of late effects such as secondary malignant neoplasms (SMN). Patients with secondary EwS are excluded from risk stratification in several studies and therefore do not benefit from new therapies. More knowledge about EwS patients with SMN or as SMN is needed to identify at-risk patients and adapt follow-up strategies.

Methods: Epidemiology and clinical characteristics of EwS patients with SMN or as SMN were analyzed in 4518 and 3874 patients treated in the last five and three consecutive international EwS trials, CESS81, CESS86, EICESS92, Euro-E.W.I.N.G.99, and EWING2008, respectively.

Results: Of the patients 96 developed SMN after primary EwS, with solid tumors detected more frequently than hematologic neoplasms (55.2% and 44.8%, respectively). The median latency between EwS and first SMN was 4.9 years (range, 0.1–28.1 years), with a significant difference of 6.1 years between earlier development of hematologic malignancies compared with solid tumors (P < 0.001). The clinical characteristics of the primary EwS did not differ between patients with and without SMN. All patients received multimodality therapy, with 80.2% receiving adjuvant radiotherapy.

There were 44 cases of secondary EwS reported, preceded by a heterogeneous group of malignancies, mainly acute lymphoblastic leukemia (n = 7) and lymphomas (n = 7). Two cases (7.6%) occurred in the radiation field of the primary tumor. The median age at diagnosis of secondary EwS was 21.4 years (range, 5.9–72 years) compared with 10.9 years (range, 0.9–53.5 years) for primary EwS. The 3-year OS/EFS was 0.70 (SE = 0.09)/0.55 (SE = 0.10) for localized patients and 0.33 (SE = 0.12)/0.27 (SE = 0.11) for metastatic patients (OS: P = 0.01). Survival in secondary EwS did not differ between hematologic or solid primary malignancies.

Conclusion: SMN after EwS remains a rare but severe event and requires a structured follow-up system. EwS as SMN accounts for approximately 1% of all reported EwS, and its risk-adjusted treatment should be curative, especially in localized tumors.

NUT carcinoma in children: the European cooperative study group for pediatric rare tumors (EXPERT) European experience

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Background and Aims: NUT carcinoma (NC) is a rare, likely underdiagnosed and highly aggressive tumor defined by the presence of a somatic NUTM1 rearrangement. The tumor occurs mainly in adolescents and young adults. We analyzed clinical, radiological, and biological features of pediatric patients (≤ 18 years) with NC.

Methods: This international retrospective multicenter study was based on review of medical records of 25 children with NC showing specific rearrangement or positive anti-NUTM1 nuclear staining.

Results: A total of 25 patients with a median age of 14.5 years (range 3.9–18 years) were analyzed. Thoracic/mediastinal tumors were the primary location in 15 patients, head and neck in 8 cases. One patient exhibited a multifocal tumor with unknown primary, one patient a primary within the pancreas. Of the patients 17 (68%) presented with regional lymph node involvement, 18 patients (72%) with distant metastases, in most cases to the lungs (40%), distant lymph nodes (36%) and bone marrow (28%). Approximately half of patients were initially misdiagnosed and diagnosis was corrected following NUT immunochemistry or gene sequencing. Chemotherapy was administered to all patients, 9 patients underwent major surgery and 20 radiotherapy. Median overall survival was 0.75 years (range 0.2–11 years), median event-free survival 0.4 years (range 0.1–11 years). Two patients are currently being treated for subsequent relapses (1.9 and 1.5 years after diagnosis) and three long-term survivors (11, 9.1 and 6.6 years after diagnosis) are on record. These cases were associated with cervical disease and non-metastatic disease with BRD3-NUT fusion.

Conclusion: As in adults, despite potential temporary response to chemotherapy, only a minority of children and adolescents with NUT carcinomas achieve long-term remission employing multimodal treatment. Early diagnosis of undifferentiated or poorly differentiated carcinomas to identify the specific rearrangement of the NUTM1 gene is necessary to initiate optimal diagnostic and therapeutic approaches. Novel multimodal treatments are currently under development.
Gene editing of the immune checkpoint receptor NKG2A improves the efficacy of CD33-CAR-NK cells for treatment of acute myeloid leukemia

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**Problem/Background:** CD33-targeting chimeric antigen receptor (CAR)-T cells already showed utility for the treatment of AML; however, natural killer (NK) cells possess certain advantages, such as an intrinsic killing capacity against AML and the possibility to be safely administered to HLA-mismatched recipients without severe side effects. Recently, we reported on the successful generation of primary CD33-CAR-NK cells (Albininger et al., *Blood Cancer J* 2022). However, CAR-NK cell function can be impaired by expressing high levels of inhibitory receptors (Bexte et al., *Oncoimmunology* 2022). Subsequently, we evaluated whether CAR-NK functionality can be improved by knocking out (KO) the inhibitory receptor NKG2A.

**Study design:** CD33-targeting CAR-NK cells were generated by lentiviral transduction. The KO of the NKG2A-encoding KLRCL locus was performed using CRISPR-Cas9 technology. The CD33-CAR and NKG2A expression as well as cytotoxicity were analyzed using flow cytometry. The in vivo efficacy was evaluated in OCI-AML2 (GFP+, Luc+) xenografted NSG-SGM3 mouse models.

**Results:** Lentiviral transduction resulted in up to 60% CD33-CAR-positive NK cells, while KLRCl gene disruption resulted in 50% reduction of NKG2A expression. Cite-Seq and qPCR analysis revealed a distinct gene regulation pattern in CD33-CAR- and CD33-CAR-NKG2A-KO-NK cells including more mature, activated and APC-like NK cells and CAR-KO-NK cells showing significantly higher elimination of CD33+/HLA-E-OCI-AML2 cells in vitro cytotoxicity assays compared to NKG2A-KO or CD33-CAR-NK cells. Furthermore, the administration of two low doses of 3 x 10⁶ CAR-KO-NK cells to AML-xenografted mice led to a strong reduction of leukemic burden and a complete elimination of AML and leukemia-initiating cells in the bone marrow, which was confirmed by bone marrow re-engraftment analysis.

**Conclusion:** Removing an inhibitory immune checkpoint receptor in CAR-NK cells showed a highly beneficial effect for the treatment of AML. This double genetic modification has the potential to enable NK cells to bypass the suppressive cancer cell contact in a broad range of malignant diseases.

**During der Pandemie wird die Diagnose pädiatrischer Malignome durch das regionale Pandemiegeschehen beeinflusst**

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**Fragenstellung:** Die COVID-19-Pandemie hat das tägliche Leben erheblich beeinträchtigt und massive Auswirkungen auf das Gesundheitssystem gezeigt, wobei enorme regionale Unterschiede beobachtet wurden. Mit dieser retrospektiven Studie sollte untersucht werden, ob die Pandemie die daraus resultierenden gesellschaftlichen Veränderungen auch einen Einfluss auf die Diagnose pädiatrischer Malignome hatte.

**Studiendesign:** Diese retrospektive Kohortenstudie umfasst die Kinderkrebsfälle in Bayern im Jahre 2016–2021 und die regionalen SARS-CoV-2-Geschehnisse der ersten beiden Jahre der Pandemie (2020–2021). Neu diagnostizierte Kinderkrebsfälle wurden aus allen Kinderkrebszentren in Bayern gemeldet, somit wurden 100% der Neuerkrankungen der letzten 6 Jahre in die Auswertung einbezogen. Klinische Daten aus den Jahren vor der Pandemie wurden mit Diagnosen verglichen, die während der Pandemie gestellt wurden. Die offiziellen bayrischen SARS-CoV-2-Meldungen des Bayerischen Landesamtes für Gesundheit und Lebensmittelsicherheit wurden abgefragt und mit den Daten zu Pandemie-Ereignissen in Bayern (Quelle: Corona-Datenplatform) parallel ausgewertet. Durch dieses Design konnte eine flächendeckende, umfassende Analyse des Pandemiegeschehens in Bayern durchgeführt werden.

**Ergebnisse:** In Verbindung mit erhöhten SARS-CoV-2-Infektionszahlen im Frühjahr wurden signifikant verringerte Odds Ratios für pädiatrische Krebsdiagnosen im Mai der Pandemiejahre beobachtet. Darauf folgte 2 Monate später ein Anstieg der Diagnosen metastasierter Erkrankungen. Darüber hinaus wurden die Zeit bis zur Diagnosestellung („time to diagnosis“) während der Pandemie erheblich verlängert und die ambulanten Kontakte (basierend auf Daten eines repräsentativen Zentrums) während der Pandemie erheblich reduziert, obwohl die Verfügbarkeit von Konsultationen gleich blieb.

**Schlussfolgerung:** Wir fanden heraus, dass die COVID-19-Pandemie Diagnosen pädiatrischer Malignome in Bezug auf Inzidenz und Time to diagnosis beeinflusste. Diese Veränderungen könnten aus dem Zögern und der Sorge von Familien resultieren, Ärzte zu konsultieren, und aus möglichen gesellschaftlichen Veränderungen, die während Phasen öffentlicher Einschränkungen auftraten. Diese Analyse zeigt, dass Kinder während einer Pandemie eine vulnerable Bevölkerungsgruppe darstellen. Es muss Bewusstsein geschaffen werden, um Kinder mit schweren Erkrankungen wie Krebs frühzeitig zu identifizieren und eine Verzögerung ihrer medizinischen Versorgung während möglicher zukünftiger Pandemien zu verhindern.
Characterization of IG-MYC breakpoints and their application for quantitative minimal disease monitoring in high-risk pediatric Burkitt’s lymphoma and leukemia

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MYC

Results:

Genomic capture high-throughput sequencing. MDD was analyzed in all tumor material applying a long-distance PCR and Sanger sequencing or ble.

with sporadic BL/ B- AL at the highest risk of relapse is essential. Survival of these patients at relapse is 20%. Early identification of children with minimal disseminated disease (MDD) or bone marrow before the second course in risk groups R3/R4 treated in NHL-BFM studies registries between 2000 and 2017, available tumor material and either initial bone marrow for quantification of minimal disseminated disease (MDD) or bone marrow before the second course for minimal residual disease (MRD) by digital PCR were eligible. IG-MYC breakpoints or clonal IGH rearrangements were analyzed from tumor material applying a long-distance PCR and Sanger sequencing or genomic capture high-throughput sequencing. MDD was analyzed in all 93 BL patients and MRD before the second course in 48/50 B-AL patients.

Results: IG-MYC breakpoints could be sequenced from 128 (90%) of 143 children with BL/B-AL in risk groups R3/ R4 treated in NHL-BFM studies/registries between 2000 and 2017, available tumor material and either initial bone marrow for quantification of minimal disseminated disease (MDD) or bone marrow before the second course for minimal residual disease (MRD) by digital PCR were eligible. IG-MYC breakpoints or clonal IGH rearrangements were analyzed from tumor material applying a long-distance PCR and Sanger sequencing or genomic capture high-throughput sequencing. MDD was analyzed in all 93 BL patients and MRD before the second course in 48/50 B-AL patients.

Results:

Background/objective: Children and adolescents with Burkitt’s lymphoma (BL) and leukemia (B-AL) have a cure rate exceeding 90% with risk-adapted NHL-BFM short-pulse treatment. The relapse risk of children in risk groups R3/R4 (stages III/IV with LDH >500U/l and/or CNS involvement) exceeds 15% with chemotherapy. Relapses occur early and survival of these patients at relapse is 20%. Early identification of children with sporadic BL/B-AL at the highest risk of relapse is essential. IG-MYC breakpoints may bear a prognostic value and can serve as minimal disease markers.

Design/methods: A total of 143 children with BL/B-AL in risk groups R3/R4 treated in NHL-BFM studies/registries between 2000 and 2017, available tumor material and either initial bone marrow for quantification of minimal disseminated disease (MDD) or bone marrow before the second course for minimal residual disease (MRD) by digital PCR were eligible. IG-MYC breakpoints or clonal IGH rearrangements were analyzed from tumor material applying a long-distance PCR and Sanger sequencing or genomic capture high-throughput sequencing. MDD was analyzed in all 93 BL patients and MRD before the second course in 48/50 B-AL patients.

Results: IG-MYC breakpoints could be sequenced from 128 (90%) of 143 children with BL/B-AL R3/R4. Chromosome 8 breakpoints >100 kb 3’ of results in abnormal proliferation of hematopoietic cells due to cytokine hypersensitivity and activation of JAK/STAT signalling.

Patients and methods: Genomic DNA was extracted and sequenced using standard protocols. Additionally, induced pluripotent stem cell (iPSC) derived JMML-like hematopoietic progenitor cells (HPCs) with different mutational backgrounds were analyzed in cell proliferation assays after exposure to multiple different JAK inhibitors.

Results: We describe the clinical characteristics of four JMML patients with initiating mutations in SH2B3. Furthermore, we found that JMML-like HPCs with alterations in SH2B3 were more sensitive to chemical JAK inhibition compared to HPCs not harboring mutations in SH2B3.

Discussion: Here, we report four patients with JMML with initiating mutations in SH2B3. The mutations identified result in a truncated protein or affect the SH2 domain. Interestingly, copy neutral loss of heterozygosity of SH2B3 in one patient, also known as uniparental isodisomy, is a mechanism that has been observed in other genes (e.g. CBL and NF1) in JMML.

As in vitro data showed that loss of LNK results in increased JAK/STAT signalling, we hypothesized that these patients may benefit from JAK inhibitor therapy. Our data from iPSC-derived JMML-like HPCs shows that those cells with an additional SH2B3 mutation are more sensitive to JAK inhibitors, specifically ruxolitinib, a JAK1/2 inhibitor that is already approved or under investigation in multiple clinical trials. In summary, we expand the list of initiating mutations in JMML to include SH2B3 and raise the possibility of targeting the JAK pathway in these patients.

Introduction: Juvenile myelomonocytic leukemia (JMML) is a rare and aggressive myelodysplastic/myeloproliferative disorder in toddlers and 95% of patients have mutations detected in the Ras/MAPK signalling pathway. Other mutations in genes encoding for proteins upstream of the Ras pathway have also been described; one of these is LNK which is encoded by SH2B3. Loss of function mutations in SH2B3 result in abnormal proliferation of hematopoietic cells due to cytokine hypersensitivity and activation of JAK/STAT signalling.

Patients and methods: Genomic DNA was extracted and sequenced using standard protocols. Additionally, induced pluripotent stem cell (iPSC) derived JMML-like hematopoietic progenitor cells (HPCs) with different mutational backgrounds were analyzed in cell proliferation assays after exposure to multiple different JAK inhibitors.

Results: We describe the clinical characteristics of four JMML patients with initiating mutations in SH2B3. Furthermore, we found that JMML-like HPCs with alterations in SH2B3 were more sensitive to chemical JAK inhibition compared to HPCs not harboring mutations in SH2B3.

Discussion: Here, we report four patients with JMML with initiating mutations in SH2B3. The mutations identified result in a truncated protein or affect the SH2 domain. Interestingly, copy neutral loss of heterozygosity of SH2B3 in one patient, also known as uniparental isodisomy, is a mechanism that has been observed in other genes (e.g. CBL and NF1) in JMML. As in vitro data showed that loss of LNK results in increased JAK/STAT signalling, we hypothesized that these patients may benefit from JAK inhibitor therapy. Our data from iPSC-derived JMML-like HPCs shows that those cells with an additional SH2B3 mutation are more sensitive to JAK inhibitors, specifically ruxolitinib, a JAK1/2 inhibitor that is already approved or under investigation in multiple clinical trials. In summary, we expand the list of initiating mutations in JMML to include SH2B3 and raise the possibility of targeting the JAK pathway in these patients.

Rituximab clearance in pediatric patients with mature aggressive B-cell non-Hodgkin lymphoma

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Background: In recent decades, treatment of pediatric patients with mature aggressive B-cell non-Hodgkin lymphoma (B-NHL) has been systematically improved to overall survival rates of 80–90%. However, patients with refractory/relapsed diseases have a poor prognosis. Current chemotherapy regimens in combination with B-cell targeting agents, like the anti-CD20 antibody rituximab improve the outcome for high-risk patients. To optimize rituximab efficacy in children with B-NHL by dose adjustment

LKN/SH2B3 as a novel driver in juvenile myelomonocytic leukemia

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we analyze potential parameters influencing the individual clearance of rituximab.

**Design/methods:** A total of 144 children enrolled in the trial B-NHL 2013 (EudraCT number 2013-003253-21) were monitored for rituximab levels at day 5 after the first rituximab infusion. Rituximab concentrations were determined with an ELISA assay established according to the method of Hampson et al., 2010. The concentrations were correlated to patient-specific parameters registered to the NHL-BFM data center.

**Results:** Rituximab levels were characterized by interindividual variability. Patients of high risk groups (R3/R4) had lower rituximab levels at day 5 after first rituximab dose compared to patients of lower risk groups (R1/R2) (mean R1/R2: 119 µg/ml versus mean R3/R4: 74 µg/ml). Among patients of risk group R4, children with Burkitt’s lymphoma (B-AL, defined as blasts in bone marrow >25%) had lower serum levels than children with Burkitt’s lymphoma (BL) R4 stages III/IV (mean B-AL/R4: 43 µg/ml versus mean BL/R4: 72 µg/ml).

In the lower risk groups (R1/R2), patients with DLBCL had a faster clearance than those with BL (mean DLBCL/R1/R2: 93 µg/ml versus mean BL/R1/R2: 139 µg/ml). Among the patients of risk group R1/R2, there was a tendency for younger children to have higher levels than older children (mean ≤10 years R1/R2: 133 µg/ml versus mean >10 years R1/R2: 93 µg/ml). In the high risk group, there was no significant difference between age groups.

**Conclusion:** Besides the already reported association of rituximab clearance with tumor burden, we observed additional individual parameters, which potentially affect the clearance and might be useful for concepts of dose adjustment.

**Clinical presentation, treatment and outcome after childhood-onset craniopharyngioma with special respect to age at diagnosis**

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**Background:** Craniopharyngiomas (CP) are rare malformational tumors. Clinical presentation and outcome of pediatric CP patients with specific respect to age at diagnosis (AaD) is not clear. The aim of this study was to determine clinical presentation and outcome in CP patients diagnosed at different AaD.

**Study design:** In this study 709 patients diagnosed with adamantinomatous CP were recruited in 1999–2021 in HIT-ENDO, KRANIOPHARYNGEOM 2000/2007/Registry2019 and prospectively observed. The AaD was categorized as infants and toddlers (0–2 years), early childhood (2–5 years), middle childhood (6–11 years) and early adolescence (12–18 years). Overall and event-free survival (EFS), functional capacity (FMH) and quality of life (QoL) (PEDQOL) were assessed after a median follow-up of 8.37 years. Multivariable cox and logistic regression were applied to assess EFS and obesity at last visit depending on AaD, hypothalamic involvement (HI), and hypothalamic lesion (HL). Linear mixed models were used to determine the effect of AaD, HI and HL on selected QoL domains.

**Results:** Severe obesity (BMI ≥3 SDS) was prevalent in 45.4% at last visit. Lower EFS was observed in children with AaD <6 years compared to children with AaD between 6 and 18 years. Reduced functional capacity (FMH) percentiles were associated with BMI-SDS at last visit (rho = –0.125, p = 0.006) and AaD <2 years. Posterior HI and lesions HL are independent risk factors for events (HR = 2.94, p < 0.001), regardless of extent of resection and obesity at last visit (OR = 2.51, p < 0.001). Patients with posterior HI and HL reported worse scores on PEDQOL body image and emotional function domains.

**Conclusion:** Hypothalamic syndrome with severe obesity is a frequent sequela in almost half of all patients. Diagnosis of CP at an age <6 years, may help patients to adapt early to disabilities, but may lead to a higher probability of CP relapse. Not AaD but posterior HL may be the contributing factor to severe obesity and a reduced QoL.

**Discovery of a new congenital syndrome with severe neutropenia and neurological involvement due to autosomal recessive COPZ1 mutation**

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**Introduction:** We have identified a family with two siblings suffering from severe congenital neutropenia (CN), autism spectrum disorder with neurological and psycho-emotional disturbance and no mutations in known neutropenia-associated genes. We aimed to identify disease-causing gene mutation by applying our in-house NGS-based approach, followed by the functional validation of candidate variants.

**Results:** We performed whole exome sequencing of DNA from affected siblings and their parents and identified a new homozygous stop-codon mutation ENST00000262061: exon7/9:c.A445C>T:p.Gln141Ter in the COPZ1 gene in both siblings. COPZ1 encodes a subunit of the cytoplasmic coatomer protein complex I (COPI), which is involved in intracellular protein trafficking, endosome maturation, lipid homeostasis, and autophagy. We introduced the stop-codon and frameshift mutations in exon 7 of COPZ1 in healthy donors’ hematopoietic stem cells (HSCs), induced pluripotent stem cells (iPSCs) and zebrafish embryos using CRISPR/Cas9 gene editing. We found that COPZ1 mutated HSCs and iPSCs have severely diminished granulocytic differentiation in vitro in liquid culture and CFU assays (HSCs) or embryoid body-based granulocytic differentiation (iPSCs), as compared to the control edited cells. In line with these findings, the number of neutrophils in COPZ1mutant zebrafish embryos was markedly lower than in the wild-type group. To elucidate the molecular mechanism of defective granulopoiesis downstream of mutated COPZ1, we performed RNA sequencing of COPZ1 mutated and control edited HSCs. We detected pathological activation of JAK/STAT3, unfolded protein response (UPR), and autophagy pathways in COPZ1 mutated cells but not in control samples. Interestingly, these pathways are known to be dysregulated in CN patients with other genetic backgrounds.

**Conclusion:** This is the first report on a new severe congenital neutropenia syndrome caused by homozygous stop-codon COPZ1 mutation inherited in autosomal recessive mode. We are currently investigating molecular mechanisms and possible therapeutic tools for the COPZ1-associated neutropenia syndrome.
Hier steht eine Anzeige.

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