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Prof. Dr. med. Martin Schrappe

Low-grade glioma

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

Romain Sigaud^{1,2}, Thomas K. Albert³, Caroline Heß^{1,2,4}, Thomas Hielscher⁵, Nadine Winkler^{1,2,6}, Carolin Walter⁷, Florian Selt^{1,2,8}, Daniela Kocher^{1,2,9}, Leo Nonnenbroich^{1,2}, Diren Usta^{1,2,8}, Jonas Ecker^{1,2,8}, Angela Brenttrup¹⁰, Martin Hasselblatt¹¹, Christian Thomas¹¹, David Capper^{12,13}, Ulrich W. Thomale¹⁴, Pablo H. Driever¹⁵, Michèle Simon¹⁵, Arend Koch¹³, Felix Sahm^{1,16,17}, Stefan Hamelmann^{16,17}, Nada Jabado^{18,19}, Augusto F. Andrade²⁰, Nettekote Schouten^{21,22}, Eelco Hoving²¹, Cornelis M. van Tilburg^{1,2,8}, Stefan Pfister^{1,8,23}, Olaf Witt^{1,2,8}, Kornelius Kerl³, David T.W. Jones^{1,2,4}, Till Milde^{1,2,8}

¹ Hopp Children's Cancer Center Heidelberg (KiTZ), Heidelberg, Germany; ² Clinical Cooperation Unit Pediatric Oncology, German Cancer Research Center (DKFZ) and German Consortium for Translational Cancer Research (DKTK), Heidelberg, Germany; ³ Department of Pediatric Hematology and Oncology, University Children's Hospital Münster, Münster, Germany; ⁴ Faculty of Biochemistry, Heidelberg University, Heidelberg, Germany; ⁵ Division of Biostatistics, German Cancer Research Center (DKFZ) and German Consortium for Translational Cancer Research (DKTK), Heidelberg, Germany; ⁶ Faculty of Neuroscience, Heidelberg University, Heidelberg, Germany; ⁷ Institute of Medical Informatics, Münster, Germany; ⁸ KiTZ Clinical Trial Unit (ZIPO), Department of Pediatric Hematology and Oncology, Heidelberg University Hospital, Heidelberg, Germany; ⁹ Faculty of Biosciences, Heidelberg University, Heidelberg, Germany; ¹⁰ Neurosurgery Dept., University Hospital Münster, Münster, Germany; ¹¹ Institute of Neuropathology, University Hospital Münster, Münster, Germany; ¹² Berlin Institute of Health, Anna-Louisa-Karsch-Straße 2, 10178, Berlin, Germany; ¹³ Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Department of Neuropathology, Berlin, Germany; ¹⁴ Charité – Universitätsmedizin Berlin, Corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Department of Pediatric Neurosurgery, Berlin, Germany; ¹⁵ Charité – Universitätsmedizin Berlin, Corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Department of Pediatric Oncology and Hematology, Berlin, Germany; ¹⁶ Department of Neuropathology, Heidelberg University Hospital, Heidelberg, Germany; ¹⁷ Clinical Cooperation Unit Neuropathology, German Consortium for Translational Cancer Research (DKTK), German Cancer Research Center (DKFZ), Heidelberg, Germany; ¹⁸ Division of Hematology-Oncology, Department of Pediatrics, McGill University Health Centre, Montreal, Quebec, Canada.; ¹⁹ Department of Child Health and Human Development, Research Institute of the McGill University Health Centre, Montreal, Quebec, Canada.; ²⁰ Department of Human Genetics, McGill University, Montreal, QC, Canada.; ²¹ Princess Máxima Center for Pediatric Oncology, Utrecht, The Netherlands.; ²² Department of Pediatric Oncology, Amsterdam UMC, Emma Children's Hospital, University of Amsterdam, Amsterdam, The Netherlands.; ²³ Division of Pediatric

Neurooncology, German Cancer Consortium (DKTK), German Cancer Research Center (DKFZ), Heidelberg, Germany; ²⁴ Division of Pediatric Glioma Research, German Cancer Research Center (DKFZ), Heidelberg, Germany

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 non-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

Pablo Hernaz Driever^{1,2}, Svea Horn¹, Nina A. Herz¹, Michele Simon^{1,2}, Arend Koch³, David Capper^{3,18}, Felix Sahm^{4,16}, Brigitte Bison⁵, Lars Behrens⁵, Ulrich-Wilhelm Thomale⁶, Ahmad El-Damaty⁷, Martin U. Schuhmann⁸, Semi Harrabi⁹, Rudolf Schwarz¹⁰, Ulrich Schuller¹¹, Christian Hagel¹¹, Rene Schmidt¹², David T.W. Jones^{13,17}, Till Milde^{13,14,15}, Cornelis M. van Tilburg^{13,14,15}, Olaf Witt^{13,14,15}

¹Charite – Universitatsmedizin Berlin, corporate member of Freie Universitat Berlin, Humboldt-Universitat zu Berlin, HIT-LOGGIC-Register, German Registry for children and adolescents with low-grade glioma, Berlin, Germany; ²Charite – Universitatsmedizin Berlin, corporate member of Freie Universitat Berlin, Humboldt-Universitat zu Berlin, Department of Pediatric Oncology/Hematology, Berlin, Germany; ³Charite – Universitatsmedizin Berlin, corporate member of Freie Universitat Berlin, Humboldt-Universitat zu Berlin, Department of Neuropathology, Berlin, Germany; ⁴Clinical Cooperation Unit Neuropathology, German Cancer Research Center (DKFZ), Heidelberg, Germany; ⁵Diagnostic and Interventional Neuroradiology, Faculty of Medicine, University Hospital Augsburg, Augsburg, Germany; ⁶Charite – Universitatsmedizin Berlin, corporate member of Freie Universitat Berlin, Humboldt-Universitat zu Berlin, Department of Pediatric Neurosurgery, Berlin, Germany; ⁷Neurosurgery, UniversitatsKlinikum Heidelberg, Heidelberg, Germany; ⁸Division of Pediatric Neurosurgery, Department of Neurosurgery, University Hospital of Tuebingen, Eberhard Karls University of Tuebingen; ⁹Department of Radiation Oncology, Heidelberg University Hospital, Heidelberg; ¹⁰Department of Radiation Oncology, Medical Center Hamburg-Eppendorf, Hamburg Germany; ¹¹Institute for Neuropathology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; ¹²University of Muenster, Institute of Biostatistics and Clinical Research, Muenster, Germany; ¹³Hopp Children's Cancer Center Heidelberg (KITZ), Heidelberg, Germany; ¹⁴Clinical Cooperation Unit Pediatric Oncology, German Cancer Research Center (DKFZ), Heidelberg, Germany, and German Cancer Consortium (DKTK); ¹⁵Department of Pediatric Oncology, Hematology, Immunology and Pulmonology, Heidelberg University Hospital, Heidelberg, Germany; ¹⁶Department of Neuropathology, Institute of Pathology, Heidelberg University Hospital, Heidelberg, Germany; ¹⁷Division Pediatric Glioma Research, German Cancer Research Center (DKFZ), Heidelberg, Germany; ¹⁸German Cancer Consortium (DKTK), Partner Site Berlin, German Cancer Research Center (DKFZ), Heidelberg, Germany

Background: The national registry HIT-LOGGIC Register aims at ensuring high quality of clinical management 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, 850k DNA methylation analysis, gene panel sequencing and if possible, RNA sequencing.

Results: The registry initiated its work in 2019. 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

Emily C. Hardin^{1,2,3,4}, Simone Schmid⁸, Alexander Sommerkamp^{1,10}, Carina Bodden^{1,2}, Anna-Elisa Heipertz^{1,2,3,4}, Philipp Sievers^{5,7}, Dominik Sturm^{1,2,3}, Andrea Wittmann^{1,10}, Ashwyn A. Perera^{1,4,10}, Till Milde^{1,2,3}, Stefan M. Pfister^{1,3,6}, Andreas von Deimling^{5,7}, Pablo Hernaz Driever⁹, Arend Koch⁸, Darren Hargrave¹¹, Olaf Witt^{1,2,3}, David Capper^{8,12}, Felix Sahm^{5,7}, David T.W. Jones^{1,10}, Cornelis M. van Tilburg^{1,2,3}

¹Hopp Children's Cancer Center Heidelberg (KITZ), Heidelberg, Germany; ²Clinical Cooperation Unit Pediatric Oncology, German Cancer Research Center (DKFZ), Heidelberg, Germany, and German Cancer Consortium (DKTK); ³Department of Pediatric Oncology, Hematology, Immunology and Pulmonology, Heidelberg University Hospital, Heidelberg, Germany; ⁴Heidelberg Medical Faculty, University of Heidelberg, Germany; ⁵Clinical Cooperation Unit Neuropathology, German Cancer Research Center (DKFZ), Heidelberg, Germany; ⁶Division of Pediatric Neurooncology, German Cancer Research Center (DKFZ), Heidelberg, Germany; ⁷Department of Neuropathology, Institute of Pathology, Heidelberg University Hospital, Heidelberg, Germany; ⁸Charite – Universitatsmedizin Berlin, corporate member of Freie Universitat Berlin, Humboldt-Universitat zu Berlin, and Berlin; Institute of Health, Department of Neuropathology, Berlin, Germany; ⁹Charite – Universitatsmedizin Berlin, corporate member of Freie Universitat Berlin, Humboldt-Universitat zu Berlin, and Berlin; Institute of Health, HIT-LOGGIC German Registry for children and adolescents with low-grade glioma, Berlin, Germany; ¹⁰Division Pediatric Glioma Research, German Cancer Research Center (DKFZ), Heidelberg, Germany; ¹¹Great Ormond Street Hospital for Children NHS Trust London, London, UK; ¹²German Cancer Consortium (DKTK), Partner Site Berlin, German Cancer Research Center (DKFZ), Heidelberg, Germany

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, 850k 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:VGLL1, GOPC: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 current pipelines, 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

Daniel Picard^{1,2,3}, Jörg Felsberg³, Maïke Langini^{1,2,3,4,5}, Paweł Stachura², Nan Qin^{1,2,3}, Jasmin Bartl^{1,2,3}, Florian Sel^{7,8,9}, Romain Sigaud^{7,8,9}, Frauke-D Meyer^{1,2,3}, Sarah Göbbels^{1,2,3}, Anja Stefanski^{4,5}, Kai Stühler^{4,5}, Lucia Roque¹⁰, Aleksandra A Pandya², Christiane Knobbe-Thomsen³, Till Milde^{7,8,9}, Arndt Borkhardt^{1,2}, Guido Reifenberger^{1,3}, Gabriel Leprivier³, Cláudia C. Faria^{11,12} and Marc Remke^{1,2,3}

¹ Department of Pediatric Neuro-Oncogenomics, German Cancer Research Center (DKFZ), Heidelberg, Germany and German Cancer Consortium (DKTK), partner site Essen/Düsseldorf, Düsseldorf, Germany; ² Department of Pediatric Oncology, Hematology, and Clinical Immunology, Medical Faculty, University Hospital Düsseldorf, Düsseldorf, Germany; ³ Department of Neuropathology, Heinrich Heine University Düsseldorf, Medical Faculty, Düsseldorf, Germany; ⁴ Molecular Proteomics Laboratory, Biological Medical Research Centre (BMFZ), Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany; ⁵ Institute for Molecular Medicine, University Hospital Düsseldorf, Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany; ⁶ Institute of Pharmaceutical and Medicinal Chemistry, Heinrich Heine University Düsseldorf, Düsseldorf, Germany; ⁷ Hopp Children's Cancer Center Heidelberg (KiTZ), Heidelberg, Germany; ⁸ Clinical Cooperation Unit Pediatric Oncology, German Cancer Research Center (DKFZ) and German Consortium for Translational Cancer Research (DKTK), Heidelberg, Germany; ⁹ KiTZ Clinical Trial Unit (ZIPO), Department of Pediatric Hematology and Oncology, Heidelberg University Hospital, Heidelberg, Germany; ¹⁰ Unidade de Investigação em Patobiologia Molecular (UIPM)–IPOLFG, Portuguese Cancer Institute, Lisbon, Portugal; ¹¹ Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, da Universidade de Lisboa, Lisbon, Portugal; ¹² Department of Neurosurgery, Hospital de Santa Maria, Centro Hospitalar Lisboa Norte, Lisbon, Portugal

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)

Olaf Witt^{1,2,3}, Cornelis van Tilburg^{1,2,3}, Till Milde^{1,2,3}, David Jones^{1,4}, Stefan Pfister^{1,3,5}, Felix Sahm^{6,7}, David Capper⁸, Arend Koch⁸, Pablo Hernàiz Driever⁹

¹ Hopp Children's Cancer Center Heidelberg (KiTZ), Heidelberg, Germany; ² Clinical Cooperation Unit Pediatric Oncology, German Cancer Research Center (DKFZ) and German Consortium for Translational Cancer Research (DKTK), Heidelberg, Germany; ³ KiTZ Clinical Trial Unit (ZIPO), Department of Pediatric Hematology and Oncology, Heidelberg University Hospital, Heidelberg, Germany; ⁴ Pediatric Glioma Research Group, German Cancer Research Center (DKFZ), Heidelberg, Germany; ⁵ Division of Pediatric Neurooncology, German Cancer Research Center (DKFZ) and German Consortium for Translational Cancer Research (DKTK), Heidelberg, Germany; ⁶ Department of Neuropathology, Institute of Pathology, Heidelberg University Hospital, Heidelberg, Germany; ⁷ Clinical Cooperation Unit Neuropathology, German Consortium for Translational Cancer Research (DKTK), German Cancer Research Center (DKFZ), Heidelberg, Germany; ⁸ Charité-Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Department of Neuropathology, Berlin, Germany; ⁹ Charité – Universitätsmedizin Berlin, Corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Department of Pediatric Oncology and Hematology, Berlin, Germany

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

S. Botzenhardt¹, M. R. Wette¹, T. Steinmeier¹, Y.-L. Lin¹, N. Journy², A. Dumas², S. Bolle³, Y. Lassen-Ramshad⁴, K. Haustermans⁵, L. Brualla¹, C. Demoor-Goldschmidt^{6,7}, L. Walsh⁸, S. Haghdoust^{9,10,11}, I. Thierry-Chef^{12,13,14}, B. Timmermann^{1,15}

¹Department of Particle Therapy, University Hospital Essen, West German Proton Therapy Centre Essen (WPE), West German Cancer Centre (WTZ), Deutschland; ²French National Institute of Health and Medical Research (INSERM), Paris, Frankreich; ³Gustave Roussy (GR) Paris, Frankreich; ⁴Danish Centre for Particle Therapy, Aarhus University Hospital (AUH), Aarhus, Dänemark; ⁵PARTICLE – Particle Therapy Interuniversity Center Leuven, KU Leuven; ⁶Department of Radiotherapy, Centre for Research in Epidemiology and Population Health, Cancer and Radiation Team, University of Paris-Sud, Villejuif, Frankreich; ⁷Centre Régional François Baclesse (CRFB), Caen, Frankreich; ⁸Department of Physics, Science Faculty, University of Zürich, Zurich, Schweiz; ⁹Advanced Resource Center for HADrontherapy in Europe (ARCHADE), 14076, Caen Frankreich; ¹⁰University of Caen Normandie, Cimrap-Aria, Campus Jules Horowitz, 14076 Caen, Frankreich; ¹¹Centre for Radiation Protection Research, Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University, 106 91 Stockholm, Schweden; ¹²Institute for Global Health of Barcelona (ISGlobal), Barcelona, Spanien; ¹³Universitat Pompeu Fabra (UPF), Barcelona, Spanien; ¹⁴Ciber Epidemiologia y Salud Pública (CIBERESP), Madrid, Spanien; ¹⁵German Cancer Consortium (DKTK)

Fragestellung: Eine Strahlentherapie (Radiotherapie, RT) wird regelmäßig bei Krebserkrankungen eingesetzt. Gesundheitliche Risiken müssen dabei gegenüber dem Nutzen abgewogen werden. Bislang fehlen noch Erkenntnisse aus einem internationalen Register zu Akut- und Spätfolgen der modernen RT bei Kindern und Jugendlichen.

Studiendesign: Das EU-geförderte HARMONIC-Projekt (Grant-No. 847707) erarbeitet Instrumente und Datenbanken, die klinische, biologische und DICOM-Daten im Zusammenhang zur RT erfassen. Im ersten Schritt sollen Daten von rund 3000 Kindern aus 5 europäischen Zentren analysiert werden. Dabei werden im Hinblick auf die Wirkungen der RT insbesondere 5 Bereiche untersucht: das endokrine, kardiovaskuläre und neurovaskuläre System sowie die Häufigkeit von Zweitumoren und die Lebensqualität. Zur Konzipierung der Instrumente und für die Analysen werden internationale Expertennetze, vorhandene Literatur und Studien miteinbezogen sowie eine Verknüpfung mit (inter-)nationalen Strukturen und Krebsregistern geschaffen. Dabei wird die Förderphase zur Validierung und ggf. zur Optimierung der Instrumente genutzt.

Ergebnisse: Es wurden alle für das Projekt zu dokumentierenden Parameter festgelegt. Dabei erfolgte die Kategorisierung der Parameter gemäß der *European Organisation for Research and Treatment of Cancer/European Particle Therapy Network* als „optional“ oder „verpflichtend“. Die Untersuchungen erfolgen vor, während und bis 120 Monate nach der RT. Zudem wurden Kriterien der Dosimetrie, Biomarker und standardisierten Prozesse definiert. Als klinische Datenplattform wurde die Webapplikation *Research Electronic Data Capture (REDCap)* ausgewählt, und für die Speicherung der dosimetrischen Daten wird die Software der Fa. *Aquilab* genutzt. Elektronische Case Report Forms wurden erstellt und deren Eingabe überprüft. Tests zur Rekonstruktion von In- und Out-of-field-Dosierung wurden durchgeführt. Auf nationaler Ebene wurden Ethikvoten eingeholt und mit der Datensammlung begonnen. Bislang wurden insgesamt 1148 Patienten retrospektiv und 84 prospektiv eingeschlossen.

Schlussfolgerung: Das HARMONIC-Projekt pilotisiert eine paneuropäische Struktur zur RT-Dokumentation und ihrer Gesundheitsfolgen bei Kindern und Jugendlichen mit Krebs. Alle grundlegenden Schritte wurden erreicht, und die klinische Nutzung des Registers hat begonnen. HARMONIC schafft u. a. die Grundlage zur Quantifizierung von Dosis-Volumen-Effekten der RT und wird dabei Möglichkeiten zur Verbesserung der Versorgung und der Lebensqualität der Betroffenen aufzeigen.

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

Sebastian Zahnreich¹, Kamran Yusufi¹, Alicia Poplawski², Johanna Mirsch³, Thomas Hankeln⁴, Peter Scholz-Kreisel^{2,5}, Maria Blettner², Danuta Galetzka¹, Claudia Spix⁶, Manuela Marron⁷, Heinz Schmidberger¹

¹Department of Radiation Oncology and Radiotherapy, University Medical Centre Mainz, Mainz, Germany; ²Institute of Medical Biostatistics, Epidemiology and Informatics, University Medical Centre of the Johannes Gutenberg University Mainz, Mainz, Germany; ³Radiation Biology and DNA Repair, Technical University of Darmstadt, Darmstadt, Germany; ⁴Institute of Organismic and Molecular Evolution, Molecular Genetics and Genome Analysis, Johannes Gutenberg-University Mainz, Mainz, Germany; ⁵Federal Office for Radiation Protection, Munich (Neuherberg), Germany; ⁶Division of Childhood Cancer Epidemiology, Institute of Medical Biostatistics, Epidemiology and Informatics, University Medical Centre of the Johannes Gutenberg University Mainz, Mainz, Germany; ⁷Department of Epidemiological Methods and Etiologic Research, Leibniz Institute for Prevention Research and Epidemiology – BIPS, Bremen, Germany

Purpose: The etiology of most sporadic pediatric first primary neoplasms (FPNs) and proneness to therapy-associated second primary neoplasms (SPNs) in childhood cancer survivors are 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 KiKme (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 as 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.

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

Maximilian Deng^{1,2,3,4}, Olaf Witt^{5,6}, Andreas von Deimling⁷, Stefan Pfister^{5,6,8}, Klaus Herfarth^{1,2,3,4}, David Jones^{5,9}, Felix Sahm^{5,7}, Jürgen Debus^{1,2,3,4}, Semi Harrabi^{1,2,3,4}

¹ Department of Radiation Oncology, Heidelberg University Hospital, Heidelberg, Deutschland; ² Heidelberg Institute of Radiation Oncology (HIRO), Heidelberg, Deutschland; ³ National Center for Tumor Diseases (NCT), Heidelberg, Deutschland; ⁴ Heidelberg Ion-Beam Therapy Center (HIT), Department of Radiation Oncology, Heidelberg University Hospital, Heidelberg, Deutschland; ⁵ Hopp Children's Cancer Center Heidelberg (KITZ), Heidelberg, Deutschland; ⁶ Department of Pediatric Oncology, Hematology, Immunology and Pulmonology, University Hospital Heidelberg, Heidelberg, Deutschland; ⁷ Department of Neuropathology, Heidelberg University Hospital and CCU Neuropathology, German Consortium for Translational Cancer Research (DKTK), German Cancer Research Center (DKFZ), Heidelberg, Deutschland; ⁸ Division of Pediatric Neurooncology, German Cancer Consortium (DKTK), German Cancer Research Center (DKFZ), Heidelberg, Deutschland; ⁹ Division of Pediatric Glioma Research, German Cancer Research Center (DKFZ), Heidelberg, Deutschland

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 2 pädiatrischen Patienten mit DGONC nach postoperativer Protonentherapie exploriert, einhergehend mit umfassender molekularer Aufarbeitung.

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

Ergebnisse: Zwei Patienten (DGONC-1/DGONC-2) wurden mit einem kalibrierten Score >0,9 der Methylierungskategorie DGONC zugeteilt. Nach vollständiger Resektion wurde DGONC-1 initial als „primitiver neuroektodermaler Tumor WHO Grad 4“ diagnostiziert und postoperativ via Protonentherapie mit einer kumulativen Gesamtdosis von 52,4 Gy(RBE), aufgeteilt in 27,2 Gy(RBE) in 1,6 Gy(RBE) Einzeldosis auf die kraniospinale Achse, und 25,2 Gy(RBE) in 1,8 Gy(RBE) Einzeldosis als Boost auf die Primärtumorregion. DGONC-2 erhielt die initiale Diagnose eines anaplastischen Oligodendroglioms WHO Grad 3, sodass nach vollständiger Resektion eine Protonentherapie auf die Tumorregion mit 59,4 Gy(RBE) in 1,8 Gy(RBE) mit paralleler Chemotherapie mit Temozolomid sowie eine Temozolomidhaltungstherapie erfolgte. Beide Patienten zeigten eine komplette Remission nach 6 bzw. 8 Jahren nach der Erstdiagnose.

Schlussfolgerung: Vorherige Arbeiten zeigen auf, dass DGONC einen klinischen Verlauf aufweisen, welcher sich ähnlich zu glialen Tumoren der WHO-Grade 2 und 3 verhält. Die Protonenradiotherapie stellt eine vielversprechende Therapieoption in Patienten mit DGONC dar, insbesondere in Abwesenheit von möglichen molekularen Targets. Die Sammlung von umfassenden integrierten, molekulargenetisch-radioonkologischen Fallserien ist geplant, um die Indikation bzw. das Therapieansprechen nach Radiotherapie für weitere, (seltene) molekular-definierte Hirntumorentitäten zu explorieren und neue risikoadaptierte Therapiekonzepte zu entwickeln.

Treatment of childhood-onset craniopharyngioma patients using proton beam therapy versus photon-based radiation therapy in the prospective KRANIOPHARYNGEOM 2007 trial

Carsten Friedrich¹, Svenja Boekhoff¹, Panjarat Sowithayasakul^{1,2}, Maria Eveslage³, Julia Beckhaus¹, Brigitte Bison⁴, Beate Timmermann⁵, Hermann L. Müller¹

¹ Department of Pediatrics and Pediatric Hematology/Oncology, University Children's Hospital, Carl von Ossietzky University Oldenburg, Klinikum Oldenburg AöR, 26133 Oldenburg, Germany; ² Department of Pediatrics, Faculty of Medicine, Srinakharinwirot University, 26120 Bangkok, Thailand; ³ Institute of Biostatistics and Clinical Research, University of Münster, 48149 Münster, Germany; ⁴ Department of Neuroradiology, University Hospital Augsburg, 86156 Augsburg, Germany; ⁵ Department of Particle Therapy, University Hospital Essen, West German Proton Therapy Centre Essen (WPE), West German Cancer Centre (WTZ) and German Cancer Consortium (DKTK), Germany, 45147 Essen, Germany

Background: Proton beam therapy (PBT) compared to photon-based radiotherapy (XRT) offers the benefit to administer lower radiation doses to critical organs thereby possibly minimizing the risk of sequelae in patients with residual craniopharyngiomas (CP) after hypothalamus-sparing surgery. The validation in large CP patient cohorts is still pending.

Study design: Of 290 childhood-onset CP patients registered in 2007–2019 in the prospective multicenter trial KRANIOPHARYNGEOM 2007, 99 (34%) received external RT (65% PBT, 35% XRT). Outcome was compared between the different groups in terms of overall (OS) and event-free survival (EFS), quality of life (QoL using PEDQOL), functional capacity (FMH), and auxological data (BMI and height SDS) 1, 3 and 5 years after irradiation or CP diagnosis.

Results: PBT became the predominant irradiation technique during the study period (used in 23% and 77% of all irradiated patients registered within the first and second half of the enrolment period, respectively). PBT as well as XRT were associated with high ($p < 0.001$) EFS (PBT: 0.917 ± 0.040 ; XRT: 0.940 ± 0.041) compared to non-RT (EFS: 0.669 ± 0.044). The OS was similar in all groups. No differences between PBT, XRT and non-RT CP patients concerning functional capacity and anthropometric parameters (height SDS, BMI SDS) were found. Only in the PEDQOL domain „physical function“, proxy assessed QoL was lower 1 year after PBT when compared to XRT treated and non-irradiated CP patients.

Conclusion: PBT is similarly efficient in preventing relapses in childhood-onset CP patients. During follow-up, clinically relevant differences between PBT and XRT in terms of QoL, functional capacity and degree of obesity as a marker of hypothalamic syndrome were not detectable. While PBT is increasingly applied, studies on larger CP cohorts with longer follow-up after RT are warranted, to analyze, whether it can prevent sequelae such as hypothalamic syndrome and severe obesity compared to XRT.

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

Oertel M¹, Wolters H¹, Rehn S¹, Hasselblatt M², Elsayad K¹, Kerl K³, Sträter R³, Scobiala S¹, Rössig C³, Eich HT¹

¹ Klinik für Strahlentherapie – Radioonkologie, Universitätsklinikum Münster, Münster, Deutschland; ² Institut für Neuropathologie, Universitätsklinikum Münster, Münster, Deutschland; ³ Pädiatrische Hämatologie und Onkologie, Klinik für Kinder- und Jugendmedizin, Universitätsklinikum Münster, Münster

Fragestellung: Hirnstammgliome finden sich v. a. bei Kindern und können mit schwerwiegenden neurologischen Defiziten einhergehen. Die Strahlentherapie (RT) hat eine wichtige Rolle für die lokale Kontrolle und Verlängerung des Gesamtüberlebens, obwohl genaue Parameter wie Dosis und Fraktionierung bisher nicht eindeutig definiert sind. Die vorliegende Analyse untersucht die Wirksamkeit der RT in einem monozentrischen Kollektiv pädiatrischer Patient*innen.

Studiendesign: Diese retrospektive Beobachtungsstudie schloss 22 Kindern mit strahlentherapeutischer Behandlung eines Hirnstammglioms 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 Symptomatik nach Komplettierung der Radiotherapie. 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 Bestrahlungsreihen 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 Hirnstammgliomen 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

Eva Rettinger

Abteilung für Stammzelltransplantation, Immunologie und Intensivmedizin, Klinik für Kinder- und Jugendmedizin, Universitätsklinikum Frankfurt, Goethe Universität, Frankfurt am Main

Fragestellung: 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

Anna Eichinger¹, Michael H Albert¹, Ulrike Poetschger², Evgenia Glogova², Peter Bader³, Oliver Basu⁴, Rita Beier⁵, Birgit Burkhardt⁶, Carl-Friedrich Classen⁷, Alexander Claviez⁸, Selim Corbacioglu⁹, Hedwig E. Deubzer^{10,11}, Johann Greil¹², Bernd Gruhn¹³, Tayfun Güngör¹⁴, Kinan Kafa¹⁵, Jörn-Sven Kühl¹⁶, Peter Lang¹⁷, Bjoern Soenke Lange¹⁸, Roland Meisel¹⁹, Ingo Müller²⁰, Martin G Sauer⁵, Paul-Gerhardt Schlegel²¹, Ansgar Schulz²², Daniel Stachel²³, Brigitte Strahm²⁴, Angela Wawer²⁵, Christina Peters²⁶

¹Department of Pediatrics, Dr. von Hauner Children's Hospital, University Hospital, LMU, Munich, Germany; ²Children's Cancer Research Institute, Vienna, Austria; ³Division for Stem Cell Transplantation and Immunology, Department for Children and Adolescents, University Hospital, Goethe University, Frankfurt/Main, Germany; ⁴Department of Pediatrics III, University Hospital Essen, University Medicine Essen, Essen, Germany; ⁵Department of Pediatric Oncology and Hematology, Medizinische Hochschule Hannover, Hannover, Germany; ⁶Pediatric Hematology and Oncology, University Hospital Muenster, Muenster, Germany; ⁷Oncology and Hematology Unit, Children's Hospital, University Medicine Rostock, Rostock, Germany; ⁸Department of Pediatrics and Bone Marrow Transplant Unit, Medical University of Schleswig-Holstein, Campus Kiel, Kiel, Germany; ⁹Department of Pediatric Hematology, Oncology and Stem Cell Transplantation, University of Regensburg, Regensburg, Germany; ¹⁰Department of Pediatric Hematology and Oncology, Charité – Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin and Humboldt-Universität Berlin, Berlin, Germany; ¹¹Experimental and Clinical Research Center (ECRC) of the Charité and the Max-Delbrück-Center for Molecular Medicine (MDC) in the Helmholtz Association, Berlin, Germany; ¹²Department of Pediatric Oncology, Hematology and Immunology, University of Heidelberg, Heidelberg, Germany; ¹³Department of Pediatrics, Jena University Hospital, Jena, Germany; ¹⁴Department of Hematology/Oncology/Immunology, Gene-therapy, and Stem Cell Transplantation, University Children's Hospital Zurich – Eleonore Foundation & Children's Research Center (CRC), Zurich, Switzerland; ¹⁵Department of Pediatrics, University of Halle, Halle, Germany; ¹⁶Department of Pediatric Oncology, Hematology and Hemostaseology, University of Leipzig, Leipzig, Germany; ¹⁷University Children's Hospital, Tübingen, Germany; ¹⁸Department of Pediatrics, University Hospital Carl Gustav Carus, Technical University of Dresden, Dresden, Germany; ¹⁹Division of Pediatric Stem Cell Therapy, Department of Pediatric Oncology, Hematology and Clinical Immunology, Medical Faculty, Heinrich-Heine-University, Düsseldorf, Germany; ²⁰Division of Pediatric Stem Cell Transplantation and Immunology, Clinic for Pediatric Hematology and Oncology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; ²¹Department of Pediatric Hematology and Oncology, University Children's Hospital Würzburg, Würzburg, Germany; ²²Department of Pediatrics, University Medical Center Ulm, Ulm, Germany; ²³Children's Hospital, Universitätsklinikum Erlangen, Erlangen, Germany; ²⁴Division of Pediatric Hematology and Oncology, Department of Pediatrics and Adolescent Medicine, Medical Center, Faculty of Medicine, University of Freiburg, Freiburg, Germany; ²⁵Department of Pediatrics and Children's Cancer Research Center, Kinderklinik München Schwabing, Technical University of Munich School for Medicine, Munich, Germany; ²⁶St. Anna Children's Hospital, University Vienna, St. Anna Children's Cancer Research Institute, Vienna, Austria

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 or 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 (0–9.9 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

Fleischhauer K, Tran TH, Meisel R, Mytilineos J, Dreger P, Kroeger N

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.

Methode: Expertenkonsensus 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). Stehen mehrere 10/10-kompatible Fremdspender zur Verfügung, sollten als klinische Auswahlkriterien Spenderalter, HLA-DP-Kompatibilität, Spendergeschlecht, Zytomegalievirusserostatus und Blutgruppe berücksichtigt werden. Findet sich kein 10/10-kompatibler Fremdspender, können alternativ haploidentische 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

Eva-Maria Demmerath¹, Richa Sharma², Sushree S. Sahoo², Hauke Busch^{3,4}, Fabian Beier⁵, Victor B. Pastor⁶, Melanie Boerries^{7,8}, Melchior Lauten⁹, Charlotte M. Niemeyer^{1,8}, Marcin W. Wlodarski^{1,2}, Miriam Erlacher^{1,8}

¹Division of Pediatric Hematology and Oncology, Department of Pediatrics and Adolescent Medicine, Medical Center, University of Freiburg, Freiburg, Germany; ²Department of Hematology, St Jude Children's Research Hospital, Memphis, TN; ³Lübeck Institute of Experimental Dermatology and Institute of Cardiogenetics, University of Lübeck, Lübeck, Germany; ⁴University Cancer Center Schleswig-Holstein, University Hospital of Schleswig-Holstein, Campus Lübeck, Lübeck, Germany; ⁵Department of Hematology, Oncology, Hemostaseology and Stem Cell Transplantation, Medical Faculty, RWTH Aachen University, Aachen, Germany; ⁶Center for Applied Bioinformatics, St Jude Children's Research Hospital, Memphis, TN; ⁷Institute of Medical Bioinformatics and Systems Medicine, Medical Center-University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany; ⁸German Cancer Consortium (DKTK), Freiburg, Germany, and German Cancer Research Center (DKFZ), Heidelberg, Germany; ⁹University Hospital Schleswig-Holstein, Department of Pediatrics, University of Lübeck, Lübeck, Germany

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 telomeroopathy. A mutation in known DC-associated 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 isodisomy (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 telomeroopathies 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

Larissa Doll, Narges Aghaallaei, Karl Welte, Julia Skokowa, Baubak Bajoghli

Department of Oncology, Hematology, Immunology and Rheumatology, Division of Translational Oncology, University Hospital Tübingen, Tübingen, Germany

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-

lished. *Jagn1*-deficient mice are not viable, and in mice with conditional KO of *Jagn1* in HSCs hematopoiesis is not affected. To overcome this limitation, we have established a *JAGN1*-CN zebrafish model.

Methods: We used two approaches to interfere with *Jagn1b* function. First, an antisense morpholino which efficiently blocked *Jagn1b* translation was used. Second, we introduced mutations into the *Jagn1b* gene by CRISPR/Cas9. Then, we explored the effect of *Jagn1b* deficiency on hematopoiesis by assessing the differentiation of neutrophils, macrophages and erythrocytes using whole mount in situ hybridization. Additionally, we used qPCR and TUNEL assay to examine the status of unfolded protein response and apoptosis in the *Jagn1b* deficient larvae.

Results: The interference with *Jagn1b* function significantly reduced the number of neutrophils without affecting monoopoiesis or erythropoiesis. Furthermore, we showed that the expression levels of *atf4a*, *atf4b* and *chop* (UPR marker) were significantly upregulated in the *Jagn1b* morphants, when compared with the wild-type counterparts. Also, the number of apoptotic cells was increased and this effect was not restricted to the hematopoietic tissues but was also seen in the skin, eyes, and nervous system.

Conclusion: Overall, we provide the first evidence that *Jagn1b* is required for neutrophil development in a vertebrate other than humans. Our zebrafish model represents a reliable tool to study the pathophysiological function of *JAGN1* and can serve as an in vivo platform to identify new therapeutic strategies.

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

Michaela Kühlen¹, Pascal Mier^{2,3}, Marina Kunstreich², Lienhard Lessel^{2,3}, Dominik Schneider⁴, Ines Brecht⁵, Denis M. Schewe³, Michael C. Frühwald¹, Peter Vorwerk², Antje Redlich^{2,3}

¹ Kinder- und Jugendmedizin, Medizinische Fakultät, Universität Augsburg, Stenglinstraße 2, 86156 Augsburg; ² MET-Register, Pädiatrische Hämatologie und Onkologie, Universitätskinderklinik, Leipziger Str. 44, 39120 Magdeburg; ³ Pädiatrische Hämatologie und Onkologie, Universitätskinderklinik, Leipziger Str. 44, 39120 Magdeburg; ⁴ Klinik für Kinder- und Jugendmedizin, Klinikum Dortmund, Beurhausstr. 40, 44137 Dortmund; ⁵ Klinik für Kinder- und Jugendmedizin, Universitätsklinikum Tübingen, Hoppe-Seyler-Str. 1, 72076 Tübingen

Fragestellung: Eine adjuvante Therapie mit Mitotane und Polychemotherapie ist für Patienten mit adrenokortikalen Karzinomen (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; 29 Mädchen) identifiziert, die Mitotane in Erst- und/oder Zweitlinientherapie erhalten haben. Die mediane Beobachtungszeit war 2,2 Jahre (Bereich: 0,04 bis 12,71 Jahre). Das Gesamt- (OS) und progressionsfreie (PFS) Überleben (3 Jahre) war 44,9 % bzw. 28,5 %. 11/43 Patienten erhielten Mitotane als neoadjuvante Therapie, 4/11 Tumoren zeigten ein partielles Ansprechen. 27/43 Patienten erhielten Mitotane als adjuvante Therapie; eine partielle Remission zeigten 5/13 Patienten mit Target-Läsionen. Das Vorhandensein von Metastasen (Hazard Ratio [HR]: 3,2; 95 %-KI: 1,2–18,6; $P=0,018$), eine Therapiedauer mit Mitotane <9 Monate (HR: 5,6; 95 %-KI: 1,9–16,9; $P=0,002$) und das Nichterreichen des Zielbereichs (HR: 28,5; 95 %-KI: 5,4–150,3; $p<0,001$) waren negative prognostische Faktoren für das PFS und OS (Metastasen: HR: 4,9; 95 %-KI: 1,6–15,5; $p=0,006$; Therapiedauer: HR: 7,0; 95 %-KI 1,9–26,0; $P=0,004$; Nichterreichen des Zielbereichs: HR: 13,5; 95 %-KI 3,6–50,3; $p<0,001$). Das Risiko eines Ereignisses nahm pro Monat der Mitotane-Therapie um 10,4 % ab ($p=0,015$). Eine erneute Behandlung mit Mitotane nach vorangegangener Erstlinientherapie war ineffektiv. Die Mitotane-Therapie wurde von 7/27 Patienten gut toleriert, 13/27 Patienten vertrugen die Therapie mäßig, und 7/27 Patienten vertrugen die Mitotane-Therapie schlecht. Bei fehlendem therapeutischen Effekt beendeten diese 7 Patienten die Therapie vorzeitig.

Schlussfolgerung: Die Dauer der Mitotane-Therapie und das Erreichen des Zielbereichs sind signifikante Faktoren für das Überleben. Die Wertigkeit von Mitotane in Kombination mit und ohne Polychemotherapie sowie die besten Indikationen müssen in randomisierten Studien geprüft werden.

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

Antje Redlich¹, Markus Luster², Kerstin Lorenz³, Marina, Kunstreich¹, Tilman R. Rohrer⁴, Kurt W. Schmid⁵, Christian Vokuhl⁶, Michael C. Frühwald⁷, Peter Vorwerk¹, Michaela Kühlen⁷

¹ MET-Register, Pädiatrische Hämatologie und Onkologie, Universitätskinderklinik, Leipziger Str. 44, 39120 Magdeburg; ² Klinik für Nuklearmedizin, Philipps-Universität Marburg, Baldinger Str., 35043 Marburg; ³ Klinik für Viszeral-, Gefäß- und Endokrine Chirurgie, Universitätsmedizin Halle, Martin-Luther Universität Halle-Wittenberg, Ernst-Grube Str. 40, 06120 Halle an der Saale; ⁴ Sektion pädiatrische Endokrinologie, Klinik für Allgemeine Pädiatrie und Neonatologie, Universität des Saarlandes, Kirrberger Str. 1, 66421 Homburg; ⁵ Institut für Pathologie, Universitätsklinikum Essen, Universität Duisburg-Essen, Hufelandstraße 55, 45147 Essen; ⁶ Sektion Kinderpathologie, Institut für Pathologie, Universitätsklinikum Bonn, Venusberg-Campus 1, 53127 Bonn; ⁷ Kinder- und Jugendmedizin, Medizinische Fakultät, Universität Augsburg, Stenglinstraße 2, 86156 Augsburg

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

Wir haben das pädiatrische Klassifikationssystem der American Thyroid Association (ATA) und das bei Erwachsenen validierte „Response-to-therapy“-Stratifizierungssystem 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 ereignisfreie (EFS) Überleben (10 Jahre) lag bei 98,9 % bzw. 78,1 %. Lymphknoten- und Fernmetastasen ($p<0,001$), postoperativ nachweisbares Thyreoglobulin ($p=0,006$), unkomplette 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. In der multivariaten Analyse imponierten das Alter <10 Jahren, die ATA-Hochrisiko-Gruppe und ein schlechtes Therapieansprechen als signifikant ungünstige Faktoren für das EFS.

Schlussfolgerung: Ein Alter <10 Jahre, ATA-Hochrisiko-Gruppe und ein schlechtes Therapieansprechen sind signifikante prognostisch ungünstige Faktoren für das EFS bei Kindern und Jugendlichen mit DTC. Hieraus ergeben sich individualisierte Möglichkeiten für eine risikoadaptierte Therapie und Nachsorge.

Pseudohypoxische Phäochromozytome und Paragangliome dominieren im Kindes- und Jugendalter

Michaela Kühlen¹, Christina Pamporaki², Marina Kunstreich³, Lienhard Lessel³, Michael C. Frühwald¹, Peter Vorwerk³, Antje Redlich³

¹ Kinder- und Jugendmedizin, Medizinische Fakultät, Universität Augsburg, Stenglinstraße 2, 86156 Augsburg; ² Medizinische Klinik III, Universitätsklinikum Dresden, Fiedlerstr. 25, 01307 Dresden; ³ MET-Register, Pädiatrische Hämatologie und Onkologie, Universitätskinderklinik, Leipziger Str. 44, 39120 Magdeburg

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

se-Signalweg und 3) Wnt-alteriert. 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 %. Lymphknotenmetastasen 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 *NF1* jeweils ein Patient). Ein Patient wurde mit Pacak-Zhuang Syndrom diagnostiziert. 96 % der PPGL waren vom pseudohypoxischen Typ (34 % TCA-Zyklus-assoziert, 66 % *VHL*/EPAS-assoziert). In der multivariaten Analyse war das Ausmaß der Tumorsektion signifikanter prognostischer Faktor für das DFS. Mittels eines „cluster approach“ konnte auch für Kinder und Jugendliche mit PPGL – basierend auf der molekularen Subgruppe, der anatomischen Lokalisation und dem Resektionsstatus – 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 gehören 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 neue Wege für die personalisierte Diagnostik, Therapie und Surveillance.

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

Verena Wiegering¹, Maria Riedmeier¹, Boris Decarolis, Sabine Frisch², Yi-Lan Lin², Antje Redlich³, Michaela Kuhlen⁴, Beate Timmermann²

¹ University Children's Hospital, Department of Pediatric Hematology, Oncology and Stem cell transplantation, University of Wuerzburg, Josef-Schneiderstr. 2, 97080 Wuerzburg, Deutschland; ² Department of Particle Therapy, West German Proton Therapy Centre Essen (WPE), West German Cancer Center (WTZ), Am Mühlenbach 1, 45147 Essen; ³ Department of Pediatric Oncology, University Children's Hospital, Otto-von-Guericke-University, Leipziger Str. 44, 39120 Magdeburg, Deutschland; ⁴ Pediatric and Adolescent Medicine, University Medical Centre Augsburg, Stenglinstr. 2, 86156 Augsburg, Deutschland

Hintergrund: Pädiatrische adrenokortikale Karzinome (ACC) sind seltene Tumoren mit einer schlechten Prognose im fortgeschrittenen Stadium. Im Vergleich zu adulten 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. Das 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 Bestrah-

lungsgebiet 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.

Analyse von Bulk- und Single-cell-RNA-Seq- und ATAC-Seq-Daten von adrenokortikalen Tumoren zur Erstellung eines molekularen Profils

Victoria Fincke¹, Maurice Loßner¹, Mateja Krulik¹, Felix Dorn¹, Michael C. Frühwald¹, Antje Redlich², Michaela Kuhlen^{*1,3}, Pascal D. Johann^{*1,3}

¹ Schwäbisches Kinderkrebszentrum, Universitätsklinikum Augsburg, 86157 Augsburg, Deutschland; ² Pädiatrische Hämatologie und Onkologie, Universitätskinderklinik, Universitätsklinikum Magdeburg, 30120 Magdeburg, Deutschland; ³ Hopp-Kindertumorzentrum (KiTZ) Heidelberg, Abteilung für pädiatrische Neuroonkologie, Deutsches Konsortium für Translationale Krebsforschung (DKTK) und Deutsches Krebsforschungszentrum (DKFZ), 69120 Heidelberg, Deutschland

Das adrenokortikale Karzinom (ACC) ist ein maligner Tumor, der sich aus Zellen der Nebennierenrinde entwickelt. Ein Teil dieser Tumoren ist hormonaktiv und wird aufgrund einer entsprechenden klinischen Symptomatik in der Regel früher entdeckt als hormoninaktive Tumoren, welche oft erst dann Symptome verursachen, wenn ihre Größe zu Gefäß- oder Organkompressionen führt. ACC des Kindes- und Jugendalters weisen häufig eine Assoziation zu bestimmten Tumordispositionssyndromen, wie dem Li-Fraumeni-Syndrom auf, oder zeigen somatische De-novo-Mutationen des *TP53*-Tumorsuppressorgens. Während lokalisierte ACC meist einer chirurgischen Therapie zugänglich sind, 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 fortgeschrittenes Krankheitsstadium, großes Tumolvolumen, unvollständige Resektion, Kortisolproduktion und bestimmte genetische Veränderungen sowie eine hohe Proliferationsrate. Molekulare Determinanten, die ein Rezidiv vorhersagen fehlen bisher.

Wir stellen die Hypothese auf, dass ein molekulares Profiling von adrenokortikalen Tumoren (ACT) dazu beitragen kann, Therapieziele aufzudecken und eine molekular basierte Risikostratifizierung einzuführen. Wir analysierten die Methylierungsdaten von 21 Tumorproben von Patienten mit ACT und führten eine RNA-Sequenzierung durch. Des Weiteren führen wir Single-cell-RNA-Seq der Tumoren mithilfe des 10x-Genomics-Systems durch, um eine mögliche intratumorale, molekulare Heterogenität aufzudecken. So könnten 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*-Varianten auf das molekulare Profil der Tumoren untersuchen.

Das Erstellen eines umfassenden molekularen Profiling soll Wissenslücken bei ACT schließen, die Prognosemöglichkeiten verbessern und Anstöße zur Entwicklung neuer Therapien liefern.

Distinct relapse pattern across ependymoma types and subtypes

Denise Obrecht¹, Melanie Schoof², Alicia Eckhardt², Martin Mynarek^{1,3}, Mark Gilbert⁴, Kenneth Aldape⁵, Terri Armstrong⁶, Vijay Ramaswamy⁶, Michael Bockmayr^{1,2,7}, Katja von Hoff⁸, Gudrun Fleischhack⁹, Jonas Adolph⁹, Stephan Tippelt⁹, Stefan Pfister^{10,11,12}, Kristian Pajtler^{10,11,12}, Dominik Sturm^{10,11,13}, Richard Drexler¹⁴, Stefan Rutkowski¹, Ulrich Schüller^{1,2,15}

¹ Pediatric Hematology and Oncology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; ² Research Institute Children's Cancer Center Hamburg, Hamburg, Germany; ³ Mildred Scheel Cancer Career Center HaTriCS4, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; ⁴ Neuro-Oncology Branch, National Institutes of Health, USA; ⁵ Laboratory of Pathology, National Cancer Institute, Bethesda, Maryland, USA; ⁶ Division of Hematology/Oncology, The Hospital for Sick Children, Toronto, Ontario, Canada; ⁷ Institute of Pathology, Charité University Medicine, Berlin, Germany; ⁸ Department of Pediatric Oncology and Hematology, Charité University Medicine, Berlin, Germany; ⁹ Pediatrics III, University Hospital of Essen, Essen, Germany; ¹⁰ Hopp Children's Cancer Center at the NCT Heidelberg (KITZ), Heidelberg, Germany; ¹¹ Division of Pediatric Neurooncology, German Cancer Research Center (DKFZ) and German Cancer Consortium (DKTK), Heidelberg, Germany; ¹² Department of Pediatric Oncology, Hematology and Immunology, Heidelberg University Hospital, Heidelberg, Germany; ¹³ KITZ Clinical Trial Unit (ZIPO), Department of Pediatric Hematology and Oncology, Heidelberg University Hospital, Heidelberg, Germany; ¹⁴ Department for Neurosurgery, University Medical Center Hamburg-Eppendorf, Germany; ¹⁵ Institute of Neuropathology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

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 $51.8 \pm 2.5\%$ (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: $58.7 \pm 14.5\%$, MO: $50.4 \pm 3.0\%$; $p = 0.5$). First relapses were in the initial tumor bed (local) in 74.5%, 20% were metastatic only, and 5.5% 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: 4.4 vs. 1.9/1.0/2.5 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 $45.0 \pm 9.9\%$, respectively), whereas PF-EPN-B and PF-SE displayed a 5-year PRS of 88.2 ± 8.0 and $90.0 \pm 9.5\%$, respectively ($p < 0.05$). Remarkably, 10-year PRS for PF-EPN-B dropped to $49.6 \pm 18.3\%$.

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

Svenja Tonn¹, Andrey Korshunov^{2,3,4}, Denise Obrecht¹, Martin Sill^{4,5}, Michael Spohn^{1,6,7}, Katja von Hoff⁸, Till Milde^{4,9,10}, Torsten Pietsch¹¹, Tobias Goschzik¹¹, Brigitte Bison^{12,13}, Björn-Ole Juhnke¹, Nina Struve^{14,15}, Dominik Sturm^{4,5,10}, Felix Sahn^{2,3,4}, Michael Bockmayr^{1,6,15,16}, Carsten Friedrich¹⁷, André O von Bueren^{18,19}, Nicolas U Gerber²⁰, Martin Benesch²¹, David T W Jones^{4,22}, Marcel Kool^{4,5,23}, Annika K Wefers^{15,24}, Ulrich Schüller^{1,6,24}, Stefan M Pfister^{4,5,10}, Stefan Rutkowski¹, Martin Mynarek^{1,15}

¹Department of Pediatric Hematology and Oncology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; ²Clinical Cooperation Unit Neuropathology (B300), German Cancer Research Center (DKFZ), and German Cancer Consortium (DKTK), Heidelberg, Germany; ³Department of Neuropathology, Heidelberg University Hospital, Heidelberg, Germany; ⁴Hopp Children's Cancer Center Heidelberg (KITZ), Heidelberg, Germany; ⁵Division of Pediatric Neurooncology (B062), German Cancer Research Center (DKFZ), and German Cancer Consortium (DKTK), Heidelberg, Germany; ⁶Research Institute Children's Cancer Center Hamburg, Hamburg, Germany; ⁷Bioinformatics Core Unit, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; ⁸Department of Pediatric Oncology and Hematology, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany; ⁹Clinical Cooperation Unit Pediatric Oncology, German Cancer Research Center (DKFZ) and German Consortium for Translational Cancer Research (DKTK), Heidelberg, Germany; ¹⁰Department of Pediatric Oncology, Hematology and Immunology, Heidelberg University Hospital, Heidelberg, Germany; ¹¹Institute of Neuropathology, DGNN Brain Tumor Reference Center, University of Bonn Medical Center, Bonn, Germany; ¹²Diagnostic and interventional Neuroradiology, Faculty of Medicine, University Hospital Augsburg, Augsburg, Germany; ¹³Neuroradiological Reference Center for the pediatric brain tumor (HIT) studies of the German Society of Pediatric Oncology and Hematology, University Hospital Wuerzburg (until 2020), University Augsburg, Faculty of Medicine (since 2021), Germany; ¹⁴Department of Radiotherapy, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; ¹⁵Mildred Scheel Cancer Career Center HaTriCS4, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; ¹⁶Institute of Pathology, Charité – Universitätsmedizin Berlin, Berlin, Germany; ¹⁷Department of Pediatrics and Pediatric Hematology/Oncology, University Children's Hospital, Klinikum Oldenburg AöR, Carl von Ossietzky University, Oldenburg, Germany; ¹⁸Division of Pediatric Oncology and Hematology, Department of Pediatrics, Gynecology and Obstetrics, University Hospital of Geneva, Geneva, Switzerland; ¹⁹CANSEARCH research platform for Pediatric Oncology and Hematology, Faculty of Medicine, Department of Pediatrics, Gynecology and Obstetrics, University of Geneva, Geneva, Switzerland; ²⁰Department of Oncology, University Children's Hospital, Zurich, Switzerland; ²¹Division of Pediatric Hematology/Oncology, Department of Pediatrics and Adolescent Medicine, Medical University of Graz, Graz, Austria; ²²Division of Pediatric Glioma Research (B360), German Cancer Research Center (DKFZ), Heidelberg, Germany; ²³Princess Máxima Center for Pediatric Oncology, Utrecht, The Netherlands; ²⁴Institute of Neuropathology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

^aContributed equally

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-sparing 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

Wolfgang Eberl¹, Ivonne Wieland², Torsten Ebeling¹, Johanna Scheer-Preiss¹

¹ Klinik für Kinder – und Jugendmedizin, Klinikum Braunschweig gGmbH; ² Medizinische Hochschule Hannover

Fragestellung: 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 Thrombosed Lokalisationen und dies bei verschiedenen 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 Vorhofthrombus, 4-mal Beinvenenthrombose, 4-mal Subklavia/Armvnenen, 3-mal primäre Lungenembolie, einmal Primärprophylaxe bei Protein-C-Mangel, einmal ausgedehnte Kavathrombose) 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 Armvnenenthrombose wurde bei vergleichbarer Symptomatik die Dosis reduziert, die Wesensveränderung war dann 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 unternahm 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 naher Zukunft bei Kindern vor der Pubertät und bei allen Patient/Innen mit onkologischen Grunderkrankungen mit Spiegelkontrollen und einer Dokumentation der Effektivität (Rekanalisierung) begleitet werden.

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

Anne Rensing-Ehl^{1a*}, Myriam Ricarda Lorenz^{2,3a}, Marita Führer^{3a}, Wolfgang Willenbacher^{4,5}, Ella Willenbacher⁴, Sieghart Sopper⁴, Mario Abinun⁶, Maria Elena Maccari^{1,7}, Christoph König^{1,8}, Pauline Haegele¹, Sebastian Fuchs¹, Carla Castro¹, Patrick Kury¹, Christian Klemann, Maximilian Heeg¹, Julian Thalhammer¹, Oliver Wegehaupt¹, Marco Fischer¹, Sarah Salou¹, Sigune Goldacker¹, Saskia Biskup⁹, Philippe Chatelain⁹, Volker Schuster¹⁰, Klaus Warnatz¹, Bodo Grimbacher¹, Andrea Meinhardt¹¹, Fabian Hauck¹², Dirk Holzinger¹³, Prasad Thomas Oommen¹⁴, Holger Hebart¹⁵, Karlheinz Seeger¹⁶, Kai Lehmbert¹⁷, Karina Butler¹⁸, Ronan Leahy¹⁹, Ilka Fuchs¹, Miriam Groß¹, Carsten Speckmann^{1,6}, Frederic Rieux-Laucat²⁰, Aude Magerus²⁰, Klaus Schwarz^{2,3a}, Stephan Ehl^{1,7a} and the AL-PID study consortium

¹Institute for Immunodeficiency, Center for Chronic Immunodeficiency (CCI), Medical Center – University of Freiburg, Faculty of Medicine, University of Freiburg, Germany; ²Institute for Transfusion Medicine, University Ulm, Ulm, Germany; ³Institute for Clinical Transfusion Medicine and Immunogenetics Ulm, German Red Cross Blood Service Baden-Wuerttemberg – Hessen, Ulm, Germany; ⁴Clinic for Internal Medicine V, Hematology and Oncology, Medical University Innsbruck, Austria; ⁵Syndena, Connect to cure, Innsbruck, Austria; ⁶Paediatric Immunology, Great North Children's Hospital, Newcastle University, Newcastle upon Tyne, UK; ⁷Division of Pediatric Hematology and Oncology, Department of Pediatrics and Adolescent Medicine, Medical Center – University of Freiburg, Faculty of Medicine, University of Freiburg, Mathildenstr. 1, 79106, Freiburg, Germany; ⁸University of Freiburg, Faculty of Biology, Schaeenzlestraße 1, D–79104 Freiburg, Germany; ⁹Center for Human Genetics, Paul-Ehrlich-Str. 23, 72076 Tuebingen, Germany; ¹⁰Children's hospital, Faculty of Medicine, University of Leipzig, 04103 Leipzig; ¹¹Center for Pediatrics and Adolescent Medicine, Department of Pediatric Hematology and Oncology, University Hospital Giessen, Germany; ¹²Division of Pediatric Immunology and Rheumatology, Department of Pediatrics, Dr. von Hauner Children's Hospital, University Hospital, Ludwig-Maximilians-Universität München, Munich, Germany; ¹³Department of Pediatric Hematology-Oncology University of Duisburg-Essen, Essen, Germany; ¹⁴Department of Pediatric Oncology, Hematology and Clinical Immunology, University Hospital, Medical Faculty, Heinrich Heine University, Düsseldorf, Germany; ¹⁵Department of Internal Medicine, Kliniken Ostalb, Stauferklinikum, 73557 Mutlangen, Germany; ¹⁶Charité Universitätsmedizin Berlin, Dept. of Ped. Oncology/Hematology, Augustenburger Pl. 1, 13353 Berlin, Germany; ¹⁷Department of Paediatric Immunology, University Medical Center Hamburg-Eppendorf, Hamburg; ¹⁸Children's Health Ireland at Crumlin, Dublin, Ireland; ¹⁹Department of Paediatric Immunology/ID, Children's Health Ireland (CHI) at Crumlin, Dublin and University of Dublin, Trinity College, Dublin, Ireland; ²⁰Université Paris Cité, Laboratory of Immunogenetics of pediatric autoimmune diseases, INSERM UMR 1163, Imagine Institute, Paris, France

^a contributed equally to this work, ^{ss} contributed equally to this work

*Anne Rensing-Ehl (anne.rensing-ehl@uniklinik-freiburg.de), Institute for Immunodeficiency, Center for Chronic Immunodeficiency (CCI), Medical Center – University of Freiburg, Breisacher Straße 115, 79106 Freiburg, Germany, phone: 0049-761-27071080, FAX: 0049-761-27077600, ORCID: 0000-0002-7031-0018

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

Nils Welter¹, Rhoikos Furtwängler¹, Christian Vokuhl², Leo Karger³, Patrick Melchior⁴, Manfred Gessler⁵, Jens-Peter Schenk⁶, Norbert Graf¹

¹Department of Pediatric Oncology and Hematology, Saarland University, Homburg, Germany; ²Section of Pediatric Pathology, University Hospital Bonn, Germany; ³St. Anna Children's Hospital and CCRI, Department of Paediatrics, Medical University Vienna, Vienna, Austria; ⁴Department of Radiation Oncology, Saarland University Hospital, Homburg, Germany; ⁵Developmental Biochemistry and Comprehensive Cancer Center Mainfranken, Theodor-Boveri-Institute/Biocenter, University of Würzburg, Würzburg, Germany; ⁶Section of Paediatric Radiology, University Hospital, Heidelberg, Germany

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 metastasized (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 (0.4% increase) and favorable in BWS (86.9% reduction). The event-free survival (EFS) of patients with BWS was significantly ($p=0.002$) worse than in others.

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.

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

Franziska Auer¹, Pia Michler², Ulrike Anne Friedrich², Maria Prazenicova², Miriam Erlacher³, Gudrun Göhring⁴, Friedrich Stölzel⁵, Julia Hauer¹

¹Technische Universität München (TUM), Forschungszentrum für krebskranke Kinder, Kinderklinik, München; ²Universitätsklinikum Carl Gustav Carus Dresden, Klinik für Pädiatrische Hämatologie und Onkologie, TU Dresden, Dresden; ³Universitätsklinikum Freiburg, Klinik für Pädiatrische Hämatologie und Onkologie, Freiburg; ⁴Medizinische Hochschule Hannover, Institut für Humangenetik, Hannover; ⁵Universitätsklinikum Carl Gustav Carus Dresden, Medizinische Klinik und Poliklinik I, TU Dresden, Dresden

Fragestellung: Die Thematik der genetischen Prädisposition bei pädiatrischen Krebserkrankungen gewinnt vermehrt an Interesse und Aufmerksamkeit. Bis zu 15 % der Fälle sind heutzutage nachweislich auf de 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 „Krebserkrankung im Kindesalter: Genetische Beratung indiziert?“ (DKG und GPOH) mit genomischen Sequenzanalysen bei einer unselektierten Kohorte von 139 Kindern mit einer Krebserkrankung 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 Überexpression der *POT1*-Variante im Vergleich zur Wildtypkontrolle zu erhöhten DNA-Schäden und chromosomalen Instabilitäten in Zellen führt. Protein- und mRNA-Expressionsanalysen in primären Patientenzellen bestätigten, dass die Variante physiologisch ein nichtfunktionelles *POT1*-Allel im Patienten erzeugt. Nachfolgende Telomerlängenmessungen in primären Patientenzellen sowie *POT1*-Knockdown-AML-Zellen zeigten eine Verlängerung der Telomere als hauptsächlich zugrunde liegenden funktionellen Effekt.

Schlussfolgerung: Unsere Ergebnisse unterstreichen die Wichtigkeit der Kombination von klinischen Checklisten und Keimbahnsequenzierung zum Auffinden genetischer Prädispositionen bei Kindern mit einer Krebserkrankung, speziell bei Patient*innen mit MDS. Darüber hinaus zeigen wir einen Zusammenhang zwischen *POT1* p.Q199* und Telomerlängenveränderung und bestätigen *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

Stefan K. Zöllner¹, Katja Kauertz¹, Isabelle Kaiser¹, Heribert Jürgens², Christiane Schaefer¹, Ruth Ladenstein³, Michael Paulussen⁴, Thomas Kühne⁵, Lianne Haveman⁶, Wolfgang Hartmann⁷, Thorsten Langer⁸, Andreas Ranft¹, Uta Dirksen¹

¹University Hospital Essen, Pediatrics III, Essen, Germany; ²University Children's Hospital Münster, West German Cancer Center Network, Department of Pediatric Hematology and Oncology, Münster, Germany; ³Children's Cancer Research Institute, Paediatric Oncology, Vienna, Austria; ⁴Vestische Children's and Youth Hospital Datteln, Pediatric Hematology and Oncology, Datteln, Germany; ⁵University Children's Hospital Basel, Department of Oncology/Haematology, Basel, Switzerland; ⁶Prinses Máxima Centrum voor Kinderoncologie, Pediatric Oncology, Utrecht, The Netherlands; ⁷University Hospital Muenster, West German Cancer Center Network, Gerhard Domagk Institute for Pathology, Essen, Germany; ⁸University Hospital for Children and Adolescents, Pediatric Oncology and Hematology, Lübeck, Germany

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, EICES92, 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 multichemotherapy, 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

Tim Flaadt¹, Lauriane Lemelle², Calogero Virgone³, Tal Ben-Ami⁴, Michael Abele¹, Hannah Wild¹, Tabea Blessing⁵, Michael C. Frühwald⁶, Antje Redlich⁷, Denis Kachanov⁸, Ulrich M. Lauer⁹, Andrea Ferrari¹⁰, Gianni Bisogno³, Yves Reguerres¹¹, Daniel Orbach², Ines B. Brecht¹, Dominik T. Schneider⁵

¹Pediatric Hematology and Oncology, Children's Hospital, Eberhard-Karls-Universität Tübingen, Germany; ²SIREDO Oncology Center (Care, Innovation and Research for Children and AYA with Cancer), PSL Research University, Institut Curie, Paris, France; ³Pediatric Surgery, Department of Women's and Children's Health, University of Padua, Padua, Italy; ⁴Pediatric Hematology Unit, Kaplan Medical Center, Rehovot, Israel; ⁵Clinic of Pediatrics, Dortmund Municipal Hospital, Dortmund, Germany; ⁶Swabian Children's Cancer Center, Children's Hospital, University Medical Center Augsburg, Augsburg, Germany; ⁷Pediatric Oncology Department, Otto von Guericke University Children's Hospital, Magdeburg, Germany; ⁸Dmitry Rogachev National Medical Research Center of Pediatric Hematology, Oncology and Immunology, Moscow; ⁹Department of Internal Medicine VIII, Medical Oncology and Pneumology, University of Tuebingen, Tuebingen, Germany. German Cancer Consortium (DKTK), German Cancer Research Center (DKFZ), Partner Site Tuebingen, Tuebingen, Germany; ¹⁰Pediatric Oncology Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy; ¹¹Department of Pediatric Hematology and Oncology, Félix Guyon Hospital, Réunion Island, France

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 immunohistochemistry 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 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

Nawid Albinger^{1,2,a}, Tobias Bexte^{1,2,3,a}, Leon Buchinger^{1,2}, Philipp Wendel^{1,2}, Ahmad Al-Ajami^{2,3,11}, Alec Gessner^{2,3,8}, Vinzenz Särchen⁷, Jamal Alzubi⁹, Sarah Mertlitz⁵, Olaf Penack⁵, Raj Bhayadia^{1,2,3}, Dirk Heckl^{1,2}, Jan-Henning Klusmann^{1,2,3}, Meike Vogler^{1,3}, Nina Möker⁴, Toni Cathomen⁹, Michael A. Rieger^{2,3,8,10}, Katharina Imkeller^{2,3,11} and Evelyn Ullrich^{1,2,3,10 b}

¹ Children's Hospital, Experimental Immunology, Johann Wolfgang Goethe University, Frankfurt am Main, Germany; ² Frankfurt Cancer Institute, Goethe University, Frankfurt am Main, Germany; ³ University Cancer Center (UCT), Frankfurt, Germany; ⁴ Miltenyi Biotec B.V. & Co. KG, Bergisch Gladbach, Germany; ⁵ Charité, University Berlin and Humboldt-University Berlin, Department of Department of Hematology, Oncology and Tumorimmunology, Berlin, Germany; ⁷ Institute for Experimental Cancer Research in Pediatrics, Johann Wolfgang Goethe University, Frankfurt am Main, Germany; ⁸ Department of Medicine, Hematology/Oncology, University Hospital Frankfurt, Frankfurt am Main, Germany; ⁹ Institute for Transfusion Medicine and Gene Therapy, Medical Center – University of Freiburg, 79106 Freiburg, Germany; ¹⁰ German Cancer Consortium (DKTK) partner site Frankfurt/Mainz, Frankfurt am Main, Germany; ¹¹ Neurological Institute/Edinger Institute, University Hospital Frankfurt, Frankfurt am Main, Germany; ¹² Pediatric Hematology and Oncology, Martin Luther University Halle-Wittenberg, Halle, Germany; ¹³ Institute for Experimental Cancer Research in Pediatrics

^aAuthors contributed equally, ^bContact: evelyn.ullrich@kgu.de

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 (Albinger et al., *Blood Cancer J* 2022). However, CAR-NK cell function can be impaired by expressing high levels of inhibitory receptors (Bexte et al., *Oncimmunology* 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 *KLRC1* 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 *KLRC1* 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 showed significantly higher elimination of CD33⁺/HLA-E⁺ OCI-AML2 cells in *in vitro* cytotoxicity assays compared to NKG2A-KO or CD33-CAR-NK cells. Furthermore, the administration of two low doses of 3×10^6 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.

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

Mira Reger^{1,2}, Kirsi Manz³, Theresa Kaeuferle^{1,4}, Ramona Krauss^{1,2}, Zofia Wotschofsky^{5,2}, Irene von Luettichau^{6,2}, Paul-Gerhardt Schlegel^{7,2}, Michael C. Frühwald^{8,2}, Selim Corbacioglu^{9,2}, Markus Metzler^{5,2}, Tobias Feuchtinger^{1,2,4}

¹Abteilung für Pädiatrische Hämatologie, Onkologie, Hämostaseologie und Stammzelltransplantation, Dr. von Haunersches Kinderspital, LMU Klinikum, Ludwig-Maximilians-Universität (LMU) München; ²Kinderonkologisches Netzwerk Bayern (KIONET); ³Institut für Medizinische Informationsverarbeitung Biometrie und Epidemiologie (IBE), LMU München; ⁴Deutsches Zentrum für Infektionsforschung (DZIF), München; ⁵Kinder- und Jugendklinik, Universitätsklinikum Erlangen; ⁶Klinik für Kinderheilkunde und Kinderkrebsforschungszentrum, Medizinische Fakultät der Technischen Universität (TU) München, Kinderklinik München Schwabing, TU München; ⁷Abteilung für Pädiatrische Hämatologie, Onkologie und Stammzelltransplantation, Universitätsklinikum Würzburg; ⁸Kinder- und Jugendmedizin, Schwäbisches Kinderkrebszentrum, Universitätsklinikum Augsburg; ⁹Abteilung für Pädiatrische Hämatologie, Onkologie und Stammzelltransplantation, Universitätsklinikum Regensburg

Fragestellung: 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 und die daraus resultierenden gesellschaftlichen Veränderungen auch einen Einfluss auf die Diagnose pädiatrischer Malignome hatten.

Studiendesign: Diese retrospektive Kohortenstudie umfasst die Kinderkrebsfälle in Bayern der 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 ab Auftreten der ersten SARS-CoV-2-Infektion in Bayern analysiert und mit Daten zu Pandemiemaßnahmen in Bayern (Quelle: Corona-Datenplattform) parallel ausgewertet. Durch dieses Design konnte eine flächendeckende, umfangreiche 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 beeinträchtigte. 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

Christine Damm-Welk¹, Paula Möker¹, Udo zur Stadt², Martin Zimmermann³, Malik Alawi⁴, Stephanie Mueller⁵, Jasmin Finger⁵, Fabian Knörr^{1,6}, Amambay Riquelme¹, Ilse Oschlies⁷, Wolfram Klapper⁷, Jutta Bradtke⁸, Birgit Burkhardt⁵, Wilhelm Woessmann¹

¹Pädiatrische Hämatologie und Onkologie und NHL-BFM Studienzentrale, Universitätsklinikum Hamburg-Eppendorf; ²Pädiatrische Hämatologie und Onkologie und CoALL Studienzentrale, Universitätsklinikum Hamburg-Eppendorf; ³Pädiatrische Hämatologie und Onkologie, Medizinische Hochschule Hannover und NHL-BFM Studienzentrale; ⁴Bioinformatik, Universitätsklinikum Hamburg-Eppendorf; ⁵Pädiatrische Hämatologie und Onkologie und NHL-BFM Studienzentrale, Universitätsklinikum Münster; ⁶Mildred Scheel Cancer Career Center HaTriCS4, Universitätsklinikum Hamburg-Eppendorf; ⁷Institut für Pathologie, Sektion Hämatopathologie und Lymphknotenregister, Universitätsklinikum Schleswig-Holstein, Campus Kiel; ⁸Institut für Pathologie, Justus-Liebig-Universität Gießen

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 *MYC* were detected in 7 children (5%). They correlated with *IGL* fusions and were associated with inferior survival compared to all other breakpoints ($14 \pm 13\%$ compared to $86 \pm 3\%$, $p < 0.0001$). MDD detected in 71 of 93 (76%) children with BL was not associated with event-free survival or survival. Early MRD positivity in 6 of 48 (12%) children with B-AL correlated with inferior survival ($33 \pm 19\%$ compared to $90 \pm 5\%$, $p < 0.001$).

Conclusion: Small groups of patients at very high risk for failure could be identified by sequencing of chromosome 8 breakpoints in *IGH-MYC* negative tumors and early MRD analysis in B-AL whereas the previously reported prognostic value of MDD could not be confirmed (*Leukemia* 2022, <https://doi.org/10.1038/s41375-022-01626-w>).

LNK/SH2B3 as a novel driver in juvenile myelomonocytic leukemia

Astrid Wintering¹, Anna Hecht², Julia Meyer¹, Eric Wong¹, Deborah French³, Jean Ann Maguire³, Chintan Jobaliya³, Marta Rojas Vasquez⁴, Sunil Desai⁴, Robin Dulman⁵, Eneida Nemecek⁶, Farid Chehab⁷, Mignon Loh⁸, Elliot Stieglitz^{1,9}

¹Helen Diller Comprehensive Cancer Center, University of California San Francisco, San Francisco, USA; ²Department of Hematology/Oncology, Klinikum rechts der Isar, Technische Universität München, München, Germany; ³Center for Cellular and Molecular Therapeutics, The Children's Hospital of Philadelphia, Philadelphia, USA; ⁴Department of Pediatrics, University of Alberta, Edmonton, Canada; ⁵Pediatric Hematology and Oncology, Pediatric Specialists of Virginia, Fairfax, USA; ⁶OHSU Knight Cancer Institute, Oregon Health and Science University, Portland, USA; ⁷Institute for Human Genetics, University of California San Francisco, San Francisco, USA; ⁸Ben Towne Center for Childhood Cancer Research, Seattle Children's Research Institute, and the Department of Pediatrics, Seattle Children's Hospital, University of Washington, Seattle, USA; ⁹Department of Pediatrics, Benioff Children's Hospital, University of California San Francisco, San Francisco, USA

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

Ida Tölle¹, Claudia Lanvers-Kaminsky¹, Maria Bethke¹, Gerrit Randau¹, Meike Roling¹, Annabelle Tann¹, Marcel te Vrugt¹, Karin Mellgren², Stephanie Müller¹, Birgit Burkhardt¹

¹Pediatric Hematology and Oncology and NHL-BFM study Center, University Hospital Muenster, Muenster, Germany; ²Sahlgrenska University Hospital Gothenburg, Gothenburg, Sweden

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 chemotherapeutic 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

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 leukemia (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-ALR4: 43 µg/ml versus mean BLR4: 72 µg/ml).

In the lower risk groups (R1/R2), patients with DLBCL had a faster clearance than those with BL (mean DLBCLR1/R2: 93 µg/ml versus mean BLR1/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

Julia Beckhaus¹, Carsten Friedrich¹, Svenja Boekhoff¹, Gabriele Calaminus², Brigitte Bison³, Maria Eveslage⁴, Beate Timmermann⁵, Jörg Flitsch⁶, Hermann L. Müller¹

¹ Department of Pediatrics and Pediatric Hematology/Oncology, University Children's Hospital, Carl von Ossietzky University Oldenburg, Klinikum Oldenburg AöR, 26133 Oldenburg, Germany; ² Department of Pediatric Hematology/Oncology, University of Bonn Medical Center, Bonn, Germany; ³ Department of Neuroradiology, University Hospital, Augsburg, Germany; ⁴ Institute of Biostatistics and Clinical Research, University of Münster, Germany; ⁵ Department of Particle Therapy, University Hospital Essen, Germany; ⁶ Department of Neurosurgery, University Medical Center Hamburg-Eppendorf (UKE), Hamburg, Germany.

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

Natalia Borbarán-Bravo¹, Larissa Doll¹, Benjamin Dannenmann¹, Sandro Bräuning¹, Mohammad ElGamacy^{1,2}, Alexei Maschan³, Anna Shcherbina⁴, Ekaterina Deordieva⁴, Cornelia Zeidler⁵, Baubak Bajoghly¹, Karl Welte⁶, Julia Skokowa¹ and Maksim Klimianko¹

¹Department of Hematology, Oncology, Clinical Immunology, University of Tübingen, Tübingen, Germany; ²Department of Protein Evolution, Max Planck Institute for Developmental Biology, Tübingen, Germany; ³Department of Hematopoietic Stem Cell Transplantation, Dmitry Rogachev National Medical Research Center of Pediatric Hematology, Oncology and Immunology, Moscow, Russian Federation; ⁴Oncology and Immunology Ministry of Healthcare of Russian Federation, Dmitry Rogachev National Medical Research Center of Pediatric Hematology, Moskva, Russian Federation; ⁵Department of Hematology, Oncology, and Bone Marrow Transplantation, Hannover Medical School, Hannover, Germany; ⁶University Children's Hospital Tübingen, Tübingen, Germany

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.445C>T;p.Gln141Ter in the *COPZ1* gene in both siblings. *COPZ1* encodes a subunit of the cytoplasmic coatmer 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 *COPZ1* mutant zebrafish embryos was markedly lower than in the wild-type group. To elucidate the molecular mechanism of defective granulopoiesis downstream of mutated *COPZ1*, we perform 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.

