PRECLINICAL MODELS/EXPERIMENTAL THERAPY/DRUG DISCOVERY

MODL-01. TARGETING REPLICATION STRESS IN PEDIATRIC BRAIN TUMORS

Sonja Krausert[1], Norman Mack[1], Benjamin Schwalm[1], Heike Peterziel[1], Ina Oehme[1], Cornelis M. van Tilburg[1,2], Olaf Witt[1,2], Stefan M. Pfister[1,2], Marcel Kool[1,3]; [1]Hopp-Children's Cancer Center (KiTZ) Heidelberg and German Cancer Research Center (DKFZ), Heidelberg, Germany. [2]Heidelberg University Hospital, Heidelberg, Germany. [3]Princess Máxima Center for Pediatric Oncology, Utrecht, Netherlands

Previously, we have found that Embryonal Tumors with Multilayered Rosettes (ETMR) tumor cells harboring high levels of R-loops, a potential marker for replication stress and genomic instability, are vulnerable to a combination of topoisomerase and PARP inhibitors. To follow up on this, we investigated whether other pediatric brain tumor types with high levels of R-loops, such as MYC-amplified Group 3 medulloblastoma (MB) and ZFTA-fusion positive ependymoma, are also sensitive to these inhibitors. First, we performed in vitro drug screens using HD-MB03, a Group 3 MB cell line, and the ETMR cell line BT183, and in both screens PARP inhibitors were identified as the most synergistic combination partners for the topoisomerase inhibitor Irinotecan, respectively the active metabolite SN-38. Normal Astrocytes were not sensitive to these combinations. Secondly, we performed in vivo studies using patient-derived xenograft (PDX) models injected subcutaneously or intracranially into NSG mice, and treated with the PARP inhibitor Pamiparib, Irinotecan or a combination of both. For a MYC-amplified Group 3 MB and a ZFTA-fusion positive Ependymoma model, both injected intracranially, treatment with Irinotecan or the combination led to a significant survival benefit and inhibition of tumor growth including transient tumor shrinkage, but addition of Pamiparib did not add any further benefit in vivo, even though intratumoral PARP was inhibited by at least 80%. In contrast, in the subcutaneously injected ETMR model, the combination treatment with Irinotecan and Pamiparib led to a synergistic effect and complete regression of the tumors. Further refinements of the treatment strategy as dose adaptations and the use of a pegylated version of SN-38 (PLX038A) did also not induce a synergistic effect of the drugs for the intracranial tumors. Additional in vivo studies to evaluate the differences in efficacy and whether these are tumor specific or due to incomplete brain penetrance of the drugs are ongoing.

MODL-02. A NOVEL *CRE*-CONDITIONAL *CMYC*-DRIVEN MB GROUP 3 TRANSGENIC MOUSE MODEL SHOWS TRACEABLE LEPTOMENINGEAL DISSEMINATION.

Alaide Morcavallo[1], Karen Barker[1], Colin Kwok[1], Jessica KR Boult[2], Patricia Benites Goncalves da Silva[3], Konstantin Okonechnikov[3], Marc Zuckermann[3], Chiara Gorrini[1], Thomas S Jacques[4], Simon P Robinson[2], Steven C Clifford[5], William A Weiss[6], Stefan M Pfister[3], Daisuke Kawauchi[7], Louis Chesler[1]; [1]Division of Clinical Studies, The Institute of Cancer Research, and The Royal Marsden NHS Trust, Sutton, Surrey, United Kingdom. [2]Division of Radiotherapy and Imaging, The Institute of Cancer Research, Sutton, Surrey, United Kingdom. [3]Hopp Children´s Cancer Center Heidelberg (KiTZ), Division of Pediatric Neurooncology, German Cancer Consortium (DKTK) and German Cancer Research Center (DKFZ), and Department of Pediatric Hematology and Oncology, Heidelberg University Hospital, Heidelberg, Germany. [4]Developmental Biology and Cancer Programme, UCL Great Ormond Street Institute of Child Health and Histopathology Department, Great Ormond Street Hospital for Children NHS Foundation Trust, London, United Kingdom. [5]Wolfson Childhood Cancer Research Centre, Newcastle University Centre for Cancer, Newcastle Upon Tyne, United Kingdom. [6]Department of Neurology, University of California, San Francisco, CA, USA. [7]Department of Biochemistry and Cellular Biology, National Center of Neurology and Psychiatry (NCNP), National Institute of Neuroscience, Tokyo, Japan

Medulloblastoma (MB), the most common embryonal tumour of the Central Nervous System, occurs in the cerebellum. Treatment regimens involve surgery, craniospinal radiotherapy, and chemotherapy. The greatest mortality is associated with disseminated disease, almost exclusively found in the leptomeningeal space. Unfortunately, knowledge about the aetiology of MB spread is limited and the need for kinder and efficacious therapy remains an unmet goal. Of the four molecular classified MB groups, Group3 (Gr3) MB presents with a high frequency of metastasis at diagnosis, with the worst overall survival. Gr3 MB tumours are dominated by primitive progenitor-like cells and *cMYC* deregulation; often, p53 deficiency is observed at relapse. To dissect the biology of primary and metastatic Gr3 MB, we have developed a new germline genetically engineered mouse model (GEMM), harbouring *cMYC* amplification in a Tamoxifen-inducible p53 functional background (*Trp53ERTAM* strain). A novel LSL-*cMYC-CopGFP-Luciferase* transgene was integrated into the Rosa-26 locus of the mouse genome. Transgenic mice were crossed with a strain expressing *Cre* recombinase under the Blbp promoter targeting embryonic neural progenitors, and subsequently bred to *Trp53ERTAM* mice. As result, the *cMYC* overexpression was sufficient to generate tumours. Tumour penetrance was observed in all the expected tumour bearing genotypes, with increased aggressiveness in a non-functional p53 background. Bioluminescence imaging demonstrated tumour onset in the brain and dissemination along the spinal cord. CopGFP positive tumour cells were isolated from primary and metastatic tumours. Pathological interrogation confirmed that tumours present large cell/anaplastic (LCA) histology. Analysis of preliminary transcriptional profiling data proved that tumours cluster with human Gr3 MB. Ongoing methylation profiling and multi-omics approaches will inform on the tumour cells of origin and clonal divergence of primary tumour versus metastasis. In conclusion, we have successfully developed a novel immunocompetent mouse model of metastatic Gr3 MB with which we can investigate therapeutic vulnerabilities of MB.

MODL-03. ESTABLISHMENT OF INTRAVENTRICULAR SHH INHIBITION AS A THERAPEUTIC OPTION FOR YOUNG PATIENTS WITH MEDULLOBLASTOMA

Catena Kresbach[1,2], Melanie Schoof[1,2], Lea Holst[1], Tara Leven[1,2], Timur Yorgan[3], Antonina Wrzeszcz[1], Stefan Rutkowski[2], Ulrich Schüller[1,4]; [1]Research Institute Children's Cancer Center Hamburg, Hamburg, Germany. [2]Department of Pediatric Hematology and Oncology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany. [3]Department of Osteology and Biomechanics, University Medical Center Hamburg-Eppendorf, Hamburg, Germany. [4]Institute of Neuropathology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

The prognosis of pediatric medulloblastoma is still dissatisfying today and tumor survivors often suffer from severe treatment-related morbidities. This poses an urgent need for more efficient therapies. Shh medulloblastoma is characterized by mutations in the Sonic Hedgehog (Shh) pathway, providing an elegant way of targeted therapy. The small molecule Vismodegib allosterically inhibits Smoothened (SMO), an upstream activator of Shh, and shows promising anti-tumor effects against Shh medulloblastoma. Unfortunately, Vismodegib caused severe bone deformities in preclinical studies and clinical trials, preventing its systemic application in children. In a mouse model, we established an intraventricular therapy with Vismodegib combining the benefits of targeted drug delivery and minimal systemic side effects. We compare intraventricular, oral, and placebo treatment regarding effects on survival, tumor biology, and bone morphology.*Math1-cre::Ptch1Fl/Fl* mice show a homozygous loss of *Ptch1* in *Math1*-positive cells, resulting in Shh pathway overactivation and development of Shh medulloblastomas. At postnatal day 11-13, Math1-cre::Ptch1$^{Fl/Fl}$ mice were randomized in four treatment arms: Group A (n=14) received intraventricular placebo, B (n=12) received 200 mg/kd/d oral Vismodegib, C (n=16) received 0.2 mg/kg/d intraventricular Vismodegib, and D (n=9) received 1.6 mg/kg/d intraventricular Vismodegib. Kaplan-Meier survival curves show a significant survival benefit of 1.6 mg/kg/d intraventricular Vismodegib over placebo (p=0.003). While all intraventricular treated animals develop proliferative tumors at end of observation, investigations at an early time point after completed treatment show promising anti-tumor effects with reduced or absent proliferation in the cerebellum compared to placebo. Bone histology and X-ray analysis of intraventricular treated mice show intact femoral and tibial growth plates, in contrast to orally treated mice that develop severe skeletal malformations. Based on these preliminary experimental results, we conclude that intraventricular application of a SMO-inhibitor might evolve as a promising new way of targeted treatment of Shh medulloblastoma in children.

MODL-04. DRUG SCREENING IN DISORDERS WITH ABNORMAL DNA DAMAGE RESPONSE/REPAIR (DADDR) AND *IN VIVO* VALIDATION

Anna Kolodziejczak[1,2], Florian Selt[3,4], Heike Peterziel[1,2], Nora Jamaladdin[1,2], Norman Mack[1,5], Kendra Maass[1,5], Marcel Kool[6,7], Christel Herold-Mende[8], Ahmed El Damaty[9], Ina Oehme[1,2], David T. W. Jones[1,10], Olaf Witt[3,4], Kristian W. Pajtler[4,6], Christian Kratz[11], Stefan M. Pfister[4,6], Till Milde[3,4]; [1]Hopp Children's Cancer Center (KiTZ), Heidelberg, Germany. [2]CCU Pediatric Oncology, German Cancer Research Center (DKFZ) and German Cancer Consortium (DKTK), Heidelberg, Germany. [3]Hopp Children's Cancer Center (KiTZ), CCU Pediatric Oncology, German Cancer Research Center (DKFZ) and German Cancer Consortium (DKTK), Heidelberg, Germany. [4]Pediatric Oncology, Hematology, and Immunology, Center for Child and Adolescent Medicine, Heidelberg University Hospital, Heidelberg, Germany. [5]Pediatric Neurooncology, German Cancer Research Center (DKFZ) and German Cancer Consortium (DKTK), Heidelberg, Germany. [6]Hopp Children's Cancer Center (KiTZ), Pediatric Neurooncology, German Cancer Research Center (DKFZ) and German Cancer Consortium (DKTK), Heidelberg, Germany. [7]Princess Máxima Center for Pediatric Oncology, Utrecht,

Netherlands. [8]Department of Neurosurgery, Heidelberg University Hospital, Heidelberg, Germany. [9]Pediatric Neurosurgery Division, Department of Neurosurgery, Heidelberg University Hospital, Heidelberg, Germany. [10]Pediatric Glioma Research, German Cancer Research Center (DKFZ) and German Cancer Consortium (DKTK), Heidelberg, Germany. [11]Pediatric Haematology and Oncology, Hannover Medical School, Hannover, Germany

INTRODUCTION: Disorders with Abnormal DNA Damage Response/Repair (DADDRs) are inherited conditions caused by constitutional mutations of DNA damage response and repair genes and are characterized by an increased cancer risk. Furthermore, affected individuals also show an elevated risk of secondary neoplasms as well as excessive toxicity, poor therapy response and increased mortality when treated with standard radiation and chemotherapy regimens. The main aim of this project is to screen for potential novel chemotherapeutic approaches for these cancer entities, and to employ faithful PDX models for *in vivo* validation. METHODS: *In vitro* drug screening was performed using a custom library composed of 345 compounds targeting 61 different proteins. For two specific DADDRs, Li-Fraumeni syndrome (LFS) and Constitutional Mismatch Repair Deficiency (CMMRD), two cancerous (glioblastoma and medulloblastoma) and one non-cancerous cell lines were selected to model each of these conditions. Performance of each drug was assessed based on its efficacy (sensitivity score) and genotoxicity (micronucleus assay). For DADDR PDX model establishment tumor material from DADDR patients is currently being injected orthotopically (brain tumors) or subcutaneously (non-brain tumors) into NSG mice. Following engraftment and expansion, the PDX models will be characterized molecularly and compared with original patient material. RESULTS AND OUTLOOK: *In vitro* screening revealed n=26 drugs that fulfilled the following criteria: a) favorable toxicity in cancerous cell lines compared to non-cancerous cell lines, b) little to no genotoxic effect in non-cancerous cell lines. These characteristics qualify them as potentially suitable candidates for novel therapeutic approaches specifically for DADDR patients. The hits included inhibitors of ATM/ATR, CHK1/CHK2, DHFR, mTOR and PI3K, as well as microtubule-associated compounds. Combination testing and further validation of these hits using disease-specific *in vitro* and *in vivo* PDX models is ongoing.

MODL-05 METRONOMIC INTRATHECAL DELIVERY OF CDK4/6 INHIBITORS IN PRECLINICAL MODELS OF PEDIATRIC BRAIN TUMORS

Sergio Guadix[1], Brice Martin[1], Madeline Laramee[1], Nadia Dahmane[1], Craig Thomas[2], Mark Souweidane[1,3]; [1]Department of Neurological Surgery, Weill Cornell Medicine, New York, NY, USA. [2]Division of Preclinical Innovation, National Center for Advancing Translational Sciences, National Institutes of Health, Rockville, MD, USA. [3]Department of Neurosurgery, Memorial Sloan Kettering Cancer Center, New York, NY, USA

INTRODUCTION: CDK4/6 inhibitors have shown promise against central nervous system (CNS) tumors *in vitro*. This class of drugs relies on long-term exposure. Their use in early phase clinical studies in children with CNS tumors has defined dose limitations due to systemic toxicity. We have sought to circumvent these limitations in using CDK4/6 inhibitors for pediatric CNS tumors by first demonstrating enhanced efficiency with long-term administration and exploiting prolonged intrathecal delivery (IT). METHODS: Pediatric CNS tumor cell lines were used for cell viability assays: ATRT (BT-12, BT-16), CPC (CCHE-45), diffuse midline glioma (DIPG-XIII, HSJD-007), and medulloblastoma (DAOY); the assays were conducted at 24h, 72h, and 7d post-administration of CDK4/6 inhibitors (abemaciclib, palbociclib, ribociclib). Half maximal growth inhibitory concentrations (GI50) and areas under the curve (AUC) were compared for short-term (24h, 72h) and long-term (7d) dose-response curves. Toxicity with chronic IT administration was assessed using a neurobehavioral safety profile of 7-day continuous infusion of 2.5mM palbociclib (n = 5) into the mouse lateral ventricle compared with vehicle (n = 4). RESULTS: Our results demonstrate increased CDK4/6 inhibitor potency with longer administration. The greatest reductions in short-term to long-term GI50 were observed in ATRT, CPC, and DIPG across all inhibitors. The most pronounced time-dependent efficacy was observed with palbociclib for ATRT and abemaciclib for CPC and DIPG. AUCs significantly decreased ($P < 0.05$) with increasing drug exposure time across all inhibitors. 7-day intraventricular palbociclib infusion was equivalent in safety to PBS at doses ranging from 1,000 to 10,000-fold the *in vitro* GI50. CONCLUSIONS: The efficiency of CDK4/6 inhibitors in pediatric CNS tumors is enhanced with prolonged exposure. Long-term IT administration can achieve high CNS doses without associated systemic toxicities. Translational efforts using a metronomic IT strategy are logical to explore for pediatric CNS tumors which have potential for a leptomeningeal disease pattern.

MODL-06. TARGETING C-MET IN COMBINATION WITH RADIATION IS EFFECTIVE IN MET-FUSION DRIVEN HIGH-GRADE GLIOMA

Marc Zuckermann[1,2], Chen He[3], Jared Andrews[3], Roketa Sloan-Henry[4], Brandon Bianski[5], Jia Xie[5], Yingzhe Wang[6], Nathaniel Twarog[7], Arzu Onar-Thomas[8], Kati Ernst[1,2], Lei Yang[7], Yong Li[7], James Dalton[9], Xiaoyu Li[9], Divyabharathi Chepyala[7], Xiaoyan Zhu[3], Junyuan Zhang[3], Ke Xu[10,11], Laura Hover[3], Peter J. McKinnon[4], Stefan M. Pfister[1,12], Zoran Rankovic[7], Burgess B. Freeman[6], Anang A. Shelat[7], Jason Chiang[9], David T.W. Jones[1,2], Christopher L. Tinkle[5], Suzanne J. Baker[3]; [1]Hopp Children's Cancer Center Heidelberg (KiTZ), Heidelberg, Germany. [2]Division of Pediatric Glioma Research, German Cancer Research Center (DKFZ), Heidelberg, Germany. [3]Developmental Neurobiology, St. Jude Children's Research Hospital, Memphis, USA. [4]Center for Pediatric Neurological Disease Research, St. Jude Children's Research Hospital, Memphis, USA. [5]Radiation Oncology, St. Jude Children's Research Hospital, Memphis, USA. [6]Preclinical Pharmacokinetics Shared Resource, St. Jude Children's Research Hospital, Memphis, USA. [7]Chemical Biology and Therapeutics, St. Jude Children's Research Hospital, Memphis, USA. [8]Biostatistics, St. Jude Children's Research Hospital, Memphis, USA. [9]Pathology, St. Jude Children's Research Hospital, Memphis, USA. [10]Center for Applied Bioinformatics, St. Jude Children's Research Hospital, Memphis, USA. [11]Computational Biology, St. Jude Children's Research Hospital, Memphis, USA. [12]Division of Pediatric Neurooncology, German Cancer Research Center (DKFZ), Heidelberg, Germany

Oncogenic fusion events involving c-MET have been observed in up to 12% of pediatric high-grade glioma (pHGG). MET inhibitors have displayed potent initial responses in MET rearranged tumors but acquired resistance to single agent modalities invariably occurs. To identify new treatment options against these tumors, we established two novel orthotopic mouse models including an immunocompetent, murine allograft and an intracranial patient-derived xenograft (PDX), both harboring distinct MET fusions. We analyzed the pharmacokinetic and pharmacodynamic profiles of two MET inhibitors, crizotinib and capmatinib, and examined their efficacy against tumor cell cultures derived from the aforementioned models. Capmatinib outperformed crizotinib in terms of specificity, potency and brain availability, resulting in a highly differential cellular response compared to crizotinib treatment. We evaluated the efficacy of both compounds in combination with radiotherapy (RT) and found that radiation further potentiated the inhibitory effect of capmatinib on tumor cell growth. We then utilized both models to assess the combinatorial effect of capmatinib and radiation on intracranial tumors in vivo and found that the combination therapy significantly increased overall survival in both cohorts. In the PDX model, the combination, relative to either intervention alone, induced a remarkable decrease of tumor burden, which persisted throughout the observation period in all treated animals. RNA-sequencing of capmatinib-treated tumors and tumor cell cultures revealed impaired expression of DNA repair genes. Further, we showed that capmatinib enhanced radiation-induced DNA damage, as demonstrated by increased γ-H2AX foci in treated cells, providing mechanistic insight for the cooperative effects of the combined treatment. Our results validate capmatinib as an effective inhibitor of MET in pHGG and demonstrate the outstanding efficacy of capmatinib and radiation against MET-driven pHGG in two complementary preclinical models, informing future clinical trials.

MODL-07. DNA METHYLATION-BASED BIOBANK OF MURINE MODELS FOR PEDIATRIC TUMORS

Tuyu Zheng[1,2], Martin Sill[1], Roland Imle[3,4], Ryo Shiraishi[5], Wanchen Wang[5], Alaide Morcavallo[6], Louis Chesler[6], Daisuke Kawauchi[5], Olivier Ayrault[7,8], Lutsik Pavlo[9], Stefan M. Pfister[1,10], Lena M. Kutscher[11], Ana Banito[3], David W. Jones[12], Kristian W. Pajtler[1,10], Marc Zuckermann[1]; [1]Hopp Children's Cancer Center Heidelberg (KiTZ) and Division of Pediatric Neurooncology, German Cancer Research Center (DKFZ), Heidelberg, Germany. [2]Faculty of Biosciences, Heidelberg University, Heidelberg, Germany. [3]Hopp Children's Cancer Center Heidelberg (KiTZ) and Pediatric Soft Tissue Sarcoma Research Group, German Cancer Research Center (DKFZ), Heidelberg, Germany. [4]Department of General, Visceral and Transplantation Surgery, Division of Pediatric Surgery, University Hospital Heidelberg, Heidelberg, Germany. [5]Department of Bicochemistry and Cellular Biology, National Center of Neurology and Psychiatry (NCNP), Tokyo, Japan. [6]Division of Clinical Studies, The Institute of Cancer Research, and The Royal Marsden NHS Trust, Sutton, Surrey, United Kingdom. [7]Institut Curie, PSL Research University, CNRS UMR, INSERM, Orsay, France. [8]Université Paris Sud, Université Paris-Saclay, CNRS UMR 3347, INSERM U1021, Orsay, France. [9]Division of Cancer Epigenomics, German Cancer Research Center (DKFZ), Heidelberg, Germany. [10]Department of Neuropathology, Institute of Pathology, Heidelberg University Hospital, Heidelberg, Germany. [11]Hopp Children's Cancer Center Heidelberg (KiTZ) and Developmental Origins of Pediatric Cancer