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,