debilitating long-term effects and risk of tumor recurrence. Medulloblastoma stem cells (MBSCs) are a fraction of tumor cells with high proliferation potential and the capability to adapt to adverse/restrictive conditions in tumor milieu thus driving the refractoriness to conventional therapies. Recently, high basal levels of Unfolded Protein Response (UPR) molecules have been found in tumors of different tissue-origin and are correlated with poor prognosis and low patient survival. However, little is known about the role of UPR in MB. We investigated the expression and activation of UPR players in MBSCs. Human group 3 MB (G3MB) cell lines, specifically CHLA-01, D283- and D341-Med, were grown in Vitamin A and/or FBS or in stem selective medium (B27™) for 72 h before collection. Cells were fixed, stained with proper primary antibodies and images were acquired by confocal microscopy. The analysis of the transcription factors ATF-4 and CHOP revealed their elevated nuclear expression and co-localization, which resulted to be more marked in G3MB stem-like cells than in the differentiated ones. Also the ATF-6 branch was investigated, in differentiating conditions D283 and D341-Med showed a greater activation of ATF-6, represented by its nuclear localization, in respect to stem cells, while CHLA-01 did not show differences. Conversely XBP1, the transcription factor downstream IRE1 signaling, was not expressed in the three cell lines. Lastly, a Kaplan-Meier analysis on MB patients showed a worse prognosis with a shorter survival rate of patients expressing high ATF4 transcript levels. Our results reveal, even in resting conditions, preferential activation of the PERK branch in G3MB cells grown in stem-like condition suggesting that ATF-4 might be a promising therapeutic and prognostic factor to specifically target the stem compartment in aggressive MB.

MEDB-10. COMPARING PEDIATRIC MEDULLOBLASTOMA WITH AND WITHOUT SPINAL METASTASIS

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AIM: To compare pediatric medulloblastoma with and without spinal metastasis METHODOLOGY: Pediatric medulloblastoma cases from 1999 to 2021 were retrospectively reviewed in this Institutional Review Board approved study. Imaging reports, presence of spinal drop metastases at diagnosis, degree of tumor excision, treatment given and survival status were captured. RESULTS: Brain and spine imaging at diagnosis was available in 54 medulloblastoma patients with no drop metastasis and in 7 with drop metastasis. Largest tumor dimension at presentation is 4.54 ± 0.94 cm with those with drop metastasis, similar to the 4.43 ± 0.94 cm in those without drop metastasis (p = 0.79). For the 54 medulloblastomas with no drop metastasis, 44 (81%) were completely excised, 9 (17%) partially excised and there was no follow up for 1. For the 7 medulloblastomas with drop metastasis, 3 (43%) of the primary tumours were completely excised, 3 (43%) partially excised and there was no follow up for 1. Post operative chemo/radiotherapy was given to 48 of the 54 with no drop metastasis, not given for 1 with no information available for 5. Chemo/radiotherapy was given to 6 of the 7 with drop metastasis with no information available for 1. At 1 year follow up of the 54 with no spinal drop metastasis at diagnosis , 42 remain tumour free, 3 have tumour, 4 are deceased and 5 are lost to follow up. At 1 year follow up of the 7 with drop metastasis, 2 are free of tumour, 2 have tumour and 3 are lost to follow up. Higher percentage of medulloblastomas without drop metastasis are completely excised (p<0.01). No significant difference between postoperative chemotherapy/radiation rates between groups CONCLUSION: Most medulloblastomas do not have spinal drop metastasis at diagnosis and complete excision is more frequently in those without drop metastasis.

MEDB-11. MYC OVEREXPRESSION AND SMARCA4 LOSS IN CEREBELLAR GRANULE CELL PRECURSORS COOPERATE TO DRIVE MEDULLOBLASTOMA FORMATION IN MICE

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Group 3 medulloblastoma is one of the most aggressive types of childhood brain tumors. Roughly 30 % of cases carry genetic alterations in *MYC*, *SMARCA4* or both genes combined. While overexpression of MYC has previously been shown to drive medulloblastoma formation in mice, the functional significance of *SMARCA4* mutations and their suitability as a therapeutic target remain largely unclear. To address this issue, we combined an overexpression of MYC with a loss of SMARCA4 in cerebellar granule cell precursors. Cells were isolated from 7-day-old *Math1-creERT2::Smarca4fl/fl* pups after tamoxifen-induced loss of SMARCA4. Subsequently, MYC overexpression was achieved by lentiviral transduction, and transduced cells were transplanted into immunodeficient *CD1nu/nu* recipient mice. Preliminary results show tumor formation in 5/19 transplanted mice (26 %) after 6 months. SMARCA4 loss in all tumor cells was confirmed both immunohistochemically and on a genetic level and suggests a dependency of tumor growth on SMARCA4 loss. In a next step, additional cohorts will clarify if tumor development is accelerated by or even dependent on the loss of SMARCA4 in our model. Additionally, the neoplastic potential of tumor cells will be verified with the aid of secondary recipient mice. To evaluate to what extent the generated tumors are comparable to human Group 3 medulloblastomas, tumors will be extensively analyzed on a morphological, transcriptional, and epigenetic level. Altogether, we hope to establish a suitable mouse model for *SMARCA4* mutated Group 3 medulloblastoma that will help to elucidate the role of SMARCA4 in tumor development and to identify new therapeutic targets.

MEDB-12. SEVERE DEVELOPMENTAL ABNORMALITIES AND PROLIFERATIVE CEREBELLAR LESIONS INDUCED BY COMBINED ACTIVITY OF WNT SIGNALLING AND LOSS OF SMARCA4

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Almost all medulloblastomas (MB) of the WNT subgroup are characterized by hotspot mutations in *CTNNB1*, and mouse models have convincingly demonstrated the tumor-initiating role of these mutations as well as the tumor origin in the dorsal brain stem. Around 20 % of WNT MB additionally carry *SMARCA4*mutations, but the functional role of these alterations is mostly unknown. We therefore amended previously described *Blbp-cre::Ctnnb1(ex3)Fl/+* mice by the introduction of a floxed *Smarca4* allele. In contrast to existing literature, Blbp-cre::Ctnnb1(ex3)$^{Fl/+}$ mice had a maximum life span of only 17 days, even after breeding into two different genetic backgrounds (C57BL/6J and 129S2/Sv). The mice displayed a severe developmental phenotype including a thinned cerebral cortex, hydrocephalus, missing cerebellar foliation and layering as well as non-proliferative cell accumulations in brain stem and cerebellum. An additional homozygous loss of SMARCA4 even resulted in prenatal death for most mice and caused big proliferative lesions in the cerebellum at embryonal day 14.5. These lesions appear to originate from SOX2-positive progenitor cells in the cerebellar ventricular zone. In a next experiment, cells isolated from this region will be characterized *in vitro* and will be transplanted orthotopically to evaluate their neoplastic potential *in vivo*. Altogether, we hope to elucidate how a loss of SMARCA4 and mutations of *Ctnnb1* cooperate during hindbrain development and tumor formation within this region.

MEDB-13. NEUROCOGNITIVE AND RADIOLOGICAL FOLLOW-UP OF CHILDREN UNDER 5 YEARS OF AGE TREATED FOR MEDULLOBLASTOMA ACCORDING TO THE HIT-SKK PROTOCOL

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BACKGROUND: HIT-SKK protocol is used for the treatment of low risk medulloblastomas in young children with the aim of eliminating cranial irradiation and its long-term side effects, in particular neuropsychological (NP) sequelae. This therapy includes IV and intraventricular (ITV) methotrexate (MTX) potentially responsible for leukoencephalopathy (LE) and neurocognitive disorders.The objectives are to describe the risk factors and

the course of LE, and to investigate its impact on long-term neurocognitive and behavioural outcome. METHODS: A French retrospective, multicenter study including 35 children under 5 years of age, treated between 2009 and 2017, with a median follow up of 72 months. All follow-up MRIs including assessment of the severity of the LE (Fazekas and CTCAE grading) and all NP evaluations were centrally rewieved. RESULTS: 25/34 evaluable patients presented a LE during follow up, in a median delay of 2 months (1 - 17 months) after the start of chemotherapy. Grade 2 and 3 abnormalities were correlated with higher cumulative dose of ITV -MTX (p=0,01). Full Scale IQ (FSIQ) and Wechsler indexes were in the average or low average of the reference population. FSIQ was deficient in 7/20 evaluable patients. Processing speed (PSI) was the most frequently impaired neurocognitive domain: 9/20 patients with borderline or very low score, all having received a significantly higher cumulative dose of ITV-MTX (p=0,04). A decrease in overall NP scores was observed in patients for whom grade 2 or 3 LE persisted at the end of follow-up with an average FSIQ estimated at 82.1 (SD 16.9) versus 94.2 (SD 20.6). This decrease was significant for PSI (p=0,049). LE and neurocognitive impairments were not correlated with a younger age at diagnosis. CONCLUSION: This study confirmed the responsibility of MTX, and in particular ITV-MTX therapy in the onset and, most often, persistence of LE and its association with neurocognitive disorders.

MEDB-14. CLINICAL OUTCOME OF PEDIATRIC MEDULLOBLASTOMA PATIENTS WITH LI-FRAUMENI SYNDROME

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PURPOSE: The prognosis for SHH-medulloblastoma (MB) patients with Li-Fraumeni syndrome (LFS) is poor. Due to lack of comprehensive data for these patients, it is challenging to establish effective therapeutic recommendations. We here describe the largest retrospective cohort of pediatric LFS SHH-MB patients to date and their clinical outcomes. PATIENTS AND METHODS: N=31 patients with LFS SHH-MB were included in this retrospective multicenter study. *TP53* variant type, clinical parameters including treatment modalities, event-free survival (EFS) and overall survival (OS), as well as recurrence patterns and incidence of secondary neoplasms, were evaluated. RESULTS: All LFS-MBs were classified as SHH subgroup, in 30/31 cases based on DNA methylation analysis. The majority of constitutional *TP53* variants (72%) represented missense variants, and all except two truncating variants were located within the DNA-binding domain. 54% were large cell anaplastic, 69% gross totally resected and 81% had M0 status. The 2-(y)ear and 5-(y)ear EFS were 26% and 8,8%, respectively, and 2y- and 5y-OS 40% and 12%. Patients who received post-operative radiotherapy (RT) followed by chemotherapy (CT) showed significantly better outcomes (2y-EFS:43%) compared to patients who received CT before RT (30%) ($p<0.05$). The 2y-EFS and 2y-OS were similar when treated with protocols including high-dose chemotherapy (EFS:22%, OS:44%) compared to patients treated with maintenance-type chemotherapy (EFS:31%, OS:45%). Recurrence occurred in 73.3% of cases independent of resection or M-status, typically within the radiation field (75% of RT-treated patients). Secondary malignancies developed in 12.5% and were cause of death in all affected patients. CONCLUSIONS: Patients with LFS-MBs have a dismal prognosis. This retrospective study suggests that upfront RT may increase EFS, while intensive therapeutic approaches including high-dose chemotherapy did not translate into increased survival of this patient group. To improve outcomes of LFS-MB patients, prospective collection of clinical data and development of treatment guidelines are required.

MEDB-15. DYNAMIC CHROMATIN ALTERATION INDUCES ONCOGENIC HIJACKING BY ESSENTIAL TRANSCRIPTIONAL FACTORS DURING SHH MEDULLOBLASTOMA TUMORIGENESIS

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Medulloblastoma is a malignant brain tumor that occurs in the cerebellum, most frequently in children. Medulloblastoma is molecularly classified into four major groups, and therapies are now being developed according to the nature of these groups and subgroups. However, there are currently no effective molecularly targeted drugs for most of these groups. In recent years, we have been analyzing the genomes of medulloblastomas to identify genetic mutations involved in tumorigen-