EMBR-12. TARGETING THE RNA-BINDING PROTEIN LIN28B IN GROUP 3 MEDULLOBLASTOMA
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Medulloblastoma (MB) is the most common pediatric malignant brain tumor and is currently divided into WNT, SHH, Group 3 and Group 4 subtypes. Even with multimodal chemotherapy, radiotherapy and surgery, many children with Group 3 MB do not survive. We have previously demonstrated an oncogenic role for the RNA-binding protein (RBP) LIN28B in neuroblastoma. LIN28B is a key regulator of let-7 family miRNAs, which in turn inhibit LIN28A/B and other oncoproteins. LIN28B has also been found to be upregulated in Wilms tumor, hepatocellular carcinoma and leukemic abnormalities among others. We hypothesize that LIN28B plays an important role in Group 3 MB and that a better understanding of LIN28B and LIN28B-driven networks will reveal novel therapeutic vulnerabilities. LIN28B levels are highest in Group 3 MB patients and its overexpression is associated with significantly worse survival. Here we demonstrate that down-regulation of LIN28B using shRNA results in significant reduction in cell proliferation by CellTiter-Glo and increased apoptosis by Caspase-Glo (as well as induction of cleaved PARP on immunoblots). In contrast overexpression of LIN28B increases Group 3 cell proliferation and tumor sphere formation. The LIN28B inhibitor 1632 also leads to significant reduction in G3 MB cell proliferator. In addition, we find that PDZ-binding kinase (PKB) a downstream target of LIN28B is downregulated when LIN28B is depleted. PKB knock down results in increased proliferation. Finally, we performed high throughput drug screening using our in-house semi-automated platform and identified the HDAC inhibitor Entinostat as a drug that shows promising effects on MYC-driven MB cells. Using gene-expression, transcriptional activation screening, potential drug response modulators, mainly TGFβ/Erk/MKK1 signaling including neural EGFL like 2 (NELL2), were discovered. For further validation, we stably overexpressed NELL2 and untransformed cisplatin-resistant control cells with Entinostat. Using PI staining, cell cycle status was tracked. Entinostat treatment led to modest induction of cell death in MYC-driven MB cell controls but only slightly increased cell death rate in MYC-driven MB cells with NELL2 overexpression. Conclusion: We report that the combination of genetic and pharmacological approaches is a powerful approach to study drug resistance. Our data suggest that activation of the TGFβ/Erk/MKK1 signaling pathway desensitizes MYC-driven MB cells to Entinostat. Synergistic targeting of TGFβ/Erk/MKK1 signaling and MYC could therefore provide a novel therapeutic option in this aggressive MB subtype.

EMBR-14. INFLUENCE OF MRI FEATURES ON THE SURVIVAL AND IMPACT ON INDIVIDUAL MOLECULAR SUBGROUPS: RESULT FROM 171 PATIENTS WITH MEDULLOBLASTOMA
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Background: To investigate the influence of different MRI features on survival of patients with medulloblastoma. Methods: A total of 171 patients were included in the study, including 131 pediatric and 40 adults (> 18 years). A set of 16 pre-defined semantic MRI features were analyzed using T1W (pre and post-contrast), T2W, and diffusion-weighted imaging (additional sequences as available). Patients with a definitive event (recurrence) or a minimum follow up of 12 months (in case of no recurrence) were included in the current analysis. All patients were treated and followed up according to standard institutional practice. Log-rank test was used for univariate analysis (UVA) and Cox regression for multivariate analysis (MVA). Results: The molecular subgroups were as follows: WNT-27 children, 7 adults; SHH-31 children, 29 adults; group 3-32 children, 3 adults; and group 4-41 children, 1 adult. The median follow up was 45 months (range 1 to 137 months). For all the patients, on UVA the recurrence-free survival (RFS) was significantly (p<0.05) influenced by location-ventral, brainstem involvement, contrast uptake area, contrast heterogeneity, necrosis, and calcification. Similar factors (T2W homogeneity instead of area of contrast) impacted overall survival (OS). On MVA, location-ventral, brainstem involvement, and significant features were retained in the model. Tumor location-ventral was the only feature influencing RFS and OS within the SHH subgroup. For group 3 tumors, contrast uptake area (RFS and OS) and calcification (RFS alone) had a significant influence (MVA). Within group 4 patients, contrast pattern (RFS and OS) and group 3 MB were significant factors on UVA, none on MVA. Conclusion: Several MRI features can be linked with survival in patients with medulloblastoma, with a specific impact on individual molecular subgroups. Considering the entire population, non-central location on the vertical aspect, tumors away from brainstem, calcification are risk factors associated with inferior outcomes.

EMBR-13. NOVEL SYNERGISTIC APPROACHES FOR TARGETED THERAPY OF MYC-DRIVEN MEDULLOBLASTOMA USING CRISPR/CAS9 GENE EDITING
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Introduction: Resistance to chemotherapy is a common cause of treatment failure in cancer patients and a major problem facing current cancer research. Targeted modulation of oncogenic signaling pathways may be used to systematically characterize drug resistance mechanisms across tumor entities and may help to identify new therapeutic strategies. Since the transcription factor MYC is aberrantly activated in many cancers including pediatric malignant brain tumors, like medulloblastoma (MB), our study focused on MYC-related drug resistance. Methods and Results: We performed high-throughput drug screening using our in-house semi-automated platform and identified the HDAC inhibitor Entinostat as a drug that shows promising effects on MYC-driven MB cells. Using gene-expression, transcriptional activation screening, potential drug response modulators, mainly TGFβ/Erk/MKK1 signaling including neural EGFL like 2 (NELL2), were discovered. For further validation, we stably overexpressed NELL2 and untransformed cisplatin-resistant control cells with Entinostat. Using PI staining, cell cycle status was tracked. Entinostat treatment led to modest induction of cell death in MYC-driven MB cell controls but only slightly increased cell death rate in MYC-driven MB cells with NELL2 overexpression. Conclusion: We report that the combination of genetic and pharmacological approaches is a powerful approach to study drug resistance. Our data suggest that activation of the TGFβ/Erk/MKK1 signaling pathway desensitizes MYC-driven MB cells to Entinostat. Synergistic targeting of TGFβ/Erk/MKK1 signaling and MYC could therefore provide a novel therapeutic option in this aggressive MB subtype.
EMBR-16. SMOOTHENED-ACTIVATING LIPIDS DRIVE RESISTANCE TO CDK4/6 INHIBITION IN HEDGEHOG-ASSOCIATED MEDULLOBLASTOMA

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Background: Medulloblastoma is an aggressive pediatric brain tumor that is associated with misactivation of the Hedgehog (HH) pathway. Our lab has shown that CDK6, a critical activator of the cell cycle, is a direct transcriptional target of HH signaling, and that inhibiting CDK6 in vitro reduces growth of HH-associated medulloblastoma in mice. A clinical trial exploring the efficacy of CDK6 inhibition in medulloblastoma patients is underway, but prior attempts to target the HH pathway in medulloblastoma have been encumbered by resistance to molecular monotherapy. Thus, we sought to identify mechanisms of resistance to CDK6 inhibition in HH-associated medulloblastoma. Methods: We performed orthogonal CRISPR and CRISPR interference screens in HH-associated medulloblastoma cells treated with transcriptional HH signaling inhibitor (PD184387) and RNA-sequencing with similar tumor-bed dose and adjuvant systemic chemotherapy (residual tumor <1.5cm unduly compromising survival in low-risk WNT-subgroup medulloblastoma) following which the study was terminated prematurely. All 3 children with relapse were treated with salvage CSI (35Gy/21 fractions) with complete response and are alive with controlled disease. The other 3 children have not shown any evidence of relapse for over 2 years from index diagnosis and remain on active clinico-radiological surveillance. Conclusion: In rigorously defined low-risk WNT-subgroup medulloblastoma, avoidance of upfront CSI is associated with unacceptably high risk of neuraxial failure. A successor study (FOR-WNT 2) incorporating low-dose CSI (18Gy/10 fractions) with similar tumor-bed dose and adjuvant systemic chemotherapy is currently underway.

EMBR-18. LASER INTERSTITIAL THERMAL THERAPY FOR RECURRENT MEDULLOBLASTOMA

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Background: Medulloblastoma is one of the most common malignant childhood brain tumors and is managed by maximal surgical resection followed by cranio-spinal irradiation and adjuvant chemotherapy. The estimates for survival have not significantly improved over the last two decades, and survivors have an increased risk of poor quality of life. Disease relapse occurs in around 30% of children and survival is less than 20%. Laser interstitial thermal therapy (LITT) is a minimally invasive approach that has been increasingly used to treat brain lesions, particularly for high-risk surgeries. While LITT has been described in a variety of primary brain tumors, including glioblastoma multiforme and metastatic brain tumors, to our knowledge, LITT has not been reported for recurrent medulloblastoma. Case Description: We describe a case of an 11-year-old female with recurrent medulloblastoma first treated at age 5. She initially underwent gross total resection complicated by severe postoperative syndrome followed by chemotherapy and radiation (per ACNS0332). She was unfortunately found to have a new enhancing lesion on surveillance imaging 6 years later, and a biopsy confirmed recurrent tumor. Due to morbidity from initial surgery, the family did not wish to pursue further open resection but agreed to proceed with laser ablation as an alternative. She continues on Avastin/irinotecan/ TMZ chemotherapy and surveillance MRI near 6-month intervals. LITT shows a significant reduction in tumor size and enhancement. Conclusion: Recurrent medulloblastoma is a highly aggressive tumor that conveys a poor prognosis. LITT offers a less invasive procedure that may serve as an adjunct in treating recurrent tumor or as palliation. Longer term follow-up and additional cases will help understand the efficacy of LITT in recurrent medulloblastoma.

EMBR-19. HH-DRIVEN MEDULLOBLASTOMA WITH CONCURRENT UNILATERAL RENAL AGENESIS

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Case Presentation: A 3-year-old female with insignificant past medical history presented with 6 weeks of headaches, emesis, and lethargy. MR imaging identified a heterogeneously enhancing right cerebellar hemispheric mass and obstructive hydrocephalus. Gross total resection was performed without complications; pathology revealed classical WHO grade 4 medulloblastoma (MB). MR imaging of the post-resection tumor bed was negative for recurrent disease. Treatment for standard risk medulloblastoma was initiated, comprising proton craniospinal irradiation with posterior fossa boost and concurrent vincristine, followed by adjuvant chemotherapy with vincristine, lomustine, cisplatin, and cyclophosphamide as standard of care. Next generation sequencing of the tumor tissue performed using a high

To ensure patient safety, stopping rules were devised according to group-sequential method. Results: Between July 2017 till Feb 2019, seven children of WNT-pathway medulloblastoma were treated with focal conformal RT followed by 6-cycles of adjuvant chemotherapy (cisplatin, cyclophosphamide, and vincristine). One child succumbed to acute renal failure during chemotherapy, while the other 6 patients completed all 6-cycles as planned. Three children were detected with neuraxial failure (supratentorial brainstem and/or local recurrence) without synchronous metastatic deposits. These children have not shown any evidence of relapse for over 2 years from index diagnosis and remain on active clinico-radiological surveillance.