by LIN28A expression and alterations in the *C19MC* locus. ETMRs predominantly occur in young children, have a dismal prognosis, and no definitive treatment guidelines have been established. We report on our experience in nine consecutive patients. METHODS: Between 2006 and 2017, nine patients were diagnosed with ETMR. Median age was 25 months (5–38), seven were treated for primary diagnosis, two referred with progressing tumors, seven diagnosed prospectively, two retrospectively, five were located supratentorially, three pineal, one in the brainstem. RESULTS: Seven patients had a gross total resection, one a partial resection and one a biopsy at initial diagnosis, followed by second resections at progression. Six patients were treated with intensive chemotherapy regimens including high-dose chemotherapy in three patients and all recurred after a median of 6 months (range 2–11) and all except one patient who died after high-dose chemotherapy, succumbed to their disease after a median of 13 months (range 7–28). Two patients were treated with gross total tumor resection, early focal radiotherapy and concomitant temozolomide followed by temozolomide and intrathecal therapy for one year and both are in continuous complete remission 51 and 46 months after diagnosis. CONCLUSION: Gross total resection followed by early focal radiotherapy, temozolomide, and intrathecal chemotherapy seem to be superior to intensive chemotherapy including high-dose chemotherapy. Steady progression was observed in both patients with initial biopsy and PR only despite intensive therapy. Radiotherapy at recurrence/progression was not successful.

ETMR-11. A CASE OF PRIMARY DIFFUSE LEPTOMENINGEAL PRIMITIVE NEUROECTODERMAL TUMOR

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BACKGROUND: Primary diffuse leptomeningeal primitive neuroectodermal tumor (PDL PNET) is a rare embryonal brain tumor which arises primarily in the meninges without an intraparenchymal mass. Few previous reports of this condition exist, and the clinical outcomes are poor. We herein report a case of a child with PDL PNET and present a cursory review of the literature. CASE: A 3-year-old female patient was seen at a local clinic due to vomiting, headaches, and seizures. As a head MRI revealed hydrocephalus but no mass, acute encephalopathy was initially diagnosed. She received steroid pulse therapy, but the symptoms progressed to hallucination and lethargy. Another MRI at the 1-month follow-up revealed diffuse leptomeningeal enhancement. Thereafter she was transferred to our hospital. A spine MRI revealed spinal dissemination. She underwent a dura mater biopsy, and the pathological analysis led to the diagnosis of PDL PNET. She received chemotherapy consisting of vincristine, cyclophosphamide, etoposide, cisplatin, and intrathecal methotrexate injections two months after the initial presentation. The progressive hydrocephalus was managed with external ventricular drainage. Two weeks after the first cycle of chemotherapy the hydrocephalus resolved, and the external ventricular drainage was removed. A follow-up MRI showed that the leptomeningeal enhancement decreased during the four cycles of chemotherapy without radiotherapy. The patient is scheduled to receive high-dose chemotherapy as consolidation therapy. CONCLUSION: PDL PNET is extremely rare, and its diagnosis is often delayed. Treatment of PDL PNET is very difficult due to its aggressive course, and surgical resection is impossible. Early diagnosis may help improve outcomes.

ETMR-12. EMBRYONAL TUMOR WITH MULTILAYERED ROSETTES: A SINGLE CENTER EXPERIENCE

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BACKGROUND: Embryonal tumor with multilayered rosettes (ETMR) is a rare, highly malignant tumor of the central nervous system and is usually diagnosed in children aged <2 years. Currently, because no defined treatment strategy has been reported, treatment regimens are often extrapolated from other embryonal tumors. Therefore, data collection of ETMR cases is important for further understanding EMTR. Here, we present our experience with four patients with ETMR. MATERIAL AND METHODS: Patients with a pathological diagnosis of ETMR from 1999 to 2016 at Saitama Children's Medical Center were included. Their clinical data were retrospectively analyzed. RESULTS: This study included four cases of ETMR (one male and three females). The mean age at diagnosis was 29.5 (range, 15–37) months. Presenting symptoms included seizure, hemiparesis, vomiting, and headache. The mean maximal tumor diameter was 42.5 mm. The tumor locations included frontal lobe, temporal lobe, occipital lobe, cerebellum, and brainstem. Gross total resection was achieved in two cases. Fluorescence in situ hybridization analysis demonstrated amplification of 19q13.42 chromosome region in all cases, and diffuse positive expression was observed in the immunohistochemical staining for LIN28A. Systemic postoperative chemotherapy was administered to all patients. Three patients received intrathecal therapy and three were irradiated. The mean overall survival and progression-free survival were 45.3 and 42 months, respectively. Two patients who underwent gross total resection are alive without recurrence. CONCLUSION: Complete surgical resection may be an important prognostic factor in patients with ETMR. Further prospective studies are needed to confirm these results.

ETMR-13. NFI GENES IN ETMR TUMORIGENESIS AND NEURODEVELOPMENT

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Embryonal tumors with multilayered rosettes (ETMRs) are aggressive pediatric embryonal brain tumors with a universally poor prognosis. These tumors are commonly characterized by amplification of *C19MC*, but other miRNA-related aberrations, such as *DICER* mutations or *MIR17HG* amplifications, are also observed. Nevertheless, it remains unknown how these aberrations are driving the tumorigenesis. We applied miRNA target prediction to investigate the downstream targets shared by these aberrations affecting normal brain development and tumorigenesis. The nuclear factor one (*NFI*) family of transcription factors were found to be top candidates shared by both miRNA clusters. These genes are expressed at very low levels in ETMRs, in contrast to other brain tumors. During normal brain development these genes are expressed in radial glial progenitors and are required for the transition of proliferation to differentiation. Since radial glial progenitors are the potential cell-of-origin of ETMRs, we hypothesize that downregulation of NFI is required for the proliferative, undifferentiated state of ETMRs. Indeed, mouse models with deletion of an *Nfi* family member display sustained proliferation and delayed differentiation of radial glial progenitors during development. This leads into brain overgrowth, which has also been observed in humans with intellectual disabilities caused by *NFI* haploinsufficiency. When multiple *Nfi* family members are simultaneously targeted in mice, the progenitors are retained and both neurogenesis and gliogenesis are inhibited, resulting in a neuropathology similar to that of human ETMR tumors. Hence, downregulation of *NFI* genes resulting from miRNA aberrations could contribute to the developmental state and possibly tumorigenesis of ETMRs.

ETMR-14. TREATMENT OF EMBRYONAL TUMOURS WITH MULTILAYERED ROSETTES (ETMR) WITH CARBOPLATIN-ETOPOSIDE INDUCTION AND TANDEM HIGH-DOSE CHEMOTHERAPY WITHIN THE PROSPECTIVE HIT-TRIALS AND REGISTRIES

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BACKGROUND: Embryonal tumours with multilayered rosettes (ETMR) are highly aggressive tumors, mostly occurring in infants. Published clinical data refer to retrospective cohorts of inhomogeneously treated patients. Here, we describe the outcome of patients, who were prospectively treated within the P-HIT2000-trial, the subsequent HIT2000-interim-registry and earlier HIT-trials. PATIENTS AND METHODS: Nineteen patients from the P-HIT2000-trial (2001–2011), 12 patients from the subsequent HIT2000-interim-registry (2012–2014) and 4 patients from earlier HIT-trials with centrally reviewed neuropathological and molecularly-confirmed diagnosis of ETMR were included. Outcome of 18 patients treated with carboplatin-etoposide-induction followed by tandem-high-dose chemotherapy ("CARBO-ETO+HDCT") with stage-stratified radiotherapy administered in case of persistant disease, relapse or progression were compared to patients treated with HIT-SKK chemotherapy ± radiotherapy (n=9) or other regimens (n=8). RESULTS: Median age at diagnosis was 2.9(1.0–5.3) years. Metastases at diagnosis were detected in 9 patients (26%). For the entire cohort of n=35, 5-year overall survival (OS) was 26.7%, and progression-free survival (PFS) was 18.5%. Five-year OS for patients with CARBO-ETO+HDCT, SKK chemotherapy or other regimens was 44.4%, 13.0% and 0%, respectively (p=0.006). Five-year PFS was 33.3%, 0% and 0%, respectively (p=0.119). Of 10 survivors, n=8 were treated with CARBO-ETO+HDCT; n=4 had craniospinal, n=2 local and n=4 no radiotherapy. Impact of initial gross-total-resection (p=0.231) and non-metastatic disease (p=0.097) was limited. CONCLUSIONS: We show improved survival with carboplatin-etoposide-induction followed by tandem-high-dose chemotherapy, indicating that a cure is possible for some patients. However, despite intensive treatment, outcome is unsatisfactory and innovative therapies urgently need to be included in an upfront setting.

ETMR-15. USE OF HIGH-DOSE CHEMOTHERAPY FOR TWO CHILDREN WITH EMBRYONAL TUMOR WITH MULTILAYERED ROSETTES

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Embryonal tumor with multilayered rosettes (ETMR) is new entity defined in the 4th revised edition of the WHO classification of tumors of the central nervous system. Although radical resection, radiotherapy, and multiagent chemotherapy are considered to be necessary for ETMR, the efficacy of chemotherapy for ETMR in Japan has not been established. Here, we report different clinical courses for two children with localized ETMR treated with the St. Jude medulloblastoma-96 (SJMB96) regimen, which consists of four cycles of high-dose chemotherapy with autologous peripheral blood stem cell transplantation. For both children, the diagnosis of ETMR, C19MC-altered was confirmed after gross total tumor resection. Multiagent chemotherapy was administered following cranio-spinal irradiation with local boost. One month after completion of the treatment, one patient experienced local recurrence but has been in remission for over 2 years after tumor resection and stereotactic irradiation with a CyberKnife and treatment every three weeks with bevacizumab. The other patient also experienced local recurrence after the third cycle of chemotherapy and several times thereafter. Although she again underwent tumor resection and local irradiation, her tumor grew larger and invaded. Because her prognosis was very poor, her parents choose only palliative care. Based on our experience, we believe that continuous chemotherapy at conventional doses is preferred over intensive-dose chemotherapy such as SJMB96. However, the number of reports on chemotherapy for ETMR is still small, and a prospective multicenter trial is needed to establish effective chemotherapy for ETMR.

ETMR-17. SINGLE-CELL TRANSCRIPTOME ANALYSIS OF ETMR PATIENT SAMPLES

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Brain tumors are comprised of cells with heterogeneous genetic and transcriptional states, resulting in substantial phenotypic diversity. This diversity is particularly evident in embryonal tumor with multilayered rosettes (ETMR), which shows a striking bi-phasic pattern for which it is named. A better understanding of its underlying molecular makeup is urgently needed to develop more effective therapeutic strategies that eliminate all malignant cell types underlying ETMR initiation, maintenance, progression, and relapse. Furthermore, the cellular origin of ETMR is currently poorly understood. We used plate-based single-cell RNA sequencing to assess the intratumoral heterogeneity in 6 fresh and 4 snap-frozen surgical biopsies, following a workflow that we have previously established to study pediatric high grade gliomas, medulloblastomas, and ependymomas. Computational analyses conducted on >4,000 single cells identified cellular hierarchies ranging from a proliferative, undifferentiated cell population to more differentiated, predominantly neural-like progeny in all samples. Patient-derived cell line and xenograft models partially recapitulated this hierarchy. We further integrated transcriptional programs identified in single cells with available datasets of the developing normal brain, as well as with programs identified in other pediatric brain tumor entities, to inform both putative cellular origins and ETMR-specific oncogenic pathways. These timely results provide unparalleled insights into the molecular underpinnings of the phenotypic heterogeneity observed in ETMR. Analyses aimed at further integrating malignant cell type abundances with genetic alterations and clinical annotations, and therapeutical targeting of malignant cell populations using in-vitro models are currently ongoing.

ETMR-18. TARGETING LIN28 IN ETMR WITH ODC1 INHIBITOR DFMO

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Embryonal tumor with multilayered rosettes (ETMR), is an aggressive brain tumor primarily occurring in young patients (<4 years of age) and characterized by C19MC amplification and Lin28 overexpression. These genetic hallmarks have been shown to participate in driving ETMR in a C19MC-Lin28-MYCN circuit. Reducing Lin28 disrupts this circuit and reduces cell viability in ETMR models. Investigation of therapeutic agents targeting this pathway is required to provide new treatment options for this deadly disease. We present data showing the effect of DFMO (α-difluoromethylornithine) in ETMR, an ODC1 inhibitor known to reduce Lin28 in neuroblastoma. DFMO treatment of the ETMR cell line BT-183 resulted in a significant reduction of intracellular Lin28 protein levels ($P<0.05$) as indicated by flow cytometry. In concert with this reduction in Lin28, there was a as significant reduction in viable cells ($P<0.05$), and the number of CD133+ cells were reduced 2-fold ($P<0.05$). High throughput drug testing of BT-183 identified a number of additional therapeutic agents with potential therapeutic efficacy for ETMR and combining these with cytostatic agent DFMO demonstrated the potential use of these drugs in combination. These *in vitro* data were complemented by testing of DFMO in an *in vivo* stereotaxic xenograft ETMR model, with inhibition of tumor burden monitored by bioluminescent imaging of the tumors. Together this work shows that Lin28 targeting agents such as DFMO merit further examination and integrating these types of agents into treatment strategies for ETMR may lead to better outcomes.