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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.