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Relapsed and refractory malignant rhabdoid tumors – Evaluation of new therapeutic approaches

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1. Introduction

Malignant rhabdoid tumors (MRT) are a rare pediatric tumor entity affecting rather young children, mainly in the first two years of life. They can arise in almost any anatomical location inside and outside of the central nervous system (CNS). The name “rhabdoid tumor” is derived from the typical “rhabdoid” microscopic appearance of the tumor cells, with eosinophilic cytoplasm and eccentrically located nuclei with prominent nucleoli.
MRT are usually classified as embryonal tumors and subcategorized according to anatomical location. MRT of the CNS are named atypical teratoid/rhabdoid tumors (AT/RT). Non-CNS tumors are further subdivided into rhabdoid tumors of the kidney (RTK) and extracranial, extrarenal rhabdoid tumors (eMRT). MRT can also occur synchronously with simultaneous intra- and extracranial lesions.

1.1. History and biology of malignant rhabdoid tumors

Histomorphological diagnosis of MRT has always been challenging, as the morphological pattern is very variable. For a long time, many MRT were misdiagnosed as, for example, Wilms tumors or medulloblastomas [1]. In 1978, Beckwith and Palmer described certain subcohorts of renal tumors with distinct histological features and an unfavorable prognosis [2]. In 1981, Haas *et al.* published a detailed characterization of these tumors and first introduced the term “malignant rhabdoid tumor of the kidney” due to the, in their words, “striking light microscopic resemblance to rhabdomyosarcoma” [3]. In the following years, the occurrence of MRT in many other anatomical locations was confirmed, including the first description of a rhabdoid CNS tumor in 1987 [4-7]. The entity "atypical teratoid tumor of infancy" was defined separately in the same year and would later be identified as rhabdoid tumors of the CNS. This led to the combined term "atypical teratoid/rhabdoid tumor", which Rorke *et al.* defined as a distinct entity in 1995 [8, 9]. By then, it was known that abnormalities in chromosome 22 were connected to AT/RT and eMRT; a few years later, alterations in the *SMARCB1* gene were discovered as the underlying genetic characteristic of rhabdoid tumors, and animal models showed rapid and highly penetrant tumorigenesis in *SMARCB1*-deficient mice [10-14]. This discovery enabled the use of immunohistology to determine the expression of INI1 (integrase interactor 1) as a specific and now-established diagnostic method for rhabdoid tumors inside and outside of the CNS. It also further confirmed the link between intra- and extracranial rhabdoid tumors, which are now mostly treated as separate but closely

related entities [15, 16]. To date, the most important parameter for diagnosing MRT is the proof of loss of INI1 protein expression on immunohistochemistry.

SMARCB1 abbreviates the gene name *SWI/SNF Related, Matrix Associated, Actin Dependent Regulator of Chromatin, Subfamily B, Member 1*. This gene encodes a subunit of the SWItch/Sucrose Non-Fermentable (SWI/SNF) complex, a chromatin remodeler. The chromatin remodeling complexes of the SWI/SNF family are also called BRG1/BRM-associated factor (BAF) complexes and play an important role in the regulation of cell differentiation and gene expression [17]. Furthermore, BAF complexes have been found to function as tumor suppressors; extensive cancer genome-sequencing studies have revealed a high rate of nearly 25% of all cancers harboring alterations in genes encoding subunits of the SWI/SNF complex, underlining their relevance even beyond MRT [18, 19].

Histone modifications due to altered function of the SWI/SNF complex lead to wide-ranging changes in gene expression and signaling pathways in rhabdoid tumor cells [20, 21]. Analyses of methylation profiles in MRT demonstrate an epigenetic heterogeneity, suggesting the existence of subgroups. In AT/RT, three distinct molecular subgroups have been established. They are closely correlated with specific clinical phenotypes and are proposed to be of prognostic relevance [22]. The internationally consented terms are AT/RT-TYR, AT/RT-SHH, and AT/RT-MYC, named after subgroup-specific molecular characteristics (overexpression of the enzyme tyrosinase, of sonic hedgehog pathway members or the *MYC* oncogene, respectively) [23]. Additional specific investigation of AT/RT-SHH revealed three further AT/RT-SHH subgroups with characteristic clinical and molecular features [24]. Likewise, methylation subgroups in extracranial MRT have been described, but clinical relevance is still unclear [25]. A defined cell of origin (COO) for MRT has yet to be determined, and it is proposed that the different subgroups have distinct COOs [26, 27].

Genetic analysis of histologically diagnosed MRT with no *SMARCB1* alteration detected led to the discovery of a rare subgroup of MRT. This subgroup carries an inactivated form of the *SMARCA4* gene, which causes alterations in another SWI/SNF complex subunit [28]. The presentation of *SMARCA4*-deficient MRT is similar to *SMARCB1*-deficient MRT concerning anatomical locations and growth patterns, and they are currently being treated equally. Recent investigations though suggest clinical and molecular differences. Patients with *SMARCA4* alterations are younger and have higher rates of inherited germline mutations [29]. Methylation profiles differed from the above-

introduced consensus subgroups, and a distinct AT/RT-SMARCA4 subgroup is proposed [30].

Beyond MRT, several other INI1 negative malignancies have also been identified, many predominantly affecting children. This includes CNS tumors such as chordomas and cribriform neuroepithelial tumors, as well as extracranial tumors such as epithelioid sarcomas and renal medullary carcinomas [31-34]. These tumors display unique clinical and histomorphological characteristics that are usually clearly distinguishable from MRT. Still, molecular similarities have been identified, and overlapping therapeutic vulnerabilities are suspected [35, 36].

1.2. Epidemiology of malignant rhabdoid tumors

Overall, MRT is a rare entity; incidence rates are usually reported separately for intra- and extracranial MRT. AT/RT constitute the majority of MRT cases. In the German Childhood Cancer Registry between 2009-2018 a total of 60.7% of all MRT occurred in the CNS (n=136), eMRT and RTK accounted for 29.9% (n=67) and 9.4% (n=21), respectively. Age-specific incidence rates of AT/RT were highest in children < 1 year, with 6.6 per million, and decreased to 2.6 between 1-4 years and 0.4 in 5-9 years. For eMRT, incidence rates in children < 1 year, 1-4 years, and 5-9 years were 4.3/0.7/0.2 per million and for RTK 1.4/0.2/0.1 per million, respectively. The median age at diagnosis was 17 months for AT/RT, 12 months for eMRT, and 13 months for RTK [37]. Despite the rarity in absolute numbers, MRT make up a relevant fraction of malignant tumors in small children. AT/RT, for example, account for > 40% of all embryonal CNS tumors in children below one year of age [38]. A male predominance with male-to-female ratios between 1.1 to 2 has repeatedly been reported [39].

AT/RT may arise infratentorially (60%), supratentorially (37%), or rarely spinally (2%). eMRT most often occur in the liver and the cervical and thoracic region. In both intra- and extracranial MRT, metastatic disease is often present at diagnosis [40].

1.3. Rhabdoid tumor predisposition syndrome

Repeated observation of families with multiple members affected by MRT led to the discovery that MRT can occur as part of a cancer predisposition syndrome, termed now Rhabdoid Tumor Predisposition Syndrome (RTPS) [41-43]. Germline mutations of *SMARCB1* (RTPS1) affect 25-35% of all newly diagnosed patients with MRT. The exact incidence of germline mutations in *SMARCA4* (RTPS2) is unclear due to the extreme

rarity [44-46]. Patients with RTPS are affected at a younger age and show a higher rate of synchronous tumors [47].

1.4. Diagnosis of malignant rhabdoid tumors

Due to the varying anatomical locations, there are no tumor-specific symptoms for MRT. Upon suspicion of MRT, magnetic resonance imaging is needed. Typical imaging characteristics for AT/RT include intratumoral hemorrhage and a “band-like” wavy enhancement surrounding a central cystic or necrotic area [48]. Extracranial MRT often show a heterogenous signal intensity in T2-weighted magnetic resonance sequences with a strong inhomogeneous contrast enhancement [49]. Tissue analysis from biopsy or (sub-) total resection is necessary for definitive diagnosis. In Germany, according to the EU-RHAB registry recommendations, genetic analysis of tumor and germline DNA and 850k methylation analysis for subgroup assignment in AT/RT is routinely conducted.

1.5. Therapy of malignant rhabdoid tumors

Because malignant rhabdoid tumors grow aggressively and often metastasize, patients undergo intensive, multimodal therapy regimens. There are several internationally established therapy regimens that all include surgical resection, polychemotherapy, and, depending on certain factors, utilization of radiotherapy (RT) and high-dose chemotherapy (HDCT) with autologous stem cell rescue (ASCR) for consolidation. Regardless of which therapy regimen is chosen, all currently used therapy protocols include chemotherapy, often anthracycline-based. For intracranial tumors, many protocols utilize intrathecal or intraventricular chemotherapy. See Table 1 for details on different therapy protocols and chemotherapy regimens.

In intra- as well as extracranial MRT, attempting resection of the tumor before the start of chemotherapy is the standard of care. Gross total resection is desirable, but only if feasible in a non-mutilating way. Second-look surgery after neoadjuvant chemotherapy may also be considered [50, 51]. Rhabdoid tumors are radiosensitive, and RT can be used in localized and disseminated disease stages through photons or protons. The young age at which MRT typically occurs is limiting its use, especially in tumors of the CNS due to the severe neurocognitive sequelae [52-54]. Since many MRT treatment protocols historically arose from guidelines for other entities, RT has been widely used in MRT in the last decades. Most published cohorts include at least a subgroup of patients who received RT (See Table 1). Specific treatment guidelines, especially

concerning the optimal timepoint of RT, are not available though [55]. Retrospective analyses of patients from the SEER (Surveillance, Epidemiology, and End Results) database indicate a significant survival benefit for patients with AT/RT who received RT, especially in those under 3 years of age [56, 57]. On the other hand, Lafay-Cousin *et al*. published a cohort of 18 patients in which a relevant proportion survived without RT but received consolidation with HDCT and ASCR only [58]. Due to the toxicity and long-term sequelae of RT in young children, it thus remains an open question if the clinical benefits of RT outweigh the toxicity in all age groups or if RT-free regimens can achieve similar survival rates in younger, more vulnerable patients [59]. Testing of this hypothesis is part of the currently recruiting, randomized SIOPE ATRT01 trial (EudraCT: 2018-003335-29). RTK and eMRT data from different cohorts likewise suggest a benefit of including RT in multimodal treatment, especially in higher-stage disease. However, promising outcomes of non-irradiated patients with low-stage tumors (SIOP stage 1 / IRS stage 1) have opened further questions concerning which patient groups should receive RT and which can be spared the toxicity [51, 60]. For additional or alternative consolidation, HDCT with ASCR is used in MRT treatment. For AT/RT, results from multiple cohorts and trials have established HDCT as an important component of multimodal treatment protocols [61-63]. In extracranial MRT, no evidence for a survival benefit from including HDCT in the treatment was found [64, 65]. Contrary to AT/RT, HDCT is thus currently not prioritized in the treatment of MRT outside of the CNS but remains an option if treatment with other modalities is not feasible.

Table 1 - Treatment and outcome in uniformly treated cohorts of malignant rhabdoid tumors

ACT-D: actinomycin-D; ADR: adriamycin; AR: average risk; ARA-C: cytarabine; AT/RT: atypical teratoid rhabdoid tumor; CBP: carboplatin; CDDP: cisplatin; ClTr: clinical trial; CPM: cyclophosphamide; DOX: doxorubicin; EFS: event-free survival; EPI: epirubicin; ETOP: etoposide; HCT: hydrocortisone; HDCT: high-dose chemotherapy; HDMTX: high-dose MTX; HR: high-risk; IDA: idarubicine; IFO: ifosfamide; IR: intermediate risk; i.th.: intrathecal; m: months; MTX: methotrexate; OS: overall survival; PBSC: peripheral blood stem cells; PFS: progression-free survival; PO: per os; RT: radiotherapy; RTK: rhabdoid tumor of the kidney; TEM: temozolomide; TROFO: trofosfamide; TT: thiotepa; VCR: vincristine; y: years
† Only 13 cases uniformly treated with UH-1 protocol, 40 treated with other regimens

Trial/cohort	Type	Cohort size	Age group	Chemotherapy drugs used	RT	HDCT	Outcomes	Source
AT/RT								
CCG921	ClTr	28	< 36 m	Regimen A: VCR, CDDP, CPM, ETOP Regimen B: VCR, CARBO, IFO, ETOP Maintenance: VCR, ETOP, CARBO, ETOP	11/28	No	5-year EFS: 14% 5-year OS: 29%	Geyer *et al*. 2005 [66]
DFCI	ClTr	20	0-18 y	VCR, ACT-D, CPM, CDDP, DOX, TEM i.th. chemotherapy: MTX, HCT, ARA-C	15/20	No	2-year PFS: 53% 2-year OS: 70%	Chi *et al*. 2009 [67]
Head Start III	ClTr	19	< 36 m	Induction: CDDP, VCR, ETOP, CPM, HDMTX, TEM HDCT: CARBO, TT, ETOP	5/19	5/19	3-year EFS: 21% 3-year OS: 26%	Zaky *et al*. 2014 [62]
Rhabdoid 2007	Registry study	31	0-18 y	Induction: VCR, CPM, DOX, IFO, CARBO, ETOP; i.th. chemotherapy: MTX HDCT: Individual, CARBO, TT recommended Maintenance: TROFO, IDA, ETOP	23/31	8/31	6-year EFS: 45% 6-year OS: 46%	Bartelheim *et al*. 2016 [68]
ACNS 0333	ClTr	65	0-22 y	Induction: MTX, VCR, ETOP, CPM, CDDP HDCT: CARBO, TT, ETOP	42/65	44/65	4-year EFS: 37% 4-year OS: 43%	Reddy *et al*. 2020 [61]

Trial/cohort	Type	Cohort size	Age group	Chemotherapy drugs used	RT	HDCT	Outcomes	Source
SJYC07	ClTr	52	< 36 m	IR: Induction: HDMTX, VCR, CPM, CARBO; Maintenance: PO CTX/TOPO, PO ETOP HR: Induction: HDMTX, VCR, CPM, CDDP, VBL; Consolidation: CPM, TOPO; Maintenance: PO CPM/TOPO, PO ETOP	34/52	No	IR: 5-year PFS: 31.4% 5-year OS: 43.9% HR: 5year PFS and OS: 0%	Upadhyaya *et al*. 2021 [69]
SJMB03	ClTr	22	≥ 36 m	CDDP, VCR, CPM followed by PBSC	22/22	No	AR: 5-year PFS:72.7% 5-year OS: 81.8% HR: 5-year PFS and OS: 18.2%	Upadhyaya *et al*. 2021 [69]
Extracranial/RTK								
SIOP 93-01 / SIOP 2001 (RTK)	ClTr	107	0-9 y	VCR, ACT-D, DOX, ETOP, CARBO, IFO, CPM, DOX, EPI	38/107	No	5-year EFS: 22% 5-year OS: 26%	Van den Heuvel-Eibrink *et al*. 2011 [70]
EPSSG NRSTS 2005	Observational study	100	0-11y	VCR, CPM, DOX, CARBO, ETOP	46/100	No	3-year EFS: 32.3% 3-year OS: 38.4%	Brennan *et al*. 2016 [71]
Bejjing Children	Case series	53	0-9 y	CTX, ADR, VCR, CARBO, ETOP, TOPO, IFO (UH-1 protocol) †	10/53	No	3-year EFS: 14.5% 3-year OS: 23.7%	Cheng *et al*. 2019 [72]
SFCE/EPSSG	Case series	35	0-17y	VCR, DOX, CPM, IFO, ETOP	17/35	No	2-year EFS: 42.9% 2-year OS: 47.6%	Enault *et al*. 2022 [73]
EU-RHAB	Registry	100	0-18 y	DOX, IFO, CARBO, ETOP, VCR, ACT-D, CPM HDCT: CBP, TT	56/100	21/100	5-year EFS: 35.2% 5-year OS: 45.8%	Nemes *et al*. 2021 [40]

1.6. The EU-RHAB registry

The EU-RHAB registry is a multinational registry based in Germany for MRT of all anatomical locations. It is part of the network of competence centers of the German Society of Pediatric Oncology and Hematology (GPOH). The therapy protocol according to EU-RHAB recommends a uniform therapy of rhabdoid tumors of all anatomical locations, intra- and extracranially. This includes surgical resection, chemotherapy, and, according mainly to age and individual decision, consolidation through either RT or HDCT (See Figure 1). It was preceded by a pilot trial (Rhabdoid 2007), and emerging from the results, the EU-RHAB registry was launched [68]. Since then, 556 patients have been enrolled in the registry, including 371 AT/RT, 141 eMRT, and 54 RTK. 29 synchronous tumors were registered (P. Neumayer, personal communication, 29.01.2024). As the definitive criteria for diagnosing MRT have only been available since the early 2000s, the registry now holds one of the most extensive collections of clinical data from MRT patients. Figure 1 provides an overview of the structure, therapy recommendations, and further tasks of the EU-RHAB registry. The registry offers a standardized therapy recommendation and individual counseling, centralized reference assessments, collects clinical data and biomaterial (tumor samples, DNA from tumor or peripheral blood, cerebrospinal fluid), and conducts research based on the collected data and biomaterial. The treatment protocol is available for download under www.rhabdoid.de. The EU-RHAB registry holds a positive vote of the ethics committee of the University of Münster for the collection of patient data and biomaterials, which was specifically expanded for analysis of relapsed and progressive tumors. (Registry: ID 2009-532-f-S; Relapse/Progression: ID 2018-302-f-S)

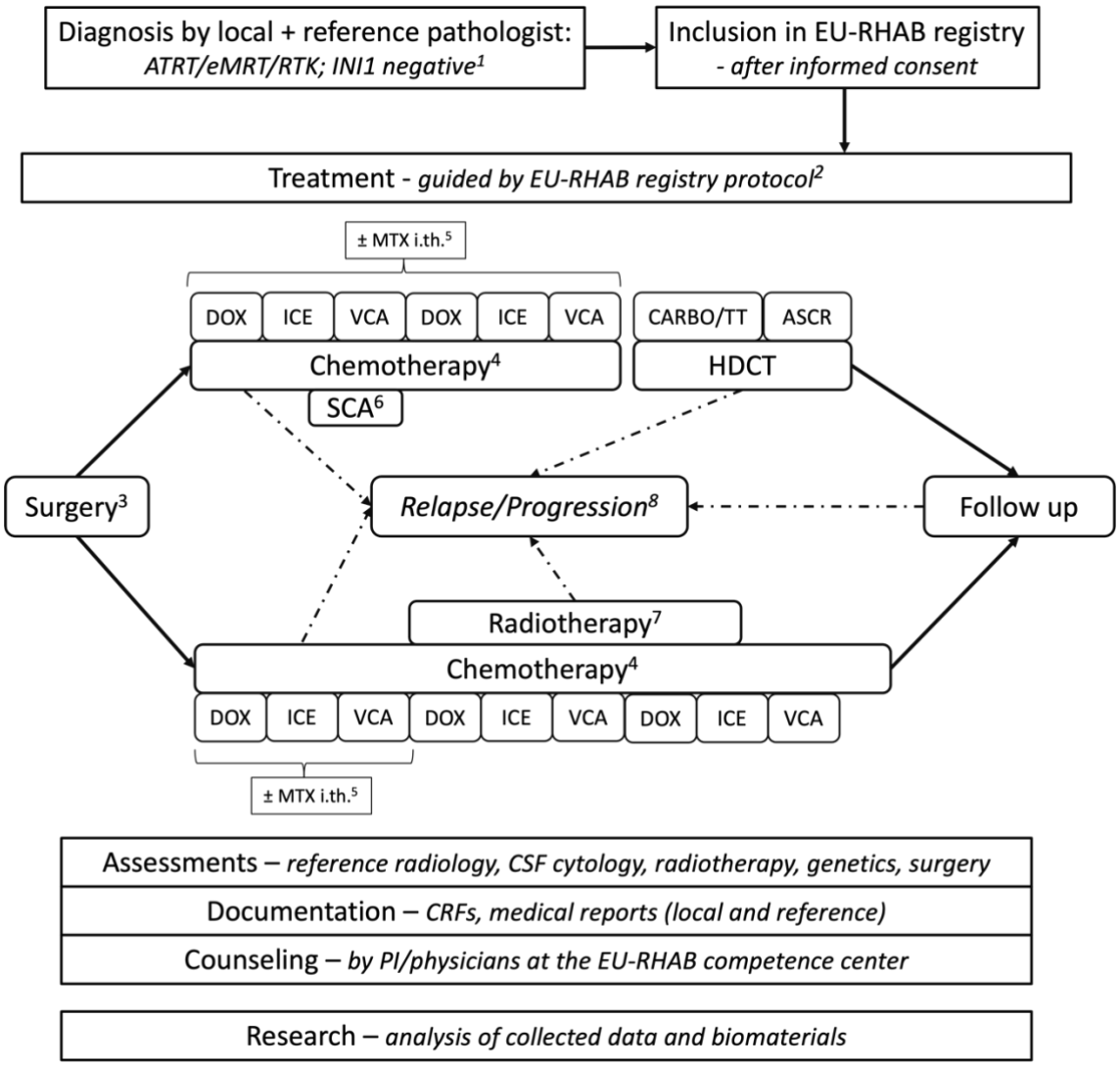

Figure 1– Organizational chart of the EU-RHAB registry

[1]Tumors with retained INI1 staining may be included, but only by suggestion of the reference pathologist. [2]The registry protocol is a recommendation, but it is not mandatory for the treating physicians to follow and may be individualized. [3]Surgery is planned before the start of chemotherapy. Second-look surgery may be considered if resection was incomplete. [4]The scheduled interval between chemotherapy elements is 14 days. During concomitant RT, no doxorubicin or actinomycin-D is given. [5]Intrathecal or intraventricular therapy is only recommended for patients with intracranial tumors. The preferred application route is via Ommaya or Rickham reservoir. No i.th. therapy shall be applied after RT. [6]Stem cell apheresis may be conducted after the first or second "ICE"-element [7]Timing of RT: Age ≥ 18 months as soon as feasible, <18 months delay until age permits (or choose HDCT for consolidation). Photon vs. proton and focal vs. craniospinal therapy may be chosen according to availability and disease status. [8]Treatment recommendations are only valid for first-line therapy. In case of relapse or progression, further therapy is managed on an individual basis at the discretion of the treating institutions, irrespective of the timepoint of relapse/progression. Recommendations for salvage therapy are made by the EU-RHAB registry but are not binding to the responsible physicians.
ASCR: autologous stem cell rescue; AT/RT: atypical teratoid, rhabdoid tumor; CARBO: carboplatin; CRF: case report form; CSF: cerebrospinal fluid; DOX: doxorubicin, eMRT: extracranial, malignant rhabdoid tumor; HDCT: high-dose chemotherapy; ICE: ifosfamide, carboplatin, etoposide; INI1: integrase interactor 1; i.th.: intrathecal; MTX: methotrexate; PI: principal investigator; RT: radiotherapy; RTK: rhabdoid tumor of the kidney; SCA: stem cell apheresis; TT: thiotepa; VCA: vincristine, cyclophosphamide, actinomycin-D

1.7. Prognosis and prognostic factors in malignant rhabdoid tumors

MRT are highly malignant tumors that are uniformly fatal if left untreated. Long-term survival, however, is possible after intensive multimodal therapy. In both AT/RT and extracranial MRT, reported outcomes have a very high range between different cohorts. Chi *et al*. reported very promising results with a 70% 2-year overall survival (OS) in 20 patients with AT/RT [67]. In the EU-RHAB pilot cohort “Rhabdoid 2007”, 6-year OS in 31 AT/RT was 46% [68]. The more recently published ACNS0333 trial saw a 4-year OS of 43% [61]. Two large cohorts from St. Jude trials (SJYC07 and SJMB03) stratified patients into risk groups according to the presence of metastasis and residual tumor. The “lower risk”-groups showed 5-year OS rates of 43.9% and 81.8%, and the “higher risk”-groups 0% and 18.2%, respectively [69].

For extracranial MRT, very limited results from prospective clinical trials are available. Patients with RTK treated in the SIOP 93-01 and SIOP 2001 trials had a 5-year OS of 26% [70]. Two cohorts of eMRT and RTK treated according to European Pediatric Soft Tissue Sarcoma Study Group recommendations have been published. Brennan *et al*. reported a 3-year OS of 38.4% in patients treated in the NRSTS 2005 study [71]. More recently, Enault *et al*. published results from French patients treated with the VDCy/IE regimen; 2-year OS was 47.6% [73]. Analysis of 100 eMRT and RTK from the EU-RHAB registry revealed a 5-year OS of 45.8% [40].

Several factors relevant to the prognosis of MRT patients have been proposed based on the results from the listed cohorts. This includes the extent of resection, anatomical location, presence of metastasis, RTPS, and molecular subgroup status. Nemes *et al.* identified two risk groups in eMRT and RTK. Patients without metastasis, who had a gross total resection and no evidence of a *SMARCB1* or *SMARCA4* germline mutation, showed a significantly superior 5-year OS. It is suggested that this model may be useful for risk stratification in future trials [40]. For AT/RT, a comparable risk stratification model is missing so far.

1.8. Innovative therapeutic approaches to malignant rhabdoid tumors

Beyond the currently used therapy approaches and modalities, there are multiple preclinical and clinical studies investigating innovative therapies based on specific molecular mechanisms or target structures and pathways that are thought to be therapeutically relevant in MRT. Details concerning active and ongoing clinical trials are listed in Table 2.

Epigenetic therapeutics

Due to the role of the SWI/SNF complex in chromatin remodeling and its impact on DNA methylation in *SMARCB1*-deficient cells, an important approach to targeted therapy of MRT is the use of epigenetic modulators [74].

EZH2 (enhancer of zeste homologue-2) is a subunit of the polycomb repressive complex 2 (PRC2), which is involved in the regulation of gene expression through histone H3K27 trimethylation. It forms an antagonistic relationship with SWI/SNF complexes, meaning that SWI/SNF suppression leads to an upregulation in EZH2 expression [75]. The EZH2-inhibitor tazemetostat showed promising effects in MRT cell lines and mice models [76, 77]. The combination of EZH2-inhibition with the bromodomain and extra-terminal domain (BET) inhibitor BET bromodomain-containing protein 4 (BRD4) led to even improved anti-proliferative effects [78]. In a phase-I trial in children, tazemetostat led to one complete response and 5/21 objective responses in patients with AT/RT. In the COG-NCI Pediatric MATCH trial, 8 AT/RT patients received tazemetostat. No objective response was reported, but 2/8 patients had stable disease [79-81]. A clinical trial combining tazemetostat with a programmed cell death protein 1 (PD-1) and a cytotoxic t-lymphocyte-associated protein-4 (CTLA4) inhibitor is currently recruiting (NCT05407441). Two of the three subgroups of AT/RT have globally hypermethylated genomes; therefore, demethylating agents such as DNA methyltransferase (DNMT) inhibitors are hoped to be effective in MRT [22]. Graf *et al*. demonstrated a possible therapeutic effect of the DNMT inhibitor decitabine in MRT in vitro and in vivo, further underlining the relevance of this approach [26]. Another important class of epigenetic modulators are histone deacetylase (HDAC) inhibitors. AT/RT samples showed significant overexpression of histone deacetylase 1; further preclinical studies saw antiproliferative effects of, e.g., panobinostat or vorinostat in MRT cells [82-85]. In a recent phase II trial (NCT04897880), 14 children with MRT received continuous low-dose panobinostat, which was well tolerated [86]. Final results of the study are still pending. Two current clinical trials explore the combination of HDAC inhibitors or DNMT inhibitors with checkpoint inhibitors (NCT03838042, NCT03445858). Results from a preclinical investigation of MRT development and differentiation suggest a therapeutic potential of combining HDAC with mammalian target of rapamycin (mTOR) inhibitors. The authors, however, raise attention to possible toxicities from therapies inducing cell differentiation in children, as seen in other entities [87].

Immunotherapy

Cancer immunotherapy has lately achieved increasing relevance in the treatment of many refractory tumors. Despite their low mutational burden, rhabdoid tumor cells show high immunogenicity and immune cell infiltration and are thus potentially susceptible to immunotherapeutic approaches [88-90]. Immune checkpoint inhibitors (ICI) downregulate a mechanism of immune evasion in cancer cells and promote T-cell-mediated antitumor immunity. Leruste *et al*. saw encouraging results of AT/RT treatment with a PD-1 inhibitor in a mouse model [88]. In three separate early-phase trials, a total of four patients with eMRT and six patients with AT/RT were treated with ICIs, including pembrolizumab, atezolizumab, and avelumab. The best overall response was a partial response in two eMRT patients [91]. The CheckMate 908-trial (NCT03130959) compared nivolumab monotherapy with the combination of nivolumab and ipilimumab and enrolled 7 patients with AT/RT. Overall, the trial saw no relevant benefit compared to historical cohorts for pediatric CNS malignancies. All AT/RT cases progressed in the first two months after enrollment [92]. Offenbacher *et al.* report a promising, ongoing remission under pembrolizumab maintenance therapy [93].

Chimeric antigen receptor (CAR)-T cells are genetically engineered to specifically target tumor-specific antigens. B7-H3 was identified as a suitable target in pediatric solid tumors. In a murine xenograft model, AT/RT B7-H3 CAR-T cells lead to good antitumor effects [94]. Multiple clinical trials are active that employ CAR-T cells to target intra- and extracranial, refractory tumors, including MRT. (Table 2)

Oncolytic viruses (OVs) are genetically engineered viruses that can selectively target and destroy tumor cells without affecting healthy tissue [95]. Several OVs have been successfully preclinically tested in MRT, including poliovirus, measles virus, herpes simplex virus, and adenovirus [96]. Clinical experience in MRT is still missing. In a recent trial in children with recurrent high-grade glioma, a recombinant polio-rhinovirus was well tolerated in the pediatric cohort [97]. A modified measles virus (MV-NIS) has been tested in 34 patients with recurrent AT/RT or medulloblastoma. The virus was directly administered into the tumor bed or subarachnoid space; results are still pending (NCT02962167). Two trials investigating the OV herpes simplex virus G207 in recurrent brain tumors are ongoing (NCT03911388, NCT02457845).

Aurora A kinase inhibitors

The aurora A kinase regulates the mitotic spindle and thus plays a relevant role in the cell cycle [98]. Aurora A kinase is overexpressed in MRT cells, making it a therapeutic target [99]. Wetmore *et al*. reported in 2015 that monotherapy with aurora A kinase

inhibitor alisertib had led to disease stabilization or regression in four patients with relapsed or refractory AT/RT; a further case of a response to alisertib monotherapy in the treatment of a relapsed AT/RT was reported in 2022. In a subsequent phase II trial, 8/30 patients with AT/RT had stable disease after relapse treatment with oral alisertib, and one patient showed a partial response. 1-year progression-free survival (PFS) and OS were 13.3% ± 5.6% and 36.7% ± 8.4%, respectively [100-102]. In a COG phase I trial, however, alisertib did not exhibit antitumor activity in four relapsed or refractory MRT (2 AT/RT, 2 eMRT) [103].

CDK4/6 inhibitors

Cyclin-dependent kinases (CDKs) are important in regulating the cell cycle and cell proliferation [104]. Cell cycle regulation is altered in *SMARCB1*-deficient cells and genetic ablation studies of cyclin D1 showed that cyclin D1 deficiency inhibited the growth of rhabdoid tumors in mice [105, 106]. Thus, CDK inhibitors are thought to have therapeutic potential in MRT. In a phase I trial of the CDK4/6 inhibitor ribociclib (LEE011), 15 patients with therapy refractory MRT (13 AT/RT, 2 eMRT) were enrolled and received escalating oral doses of ribociclib (3-weeks-on/1-week-off). Two patients with AT/RT had prolonged stable disease and stayed on treatment for 24 and 20 months. A more recent trial of ribociclib in combination with topotecan-temozolomide (TOTEM) (AcSé-ESMART trial) enrolled one patient with MRT, who showed progressive disease after one cycle [107, 108]. A trial of oral abemaciclib in children with relapsed and refractory brain tumors, including AT/RT, has recently been completed; results are not available yet (NCT02644460).

Subgroup specific therapy

The correlation of AT/RT methylation subgroups with clinical features and prognosis raises the question if there are subgroup-specific therapeutic targets. Torchia *et al*. found that subgroups are associated with distinct sensitivity to certain inhibitors. In particular, they identified an increased sensitivity of group 2 AT/RT to the tyrosine kinase inhibitors (TKIs) dasatinib and nilotinib [109]. As per the since-then-established consensus subgrouping, group 2 AT/RT belongs to AT/RT-TYR or AT/RT-MYC [23]. Tumoroid models of AT/RT-MYC and AT/RT-SHH showed differences in drug sensitivity between the subgroups. AT/RT-MYC tumoroids were consistently vulnerable to TKIs with significant differences in comparison to AT/RT-SHH for the agents lenvatinib and pazopanib. AT/RT-SHH, on the other hand, displayed a high intertumoral heterogeneity in drug sensitivity. It is hypothesized that this effect is due to the proposed further subclassification into three AT/RT-SHH subgroups [24, 110]. Analysis of tumor tissue

from relapsed and refractory AT/RT with matched samples from primary tumors for comparison revealed overall high stability in subgroup allocation. Samples from all three subgroups showed subgroup-specific alterations in gene expression, further hinting at the possibility but also the necessity of subgroup-specific targeted therapy, especially during salvage therapy [111]. There are no clinical trials yet, that use subgroup assignment for therapeutic stratification.

Metronomic therapy

Metronomic therapy is not a traditional “targeted therapy” aiming to interrupt a specific protein or pathway. The regimens usually employ continuous, low-dose application of multiple drugs, including, for example, conventional chemotherapeutics, vascular endothelial growth factor (VEGF) - inhibitors, and cyclooxygenase inhibitors. Initially, it was thought that the main effect of metronomic therapy was due to the antiangiogenetic potency [112]. In the past decade, increasing evidence indicates that the anti-tumor effects observed are at least partly also due to immunomodulatory effects and direct targeting of cancer cells [113, 114]. Feasibility trials of metronomic therapy in pediatric patients with recurrent and refractory malignancies by Kieran *et al*. and Robison *et al*. yielded encouraging results [115, 116]. Based on positive clinical experiences with these regimens, the MEMMAT trial was designed, that employs a combination of daily oral thalidomide, fenofibrate, celecoxib, and alternating cycles of oral etoposide and cyclophosphamide supplemented by bevacizumab and intraventricular therapy with etoposide and liposomal cytarabine. The official trial is still ongoing and is also open for AT/RT (NCT01356290). Results from a pilot cohort of patients with recurrent medulloblastoma treated “MEMMAT-like” showed increased OS in comparison to historical cohorts [117]. Winnicki *et al*. published a case series including eight patients with relapsed or refractory AT/RT, that were all treated according to the MEMMAT regimen outside of the official trial. Event-free survival (EFS) among the AT/RT patients was 6 months, which the authors find an “interesting” result [118]. Two further separate reports of responses to metronomic chemotherapy in relapsed or refractory AT/RT have been published. The metronomic regimens used either vinorelbine, cyclophosphamide, and celecoxib or bevacizumab, liposomal cytarabine, celecoxib, cyclophosphamide, and etoposide [119, 120].

Others

Many more targeted or innovative approaches to the treatment of MRT have been or are currently being evaluated. Ongoing clinical trials include the evaluation of mTOR (mammalian target of rapamycin) inhibitors, Exportin-1 inhibitors, glycogen synthase

kinase-3 inhibitors, local therapy with high-intensity focused ultrasound, and targeted radiotherapeutics (Table 2).

Table 2 – Ongoing clinical trials enrolling children with malignant rhabdoid tumors

* MRT exclusive; ** INI1 negative tumors exclusive
ASCR: autologous stem cell rescue; BRD9: bromodomain-containing protein 9; CAR: chimeric antigen receptor; CD40: cluster of differentiation; CDK: cyclin-dependent kinase; CNS: central nervous system; CT: chemotherapy; CTLA4: cytotoxic t-lymphocyte-associated protein-4; DNMT: DNA methyltransferase; EGFR: epidermal growth factor receptor; EZH2: enhancer of zeste homologue; GPC3: glypican-3; GSK-3β: glycogen synthase kinase-3; HDAC: histone deacetylase; Her2: human epidermal growth factor receptor 2; HSV: herpes simplex virus; Mdm2: mouse double minute 2 homolog; mTOR: mammalian target of rapamycin; Metron. CT: metronomic chemotherapy, PD-1: programmed cell death protein 1; PD-L1: programmed cell death ligand-1; PI3K: phosphoinositid-3-kinase; TIGIT: T-cell immunoreceptor with immunoglobulin and ITIM domains; XPO1: exportin 1

Target	Treatment	Trial ID	Phase	Countries	Location	Status
Epigenetic therapeutics						
EZH2 + PD-1+ CTLA 4	**Tazemetostat** and **Nivolumab** and **Ipilimumab**	NCT05407441	I/II	USA	All**	Recruiting
DNMT + PD1	**Pembrolizumab** and **Decitabine** + single course fixed-dose **hypofractionated radiotherapy**	NCT03445858	I	USA	Non-CNS	Active, not recruiting
HDAC + PD-1	**Nivolumab** and **Entinostat** (INFORM2)	NCT03838042 EUDRA CT: 2018-000127-14	I/II	Europe incl Germany, Australia	All	Recruiting
BRD9	**FHD-609** monotherapy	NCT04965753	I	USA,France, Italy, Spain	All**	Active, not recruiting
Immunotherapy						
PD1 + CTLA 4	**Nivolumab** and **Ipilimumab**	NCT04416568	II	USA	All**	Recruiting
PD1 + metron. CT	**Nivolumab** and **Metronomic CT** (cyclophosphamide, vinblastine, capecitabine)	NCT03585465	I/II	France, Belgium	All	Recruiting
TIGIT + PD-L1	**Tiragolumab** and **Atezolizumab**	NCT05286801	I/II	USA, Canada	All**	Recruiting
CD40	**APX005M** (monoclonal antibody) monotherapy	NCT03389802	I	USA	CNS	Active, not recruiting
B7H3	**B7H3(xCD19)-specific CAR T cells**	NCT04483778	I	USA	Non-CNS	Active, not recruiting

Target	Treatment	Trial ID	Phase	Countries	Location	Status
B7H3	**B7-H3-CAR T cells** after **conventional CT** (fludarabine, cyclophosphamide) *B7-H3 positive tumors only*	NCT04897321 (3CAR)	I	USA	Non-CNS	Recruiting
EGFR	**EGFR806-specific CAR T cells** *EGFR positive tumors only*	NCT03618381	I	USA	Non-CNS	Recruiting
B7H3	**B7-H3-Specific CAR T Cell Locoregional** therapy	NCT04185038	I	USA	CNS	Recruiting
EGFR	**EGFR806-specific CAR T Cell Locoregional** therapy - *EGFR positive tumors only*	NCT03638167	I	USA	CNS	Active, not recruiting
Her2	**HER2-Specific CAR T Cell Locoregional** therapy *Her2 positive tumors only*	NCT03500991	I	USA	CNS	Active, not recruiting
GPC3	**GPC3-CAR and the IL15 plus IL21 (CARE T cells)** after **conventional CT** (fludarabine, cyclophosphamide) – *GPC3 positive tumors only*	NCT04715191	I	USA	Non-CNS	Not yet recruiting
GPC3	**GPC3-CAR and the IL15 (AGAR T cells)** after **conventional CT** (fludarabine, cyclophosphamide) *GPC3 positive tumors only*	NCT04377932	I	USA	Non-CNS	Recruiting
GPC3	**GPC3-Car (GAP T cells)** after **conventional CT** (fludarabine, cyclophosphamide) *GPC3 positive tumors only*	NCT02932956	I	USA	Liver only	Active, not recruiting
Oncolytic virus	**HSV G207** infused through catheters into region(s) of tumor	NCT03911388	I	USA	CNS, cerebellar	Recruiting
Oncolytic virus	**HSV G207** infused through catheters into region(s) of tumor - alone or with a **Single Radiation Dose**	NCT02457845	I	USA	CNS	Active, not recruiting
Kinase inhibitors						
Aurora A kinase	**Alisertib** monotherapy or combination with **Radiochemotherapy**	NCT02114229 (SJATRT)	II	USA	All*	Active, not recruiting
Tyrosinkinase	**Pazopanib** and **conventional CT** (ifosfamide, carboplatin, etoposide)	NCT03628131	I/II	Korea	All	Not yet recruiting

Target	Treatment	Trial ID	Phase	Countries	Location	Status
CDK4/6 inhibitors						
CDK4/6	**Palbociclib** with **conventional CT** (temozolomide and irinotecan or topotecan and cyclophosphamide)	NCT03709680 EUDRA CT: 2021-003444-25	I	International, incl. Germany	All	Recruiting
CDK4/6	**Abemaciclib** with **conventional CT** (temozolomide and irinotecan or temozolomide only)	NCT04238819 EUDRA CT: 2019-002931-27	I	International	All	Recruiting
CDK4/6 + MEK	**Ribociclib** and **Trametinib**	NCT03434262 (SJDAWN)	II	USA	CNS	Active, not recruiting
CDK4/6	**Ribociclib** with **conventional CT** (topotecan and temozolomide)	NCT05429502 EUDRA CT: 2021-005617-14	I/II	Germany, Singapore, Spain	All	Recruiting
Others						
mTOR + metron. CT	**Sirolimus** with **Metronomic CT** (celecoxib, etoposide, cyclophosphamide)	NCT02574728 (AflacST1502)	II	USA	All	Recruiting
PI3K/mTOR	**Samotolisib** monotherapy *PI3K/mTOR mutated tumors only*	NCT03213678	II	USA	All	Active, not recruiting
Small molecule	**RRx-001** (first in class small molecule) with **conventional CT** (irinotecan and temozolomide)	NCT04525014	I	USA	All	Active, not recruiting
XPO1	**Selinexor** monotherapy	NCT02323880	I	USA	All	Active, not recruiting
Mdm2 + XPO1	**Idasanutlin** and **Selinexor**	NCT05952687	I	USA	All*	Not yet recruiting
Peri-nucleolar compartment	**Metarrestin** monotherapy	NCT04222413	I	USA	Non-CNS	Recruiting
GSK-3β	**9-ING-41** (selective GSK-3β inhibitor) monotherapy	NCT04239092	I	USA	All	Active, not recruiting

Target	Treatment	Trial ID	Phase	Countries	Location	Status
Metron. CT	Metronomic **Cyclophosphamide** or **Thalidomide** post ASCR	NCT01661400	I	USA	All	Active, not recruiting
Metron. CT	**Bevacizumab** with five oral drugs (**Thalidomide, Celecoxib, Fenofibrate, Etoposide and cyclophosphamide**), plus intrathecal **Etoposide and Cytarabine**	NCT01356290	II	USA, Europe without Germany	CNS	Recruiting
Conventional CT	Intraventricular **Methotrexate** and **Etoposide** into implanted fourth ventricle catheter	NCT02905110	I	USA	CNS, Posterior Fossa	Recruiting
Conventional CT	Umbrella trial, including randomized arm evaluating the non-inferiority of three courses of **High-Dose Chemotherapy** compared to **Focal Radiotherapy** as consolidation therapy – **Conventional Chemotherapy** according to a revised EU-RHAB regimen (See Figure 1)	EudraCT: 2018-003335-29 (SIOPE ATRT01)	III	Europe	CNS	Recruiting
Conventional CT	Flavored, oral **Irinotecan** VAL-413 (Orotecan®) with **Temozolomide**	NCT04337177	I	USA	All	Recruiting
Local therapy	Transarterial radioembolization with **Yttrium-90 (TARE-Y90)**	NCT04315883	-	USA	Liver only	Recruiting
Local therapy	Magnetic resonance **High Intensity Focused Ultrasound** ablative therapy	NCT02076906	I	USA	Non-CNS	Active, not recruiting
Radiotherapeutic	**CLR 131** monotherapy	NCT03478462	I	USA	All	Active, not recruiting

1.9. Relapse and progression in malignant rhabdoid tumors and early identification of high-risk tumors - open questions and aims of the project

Diagnosis of a MRT is for every patient affected a life-threatening diagnosis and high-risk situation, but fortunately, current therapy strategies can heal a relevant amount of these patients. On the other hand, there are no validated methods available for risk-stratification and early identification of patients who cannot be healed by current, conventional therapeutic regimens. At present, we treat all patients uniformly and closely monitor them for signs of relapse or progression. But once the relapse or progression is detected, only experimental treatment can be offered. Intense effort is therefore going into the identification and testing of new and hopefully better therapeutic agents for MRT, as demonstrated in section 1.8. At the same time, many questions remain open about the clinical characteristics, such as relapse patterns or prognostic factors in patients with relapsed or refractory (r/r) MRT, and comprehensive cohorts are missing. This work aims to retrospectively utilize the database of the EU-RHAB registry for a structured data collection and analysis of clinical data from patients enrolled in the registry who experienced relapse or progression. The underlying hypothesis is the feasibility of using the database to gain new knowledge about outcomes, prognostic factors, and therapeutic approaches in r/r MRT, even though data collection for the registry has not been specifically designed for analysis of r/r MRT. To prove the usability of data about r/r MRT from the EU-RHAB registry, a pilot cohort of patients treated with the epigenetically active agent decitabine was selected for detailed analysis to evaluate this new therapeutic approach [121]. Additionally, we aimed to improve our ability to identify those patients who cannot be healed with current therapeutic regimens as early as possible. In AT/RT, the recently established molecular subgroups open new opportunities for risk stratification and targeted therapy. However, comprehensive, both clinically and genetically well-characterized cohorts are scarce. This problem was addressed in the analysis of 143 AT/RT cases from the EU-RHAB registry [122]. Furthermore, patients with MRT include a special patient group due to the exceptionally early onset of the disease: Those patients affected as newborns or in the first months of life. In order to improve the efficacy and safety of primary and salvage therapy, better characterization of this cohort is urgently needed. To close this gap, Nemes et al. analyzed a cohort of 100 infants and newborns with MRT [123]. The three analyses together aim to enhance our understanding of r/r MRT and high-risk MRT cases that have a high probability of first-line therapy failure. The obtained results are expected to

guide the creation of novel, more efficient and risk-adapted therapeutic strategies for primary and r/r MRT.

2. Abstracts of the manuscripts on which this dissertation is based

Steinbügl M, Nemes K, Johann P, Kröncke T, Tüchert S, da Costa MJG, Ebinger M, Schüller U, Sehested A, Hauser P, Reinhard H, Sumerauer D, Hettmer S, Jakob M, Hasselblatt M, Siebert R, Witt O, Gerss J, Kerl K, Frühwald MC.
Clinical evidence for a biological effect of epigenetically active decitabine in relapsed or progressive rhabdoid tumors. **Pediatr Blood Cancer**. 2021 Dec;68(12):e29267. doi: 10.1002/pbc.29267. Epub 2021 Aug 4. PMID: 34347371.

In this publication, we characterized a cohort of 22 patients with r/r MRT who all received individual salvage therapy that included the DNMT inhibitor decitabine. We were able to demonstrate that 6/22 patients (27.3%) had a radiological response to the treatment with decitabine, defined as at least partial tumor size reduction. In survival analysis, we found prolonged OS and time to progression (TTP) in those with a radiological response. In this small cohort, this survival benefit was not statistically significant after correction for survivorship bias. We analyzed tumor methylation profiles whenever available. Results showed increased average methylation levels in patients with a radiological response to therapy with the demethylating agent. For two patients, tumor samples from before and after treatment with decitabine were available, which showed a decrease in methylation after receiving decitabine. These results support the hypothesis, that the responses found in these patients may be due to a specific effect of the epigenetic therapy. It was concluded that with the promising data suggesting anti-tumor activity in r/r MRT, decitabine should be further investigated in prospective clinical investigations, potentially in combination with other innovative agents such as immunotherapeutics [121].

Frühwald MC, Hasselblatt M, Nemes K, Bens S, *Steinbügl M*, Johann PD, Kerl K, Hauser P, Quiroga E, Solano-Paez P, Biassoni V, Gil-da-Costa MJ, Perek-Polnik M, van de Wetering M, Sumerauer D, Pears J, Stabell N, Holm S, Hengartner H, Gerber NU, Grotzer M, Boos J, Ebinger M, Tippelt S, Paulus W, Furtwängler R, Hernáiz-Driever P, Reinhard H, Rutkowski S, Schlegel PG, Schmid I, Kortmann RD, Timmermann B, Warmuth-Metz M, Kordes U, Gerss J, Nysom K, Schneppenheim R, Siebert R, Kool M, Graf N.
Age and DNA methylation subgroup as potential independent risk factors for treatment stratification in children with atypical teratoid/rhabdoid tumors. **Neuro Oncol.** 2020 Jul 7;22(7):1006-1017. doi: 10.1093/neuonc/noz244. PMID: 31883020; PMCID: PMC7339901.

In “Age and DNA methylation subgroup as potential independent risk factors for treatment stratification in children with atypical teratoid/rhabdoid tumors.” clinical and

molecular data of 143 patients with AT/RT were analyzed, including methylation profiles from 84 tumors. Five-year OS was 34.7 ± 4.5%, EFS was 30.5 ± 4.2%. 64% of patients suffered from relapse or progression. In a multivariate Cox regression model, age below 12 months and a non-TYR methylation signature were identified as independent risk factors. Patients with both characteristics showed a dismal prognosis of 0% 5-year OS. A potential combined clinical and genetic risk model is proposed with three risk groups according to age at diagnosis and methylation subgroup. Relapse or progressive disease were negative prognostic factors with only 14% 5-year OS among this subgroup and only 5% if relapse or progression happened early after diagnosis. The localization of relapse was predominantly local (75%), and less frequently distant only (12%) or combined (13%) [122].

Nemes K, Johann PD, Steinbügl M, Gruhle M, Bens S, Kachanov D, Teleshova M, Hauser P, Simon T, Tippelt S, Eberl W, Chada M, Lopez VS, Grigull L, Hernáiz-Driever P, Eyrich M, Pears J, Milde T, Reinhard H, Leipold A, van de Wetering M, Gil-da-Costa MJ, Ebetsberger-Dachs G, Kerl K, Lemmer A, Boztug H, Furtwängler R, Kordes U, Vokuhl C, Hasselblatt M, Bison B, Kröncke T, Melchior P, Timmermann B, Gerss J, Siebert R, Frühwald MC.
Infants and Newborns with Atypical Teratoid Rhabdoid Tumors (ATRT) and Extracranial Malignant Rhabdoid Tumors (eMRT) in the EU-RHAB Registry: A Unique and Challenging Population. **Cancers (Basel)**. 2022 Apr 27;14(9):2185. doi: 10.3390/cancers14092185. PMID: 35565313; PMCID: PMC9100752.

Nemes *et al*. looked specifically into the youngest patients affected by MRT – those under 6 months of age. 100 patients with intra- and extracranial MRT were analyzed; survival analysis showed 5-year OS and EFS of 23.5 ± 4.6% and 19 ± 4.1%, respectively. Several prognostic factors were identified, including sex, presence of metastasis and germline mutation, and whether patients received maintenance therapy. A high rate of venoocclusive disease was seen, suggesting increased toxicity of the treatment in this cohort. The 78 patients who suffered from relapse or progressive disease showed very poor survival with hazard ratios of 54.7 and 64.5 in those with early therapy resistance or relapse after primary therapy, respectively. Relapse and progression occurred locally in 53 patients. Combined events were found in 13 patients, and 12 patients had distant metastasis but retained complete remission at the primary site [123].

Statement concerning my own contributions to the manuscripts on which this dissertation is based

Prior to the initiation of this project, the EU-RHAB database only partially recorded relapses and progressions. The existing data was, if available at all, mostly limited to the data points collected by the standard case report forms used for the registry. This included information about tumor location at relapse or progression (local, distant, or combined local and distant), time of the event, and time of death or last available status. More detailed information was only available in an unstructured manner through personal communication, counseling requests, and medical reports.

For the purpose of my project, I manually reviewed the complete EU-RHAB database and paper-based patient source files to identify all patients with relapse or progression. I gathered all available data about events of therapy failure and the subsequent clinical course in these patients, including attempts at salvage therapy, therapy toxicity, further progressions, and imaging data. To complete missing or unclear data, institutions were individually contacted to provide as much information as possible. This data was reviewed and medically validated. The result was the first structured collection of EU-RHAB patients with r/r MRT including details on salvage therapy. However, due to the individualized nature of the therapeutic interventions and the retrospective data collection, it was unclear if meaningful analyses were possible from the dataset. I then identified a cohort of 22 patients who all received the therapeutic agent decitabine throughout salvage treatment and conducted a detailed analysis of this subset of patients as a pilot cohort. This comprised collecting raw imaging data and biomaterials, whenever available, from the treating institutions. Imaging was individually reviewed and preselected for re-analysis by the reference radiologist to identify radiological responses. Biomaterial suitable for methylation analysis was selected. A separate request for this specific analysis was approved by the ethics committee of the LMU München (Project-Nr.19-269). I conducted a preliminary statistical analysis that was reviewed and optimized by J. Gerss. Methylation analysis and processing of the results was conducted by P. Johann, who also created one figure in the manuscript. I independently created all further tables and figures and wrote the initial manuscript (See Figure 2 for exemplary tables and figures).

Through the effort described above, not only new information on salvage therapy was collected, but also data about primary therapy and the general outcome of patients in the

registry was added or refined. I was thus able to also provide important content to analyses of the two subcohorts from the EU-RHAB registry published in the manuscripts by Frühwald *et al.* and Nemes *et al.* For both works, I collected new clinical data to complete patient records and reviewed medical data to optimize data quality. I analyzed parts of the data and compiled a table describing therapy details and patient outcomes (See Figures 3 and 4). I supported the preparation of the data for statistical analysis by J. Gerss and reviewed and edited both manuscripts.

In Figure 2 - 4 important figures and tables from the manuscripts on which this dissertation is based are shown.

The following three images are copied from:

Steinbügl Mona, et al. „Clinical evidence for a biological effect of epigenetically active decitabine in relapsed or progressive rhabdoid tumors." *Pediatr Blood Cancer*. 2021; 68:e29267.
Link: https://doi.org/10.1002/pbc.29267

TABLE 2 Characteristics of responders, non-responders, and patients not evaluable for a response

		Responder		Non-responder		Not evaluable	
		n	%	*n*	%	*n*	%
Total number		6	100	12	100	4	100
Diagnosis							
	ATRT	4	67	7	58	1	25
	eMRT	2	33	3	25	3	75
	RTK	–		1	8	–	
	Synchronous	–		1	8	–	
GLM							
	No	4	67	7	58	4	100
	Yes	2	33	4	33	–	
	Not tested			1	8	–	
Methylation subgroup							
	SHH	4	67	3	25	1	25
	SHH + TYR	–		1	8	–	
	MYC	–		3	25	2	50
	Not tested	2	33	5	42	1	25
Sex							
	Male	3	50	6	50	4	100
	Female	3	50	6	50	–	
Age group							
	< 1 year	1	17	4	33	–	
	1-4 years	4	67	6	50	2	50
	5-9 years	1	17	1	8	1	25
	> 9 years	–		1	8	1	25
Metastasis at time of event		6	100	11	92	2	50
Type of event							
	Progression	3	50	4	33	–	
	Relapse	3	50	8	67	4	100

Abbreviations: ATRT, atypical teratoid rhabdoid tumor; eMRT, extracranial malignant rhabdoid tumor; GLM, germ line mutation; RTK, rhabdoid tumor of the kidney.

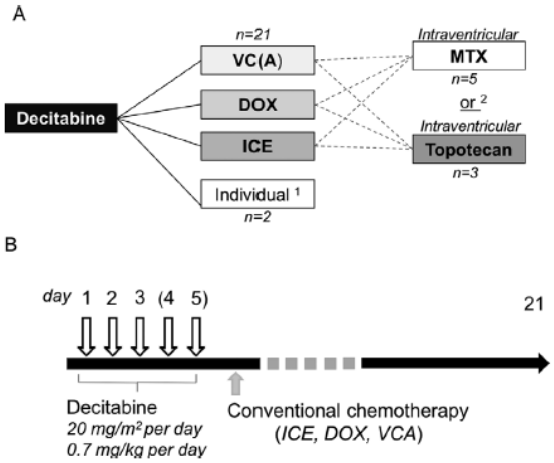

FIGURE 1 (a) Compound combinations. Decitabine was combined with different conventional chemotherapeutics. VC(A), DOX, ICE, and MTX were administered according to recommendations of the EU-RHAB registry, topotecan on an individual basis. VC(A), vincristine, cyclophosphamide (actinomycin D); DOX, doxorubicin; ICE, ifosfamide, carboplatinum, etoposide.; MTX, methotrexate. [1] One patient received four courses of decitabine in combination with intraventricular topotecan only; one patient was given a combination of liposomal doxorubicin, melphalan, and decitabine. [2] Intraventricular therapy was conducted in patients with central nervous system lesions if no contraindications were present, either with methotrexate or with topotecan. (b) Standard treatment regimen. Patients received at least two and up to five days of decitabine at the indicated doses. After completion of the decitabine prephase, conventional chemotherapy was started on the following day. Cycles restarted after day 21

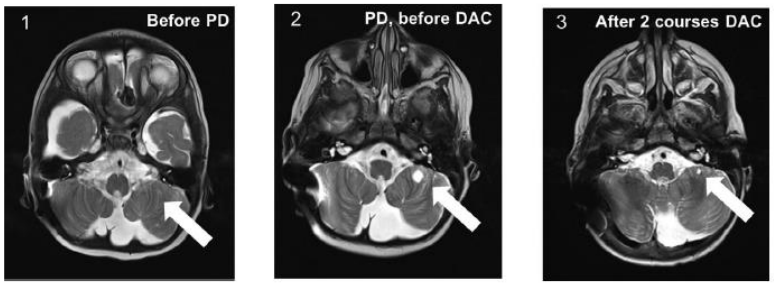

FIGURE 2 Exemplary imaging in a responder. MRI (axial T2 with contrast) of patient #10 from 2 months before initiation of decitabine therapy and during first-line therapy, showing no cerebellar lesion (1); 3 weeks before initiation of decitabine therapy showing a new metastasic lesion in the cerebellum (2) and following 2 courses of decitabine plus conventional chemotherapy showing regression of the lesion (3). DAC, decitabine; PD, progressive disease; MRI, magnetic resonance imaging

Figure *2* – Important tables and figures as published in *"Clinical evidence for a biological effect of epigenetically active decitabine in relapsed or progressive rhabdoid tumors."*, Steinbügl *et al.* 2021

Table 1 shows patient characteristics categorized according to the response to salvage therapy with decitabine. Figure 1 outlines compound combinations used in the cohort and a detailed standard treatment regimen for the incorporation of decitabine into the treatment of r/r MRT. In Figure 2, exemplary imaging of a responder illustrates how a metastatic lesion developed during standard chemotherapy and then decreased in size after intensification of the same therapy with decitabine, hinting at a specific effect of the agent. I collected and analyzed the data presented in this work. I prepared all figures and tables in the manuscript apart from figure 3 and wrote the initial manuscript.

The following three images are copied from: Frühwald, Michael C., et al. "Age and DNA methylation subgroup as potential independent risk factors for treatment stratification in children with atypical teratoid/rhabdoid tumors." *Neuro-oncology* 22.7 (2020): 1006-1017.
Link: https://doi.org/10.1093/neuonc/noz244

Table 1 Clinical characteristics of 143 eligible patients with ATRT

	Total	%
Median age, mo (range)	**29.5 (0–231)**	
Age, y, at diagnosis		
<12	50	35
12–36	73	51
>36	20	14
Origin		
Germany	110	77
Other countries	33	23
Sex		
Female	67	47
Male	76	53
Localization		
Infratentorial	**86**	**60**
Cerebellum	54	
IVth ventricle	18	
Cerebellopontine angle	2	
Brainstem	1	
Mesencephalon	5	
Tectum mesencephalii	2	
Medulla oblongata	4	
Supratentorial	**53**	**37**
Hemisphere	32	
Lateral ventricle	6	
Basal ganglia	4	
Pineal gland	5	
Suprasellar area	2	
Thalamus	1	
Ist–IIIrd ventricle	2	
Hypothalamus	1	
Infra + supratentorial	**1**	**1**
IVth ventricle + lateral ventricle, IIIrd ventricle	1	
Spinal	**3**	**2**
Synchronous tumors	**9**	
eMRT	5	56
RTK	3	33
eMRT + RTK	1	11
Stage		
M0	100	70
M1	7	5
M2	7	5
M3	24	17
M4	5	3

Abbreviations: eMRT, extracranial/extrarenal malignant rhabdoid tumor; RTK, rhabdoid tumor of the kidney.

Table 2 Treatment details of 143 eligibile patients with ATRT

	Total	%
Extent of surgical resection		
Complete	49	34
Incomplete	94	66
HDCT		
Yes	34	24
No	109	76
Completed chemotherapy according to EU-RHAB		
Yes	107	75
No	36	25
Radiotherapy#		
Yes	81	87
No	12	13
Complete remission		
Yes	76	53
After surgery	23	
After chemotherapy	53	
No	67	47
Progression		
No	52	36.5
PD on CT*	49	34
PD after CT**	42	29.5
SAE	***n* = 20**	
VOD§	11	
CNS toxicities&	5	
Severe infection (pneumonia)	1	
AML***	3	
Present status		
CR	44	31
Stable disease	10	7
Progressive disease	7	5
Death	82	57

Abbreviations: CR, complete remission; CT, chemotherapy; HDCT, high dose chemotherapy; PD, progressive disease; SAE, serious adverse event; VOD, veno-occlusive disease; AML, acute myeloid leukemia.
#Only patients >12 months at diagnosis (*n* = 93) were analyzed.
*During CT, analyzed within 4 months from diagnosis.
**After CT, <1 year from diagnosis.
§All VOD resolved.
&2 infections, 2 leukoencephalopathies, 1 central apnea.
***32, 23, and 53 months from diagnosis. Two of them died due to AML (one with SD of the ATRT and no GLM, the other with a GLM in *SMARCB1* in CR of the ATRT). The third patient continues to be in CR following GTR for ATRT and is in first CR following chemotherapy for AML which was diagnosed 4 years after the diagnosis of ATRT.
110 patients were from German speaking countries (Germany, Austria, and Switzerland), the remainder (*n* = 33) from the Czech Republic, Denmark, Norway, Sweden, Hungary, Ireland, Italy, the Netherlands, Poland, Portugal, and Spain.

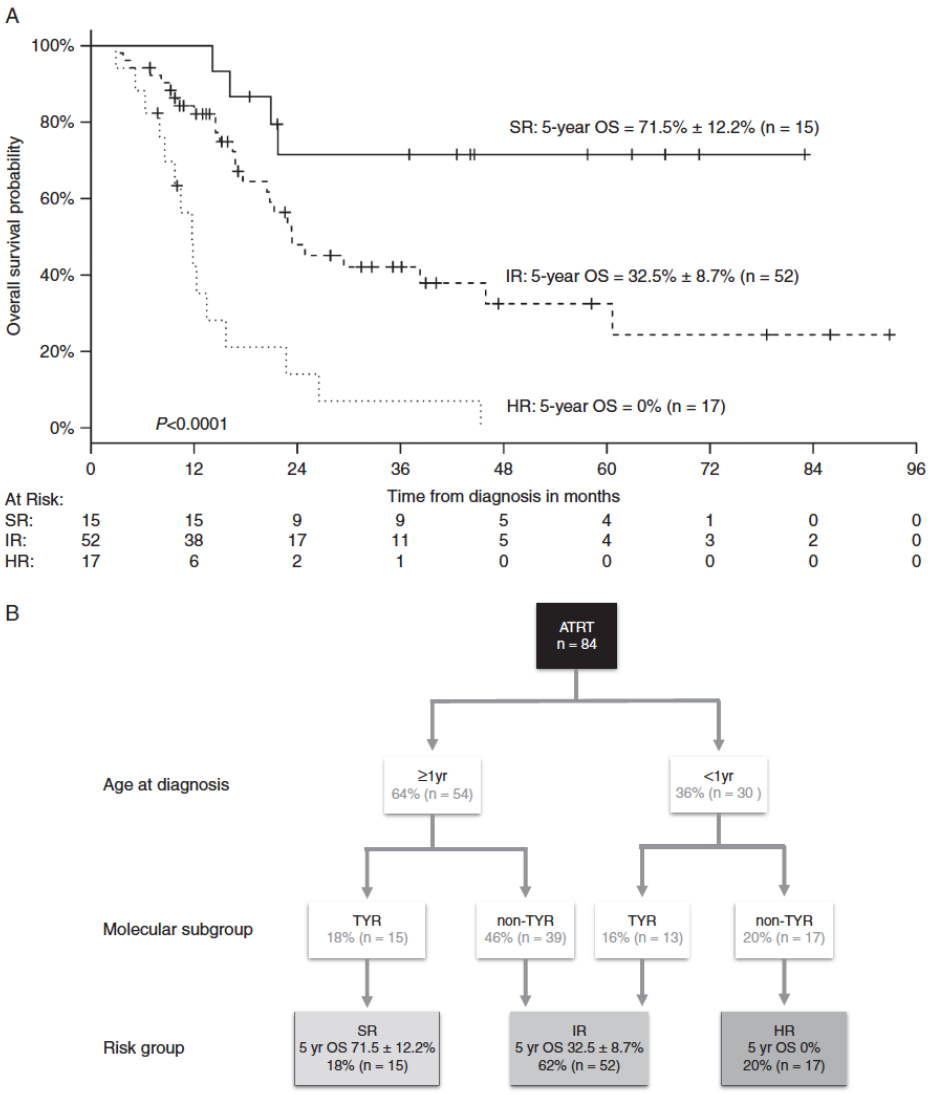

Fig. 4 A combined clinical and genetic risk model for stratification in ATRT Kaplan–Meier analyses (A). Patients with the risk factors age < or ≥1 year and features of the ATRT-TYR or non-TYR DNA-methylation subgroups were analyzed for their 5-year OS. Three risk strata were delineated: high risk (<1 y + non-TYR; 5-y OS 0%), intermediate risk (<1 y + TYR or ≥1 y + non-TYR; 5-y OS 32.5 ± 8.7%), and standard risk (≥1 y + ATRT-TYR; 5-y OS 71.5 ± 12.2). Potential risk model for the stratification of ATRT. (B) Age at diagnosis (<1 y vs ≥1 y) and DNA methylation subgroup (non-TYR vs TYR) may predict the potential risk of patients affected by ATRT independently of any other clinical or known genetic factor.

Figure 3 - Important tables and figures as published in *"Age and DNA methylation subgroup as potential independent risk factors for treatment stratification in children with atypical teratoid/rhabdoid tumors"*, Frühwald *et al. 2020*

Tables 1 and 2 demonstrate the detailed clinical data available for this cohort. Figure 4 shows the results of using this data in combination with molecular data for the development of a combined clinical and genetic risk-stratification model. Clinical data collected and validated during this dissertation project was incorporated into the clinical characteristics, treatment details, and survival analyses shown here. I analyzed parts of the data and personally contributed Table 2 "*Treatment details of 143 eligible patients with ATRT*". I supported the preparation of the data for statistical analysis and reviewed and edited the manuscript.

The following three images are copied from: Nemes Karolina, et al. “Infants and Newborns with Atypical Teratoid Rhabdoid Tumors (ATRT) and Extracranial Malignant Rhabdoid Tumors (eMRT) in the EU-RHAB Registry: A Unique and Challenging Population.” *Cancers.* 2022; 14(9):2185.
Link: https://doi.org/10.3390/cancers14092185

Table 1. Clinical characteristics of 100 infants with malignant rhabdoid tumors.

	Total
Median age [months]	3 (0–6)
Origin [*n* = 100]	
Germany/Austria/Switzerland	74
Other countries	26
Sex [*n* = 100]	
Female	49
Male	51
Localization [*n* = 117] *	
Intracranial MRT (ATRT)	62
Cerebellum	35
Cerebral hemisphere	9
Lateral, 3rd, 4th ventricle	8
Pineal gland	4
Brain stem	4
Basal ganglia	2
Extracranial MRT	55
Kidney	*15*
Liver	7
Neck	6
Orbit	4
Thorax	3
Skin	3

Table 1. *Cont.*

	Total
Retroperitoneum	2
Pelvic soft tissue	2
Cheeks	2
Pre-auricular	1
Clavicle	1
Heart	1
Pancreas	1
Abdomen	1
Adrenal gland	1
Arm	1
Hand	1
Thigh	1
Sacrum	1
Scrotum	1
Metastasis [*n* = 97] **	
M0, LN−	49
M0, LN+	5
M+	26
Synchronous tumor	17

* Anatomical localization of 100 patients, including patients with synchronous tumors (*n* = 17). ** Metastatic stage (M-stage) in *n* = 3 patients not available, M0, LN−; localized disease without loco-regional lymph node involvement, M0, LN+; localized disease with loco-regional lymph node involvement, M+; metastasis.

Table 2. Therapy outline and outcome of 100 infants with malignant rhabdoid tumors.

	Total [*n*]
Gross total resection [*n* = 100]	
Yes	32
No	65
Any radiotherapy [*n* = 100]	
Yes	24
No	76
High dose chemotherapy [*n* = 93] *	
Yes	16
No	77
Maintenance therapy [*n* = 93] *	
Yes	18
No	75
Complete remission (of all sites involved) [*n* = 100]	
Yes	34
After surgery	5
+ chemotherapy	25
+ radiotherapy	4
No	66
Progression [*n* = 100]	
No	22
PD on CT **	56
PD after CT ***	22
SAE [*n* = 17]	17
VOD	12
Encephalomalacia	1
Sinus vein thrombosis	1
Shunt failure	1
Sinus tachycardia	1
AML	1
Present status [*n* = 100]	
Complete remission	18
Stable disease	1
Progressive disease	3
Death	78

* 7 patients did not receive any chemotherapy. ** progression on chemotherapy, analyzed within 4 months from diagnosis, *** progression after chemotherapy, analyzed at 12 months from diagnosis, SAE; serious adverse event, VOD; venoocclusive disease, AML; acute myeloid leukemia.

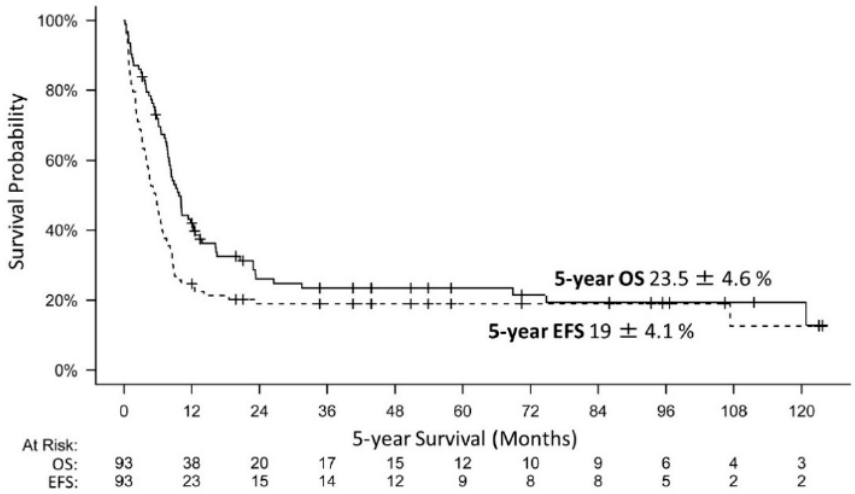

Figure 4. The 5-year overall (OS) and event free survival (EFS) of 93 patients infants with MRT. 7 patients did not receive any chemotherapy, not included in this analysis. The 5-year overall survival (OS) of 93 infants with MRT (ATRT = 41, eMRT = 28, RTK = 9, SYN = 15) was 23.5 ± 4.6%, while the 5-year event free survival (EFS) of the same cohort was 19 ± 4.1%.

Figure 4 – Important tables and figures as published in *"Infants and Newborns with Atypical Teratoid Rhabdoid Tumors (ATRT) and Extracranial Malignant Rhabdoid Tumors (eMRT) in the EU-RHAB Registry: A Unique and Challenging Population"*, Nemes *et al.* 2022

Tables 1 and 2 demonstrate the detailed clinical data available for this cohort. Figures 4 shows the results of survival analysis from the complete cohort. Clinical data collected and validated during this dissertation project was incorporated into the clinical characteristics, treatment details, and survival analyses shown here. I analyzed parts of the data and personally contributed Table 2 "*Therapy outline and outcome of 100 infants with malignant rhabdoid tumors*". I supported the preparation of the data for statistical analysis and reviewed and edited the manuscript.

3. Discussion

Malignant rhabdoid tumors represent one of the most aggressive tumor entities of early childhood. From the discovery and definition of the entity in the 1980s and 1990s to the first MRT-specific therapy regimens in the early 2000s, our knowledge about the biology of MRT and how to treat the disease efficiently has much improved in the last decades. Nevertheless, salvage treatment of a MRT not healed by first-line multimodal treatment is rarely successful and limited clinical data about these patients is available.

In this work, first results from a structured analysis of clinical data from the EU-RHAB database about r/r MRT are presented. This led to the identification, characterization, and analysis of a uniform cohort of 22 patients who all received the epigenetically active agent decitabine during therapy of a relapsed or progressive MRT. Clinical data, imaging data, and biomaterials were used to establish evidence for a biological activity of the drug in MRT and a possible clinical benefit. Thus, we were able to use the existing retrospective data to identify a therapeutic approach that will be a candidate for future clinical trials and investigations [121]. Furthermore, data collected and validated within the scope of this work was used to support the characterization of two subcohorts from the EU-RHAB registry (AT/RT and infants under 6 months of age). Both works added important aspects to our ability to identify patients who are at high risk for therapy failure and thus in need of new, innovative treatment approaches [122, 123]. Taken together, the results from the three manuscripts on which this dissertation is based improve the identification of high-risk MRT, establish a method to better characterize r/r MRT and present evidence for a potentially effective innovative therapeutic approach.

3.1. Utilizing EU-RHAB registry data is a feasible strategy to investigate relapsed and refractory malignant rhabdoid tumors

There are currently no international standards for the clinical management of primary intra- and extracranial MRT; however, substantially overlapping local and national treatment protocols are available and have been prospectively evaluated (see also Table 1). For the treatment of r/r MRT, on the other hand, there are no existing therapy guidelines, and we currently do not know how to effectively treat r/r MRT. As again demonstrated in the current outcome analyses in the AT/RT cohort and the cohort of MRT patients under 6 months of age, the prognosis after relapse or progression is bleak and long-term survival rare. To change this and to offer patients the best possible treatment, in theory, enrollment of all patients into a controlled clinical trial would be the

preferred pathway. But access to clinical trials for patients with r/r MRT is very limited and restricted to mostly single-agent and unimodal early-phase trials. At the time of writing this manuscript, four trials were open in Germany explicitly enrolling patients with r/r MRT (EudraCT numbers 2019-002931-27, 2021-005617-14, 2018-000127-14, 2021-003444-25). All trials have age- or weight restrictions (minimum 10kg, 12 months, and 24 months, respectively). With a median age of < 2 years at diagnosis for MRT, this means many patients will not be eligible at all. Internationally, especially in the US, there are more trials accessible, but still, only a minority of them have been specifically designed for rhabdoid tumors, like the evaluation of alisertib monotherapy in r/r AT/RT [101]. Furthermore, many trials only include patients with a defined target lesion measurable by RANO or RECIST criteria [124, 125]. This means that clinicians must choose between local therapy, for instance by re-resection, or enabling clinical trial participation. As a result, many patients are treated on an individual basis outside of controlled trials, as demonstrated in our cohort of 22 children with r/r MRT. Due to these circumstances, there are currently no representative cohorts of r/r MRT that help us understand patterns of disease, clinical characteristics, and outcome after failure of first-line therapy. This not only hinders the design of new clinical trials tailored to r/r MRT but also complicates the interpretation of existing data. Since most early-phase trials are single-arm, non-randomized investigations, interpretation of outcome data requires comparison with historical data to evaluate for potential survival benefits. However, this simply does not exist for either AT/RT or extracranial MRT.

In our analyses of patients treated with the agent decitabine during individualized salvage therapy, we were able to track the complete clinical course from relapse or progression mostly until the death of the patients and thus were able to capture all treatment modalities and therapy regimens used. The combination with imaging data and biomaterial enabled a comprehensive evaluation of clinical and biological effects, even though each patient was treated and followed up on a completely individual basis. Our analysis of patients treated for relapse or progression of MRT outside of controlled clinical trials thus proves that using EU-RHAB registry data is a feasible strategy to address the knowledge gap described above.

3.2. The heterogeneous epigenetic landscape of malignant rhabdoid tumors as a tool for risk stratification and targeted therapy with epigenetically active agents

Molecular analysis of genetic characteristics is becoming increasingly important in many malignancies for subclassification and therapeutic decisions. Examples of other solid, pediatric tumors where molecular subgroups with clinical and prognostic relevance have been established include medulloblastoma, glioblastoma, pineoblastoma, and ependymoma [126-129]. The former entity “primitive neuroectodermal tumors of the CNS”, was even completely reclassified into four new molecular entities [130]. MRT are a very homogeneous disease on a genetic level with few - if any - recurrent mutations other than in *SMARCB1* or *SMARCA4*. Only the discovery of epigenetic heterogeneity in MRT has recently enabled further subclassification with the identification of molecular subgroups with distinct clinical features in AT/RT [22]. The EU-RHAB registry has since then begun to systematically conduct methylation profiling of tumor samples from AT/RT. In our analysis of primary AT/RT, we were able to utilize this methylation data in combination with clinical information and establish a risk stratification model that includes subgroup status as a prognostic marker. This was further validated by similar results from the cohort of infants and newborns. In combination with preclinical studies indicating subgroup-specific therapeutic targets, this may lead to prospective therapy approaches with early, methylation-guided risk stratification into subgroup-specific treatment protocols. In our cohort of patients treated with decitabine, methylation analysis demonstrated a decrease in global methylation levels correlating with tumor size reduction after treatment with the demethylating agent. Both results show that methylation profiling should be implemented into routine diagnostic workups after the first diagnosis of MRT, but also considered in case of second-look or salvage surgery.

This is especially relevant for patients who have or will be treated with epigenetically active therapeutic agents. Evidence, albeit limited, for a therapeutic effect has already been shown for the EZH2-inhibitor tazemetostat [79-81]. In our cohort of 22 patients treated with the DNMT inhibitor decitabine, we demonstrate a possible clinical benefit and satisfactory safety profile. These results support the therapeutic potential of epigenetic therapeutics in MRT anticipated from preclinical data [131]. As promising as our observations are, we mostly observed partial or mixed responses that had no lasting effect. Similarly, many of the patients treated with tazemetostat only had stable disease or an objective (partial) response. To efficiently treat r/r, MRT monotherapies may not be effective overall, and future regimens will need to utilize combination therapies.

Especially the combination with immunotherapeutics is hoped to yield a synergistic, improved effect. Ongoing clinical trials such as NCT05407441 (nivolumab, ipilimumab and tazemetostat in *SMARCB1* or *SMARCA4*-deficient CNS tumors) and NCT03445858 (pembrolizumab and decitabine in extracranial solid tumors) are already investigating this approach.

3.3. Salvage therapy in malignant rhabdoid tumors may need not only multiagent but also multimodal approaches

First-line therapy of MRT always comprises multimodal treatment including polychemotherapy. Thorough local therapy through surgery and, if possible, RT plays an important role in the outcome [40, 61, 69, 71]. Our current findings in the AT/RT cohort further support this, as RT significantly prolonged survival in patients older than 12 months. It is, therefore, likely that for effective salvage therapy of r/r MRT, multimodal approaches are necessary as well. Among the patients treated with decitabine, for example, we reported one case in which the size reduction of the spinal metastasis enabled the planning and application of salvage re-irradiation. Yet, the role of repeated RT in MRT has not been studied systematically. Experiences from different solid pediatric malignancies show an acceptable safety profile and efficacy of re-irradiation [132-135]. Furthermore, in our cohort of infants and newborns, 9/24 patients who received RT did so following relapse. In patients too young to receive RT during primary therapy, RT during salvage therapy hence may be of even greater significance. Future clinical investigations of r/r MRT should thus not only focus on finding a potent antineoplastic agent or combination of agents. They should also incorporate the role of resection and RT, including re-irradiation, in combination with systemic therapy.

3.4. The patient population in malignant rhabdoid tumors is young and vulnerable – making primary and salvage therapy even more challenging

Treating MRT is already challenging due to the tumor's invasiveness and malignancy. Moreover, many patients are diagnosed at a very young age. Among the primary AT/RTs, 35% (50/143) were younger than one year at diagnosis. In the cohort treated with decitabine, 23% (5/22) were <12 months old at the time of salvage treatment, even though we assume older patients are usually considered better candidates for experimental treatment, thus creating a selection bias for higher age. In a significant number of cases, the tumor is even diagnosed within the first 6 months of life, as shown

in the cohort of 100 infants and newborns with MRT. Neonatal cancer is in general associated with inferior outcomes and higher treatment-associated mortality [136]. Chemotherapeutics and drugs used for supportive therapy underly age-dependent differences in pharmacokinetics and pharmacodynamics that complicate optimal dosing and application in younger children [137]. RT is commonly only applied in children aged 12 months or, preferably, older. The outcome in very young children with MRT is poor: 5-year OS and EFS in the infant and newborn-cohort were reduced in comparison to other MRT cohorts. Concordantly, among the 143 AT/RTs age below 1 year proved to be an independent negative risk factor. Regarding relapse and progression, we conclude that prospective analyses and trials in r/r MRT need to specifically observe characteristics and patterns of therapy failure in very young children below the age of 12 and/or 6 months. Potential therapy algorithms may need to stratify patients age-dependently and regard limitations and specific pharmacological requirements for this age group.

3.5. Limitations of the investigation

Reaching the aim of this project – better clinical characterization of r/r MRT – was limited by the retrospective, unstructured nature of the data available for analysis of relapses and progressions. The EU-RHAB protocol only guides therapy and assessments during primary treatment. After the failure of first-line therapy, patients always receive individual treatment guided by the respective treating institution, and assessments are carried out differently (method and timing) in every patient. Many patients were treated with a palliative approach, often meaning imaging was limited, as for many of the young patients with MRT, imaging studies via MRI are only possible under general anesthesia or sedation. Therefore, results of statistical analysis, especially when assessing the efficacy of certain therapy elements, need to be interpreted and used very carefully since selection bias and unknown cofounders likely influence results. The observations of changes in methylation levels, correlation of response rates to global hypermethylation, and our risk-stratification model correlating subgroup assignment to outcome need to be prospectively evaluated and confirmed in other independent cohorts.

All these limitations underline the need for international clinical trials for primary and r/r MRT that include the collection of biomaterials. Only with large and uniform cohorts will we be able to create more and better evidence on how to successfully treat r/r MRT. In the meantime, currently available retrospective data on r/r MRT from different smaller cohorts should ideally be pooled together for larger-scale investigations. This would not

only increase the cohort size for meaningful statistical analyses but also balance out potential confounding factors created by the individual manner of salvage therapy that might be influenced by anecdotal evidence or personal opinions.

3.6. Conclusions and outlook

Summing up, in this work we were able to further characterize r/r MRT and identify a potential therapeutic approach from retrospective registry data. Furthermore, with the cohort of 143 AT/RTs, a risk-stratification model for primary AT/RT was established with further evidence for the clinical relevance of molecular subgroups in AT/RT. With the cohort of 100 infants and newborns with MRT, an important and vulnerable patient population was characterized in detail. Despite the limitations, the results presented here prove the value of this dataset and allow the conclusion that further analyses are feasible and reasonable, and the results will help fill a gap in knowledge regarding the characteristics of relapsed and progressive MRT. Especially the analysis of the decitabine-subcohort gives important evidence that, despite the obvious limitations of the retrospective data as discussed above, robust clinical and biological observations about r/r MRT can be made that may be used for future therapy guidelines and the design of clinical investigations. Our novel insights into clinical and molecular prognostic factors in AT/RT and infants with MRT will likewise help to improve our abilities to treat all kinds of rhabdoid tumors.

The next steps for optimal utilization of the r/r MRT data collected are complete analyses of the two major subcohorts of intracranial (AT/RT) and extracranial MRT. Clinical data of r/r extracranial MRT will be correlated with methylation data from primary and relapse tumor tissue with the hypothesis that molecular data can help to identify and treat r/r MRT better (Fincke, Steinbügl *et al*., manuscript submitted to Clinical Cancer Research). In cooperation with institutions from Europe, North America, Australia, and Asia, the data will be incorporated in an analysis of data from multiple international cohorts of r/r AT/RT (Abstract submitted to the International Symposium on Pediatric Neuro-Oncology 2024, manuscript in preparation). Ideally, our findings will support investigators and clinicians in the management of patients with r/r MRT, and we will be able to provide reliable datasets that serve as historical controls and references for future clinical trials, increasing the value of newly generated data.

4. Summary

Rezidive oder Progresse maligner rhabdoider Tumoren (MRT) treten häufig auf, sind aber weiterhin nur unzureichend erforscht. Standardisierte Therapieansätze existieren für die Rezidivtherapie nicht und der Zugang zu klinischen Studien ist für die junge Patientenpopulation sehr beschränkt. Das EU-RHAB Register erforscht seit vielen Jahren MRT und deren Erstlinientherapie. In dieser Arbeit untersuchen wir die Machbarkeit der Verwendung von Registerdaten auch zur besseren Beschreibung von rezidivierten und refraktären MRT. In einer Pilotkohorte von Patienten, die als Rezidivtherapie mit dem innovativen Wirkstoff Decitabine behandelt wurden, wird gezeigt, dass eine detaillierte Auswertung möglich ist, zudem können sogar Signale für eine Wirksamkeit der Therapie gefunden werden. Die zwei größeren Kohorten von intracraniellen MRT (AT/RT) und Säuglingen mit MRT liefern neue Erkenntnisse zu Patienten mit besonders hohem Risiko für ein Therapieversagen. Basierend auf diesen Ergebnissen sind weitere Analysen des gesamten Kollektivs der im Register erfassten Rezidive und Progresse geplant. Die generierten Daten unterstützen therapeutische Entscheidungen und die Planung neuer, für MRT designter klinischer Studien.

Relapse or progression of malignant rhabdoid tumors (MRT) is a frequent event, but our knowledge on this topic is insufficient. There is no standard algorithm for salvage therapy and clinical trial access is limited for this young patient population. The EU-RHAB registry has been investigating MRT with a focus on first-line therapy. In this work, we investigate the feasibility of using registry data for better characterization of relapsed and refractory MRT. In a pilot cohort of patients treated with the innovative agent decitabine during salvage therapy, we were able to conduct a detailed analysis and even find evidence of a clinical benefit from the treatment. Analyses of two larger cohorts of intracranial MRT (AT/RT) and infants with MRT offer new insights into patients with an increased risk for failure of first-line therapy. Based on these results, we will be able to conduct a comprehensive analysis of all relapses and progressions recorded in the registry. Results will aid in clinical decision-making during salvage therapy and help the design of new clinical trials.

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6. Appendix

I List of abbreviations

ACT-D	Actinomycin-D
ADR	Adriamycin
AR	Average risk
ARA-C	Cytarabine
ASCR	Autologous stem cell rescue
AT/RT	Atypical teratoid/rhabdoid tumor
BAF	BRG1/BRM-associated factor
BET	Bromodomain and extra-terminal domain
BRD4	BET bromodomain-containing protein 4
BRD9	Bromodomain-containing protein 9
CAR	Chimeric antigen receptor
CARBO	Carboplatin
CD40	Cluster of differentiation 40
CDDP	Cisplatin
CDK	Cyclin dependent kinase
ClTr	Clinical trial
CNS	Central nervous system
COO	Cell of origin
CRF	Case report form
CSF	Cerebrospinal fluid
CT	Chemotherapy
CTLA4	Cytotoxic t-lymphocyte-associated protein-4
DNMT	DNA methyltransferase
DOX	Doxorubicin
EFS	Event-free survival
EGFR	Epidermal growth factor receptor
eMRT	Extracranial, extrarenal MRT
EPI	Epirubicin
ETOP	Etoposide
EZH2	Enhancer of zeste homologue
GPC3	Glypican-3
GSK-3β	Glycogen synthase kinase-3
HCT	Hydrocortisone
HDAC	Histone deacetylase
HDCT	High-dose chemotherapy
HDMTX	High-dose methotrexate
Her2	Human epidermal growth factor receptor 2
HR	High risk
HSV	Herpes simplex virus
ICE	Combination of ifosfamide, carboplatin, etoposide
IDA	Idarubicin
IFO	Ifosfamide
INI1	Integrase interactor 1
IR	Intermediate risk
IT	Intrathecal
M	Months
Metron. CT	Metronomic chemotherapy
Mdm2	Mouse double minute 2 homolog

mTOR	Mammalian target of rapamycin
MTX	Methotrexate
MRT	Malignant rhabdoid tumor
OS	Overall survival
OV	Oncolytic virus
PBSC	Peripheral blood stem cells
PD-1	Programmed cell death protein 1
PD-L1	Programmed cell death ligand 1
PI	Principal investigator
PI3K	Phosphoinositid-3-kinase;
PO	Per os
PFS	Progression-free survival
r/r	Relapsed or refractory
RT	Radiotherapy
RTPS	Rhabdoid tumor predisposition syndrome
RTK	Rhabdoid tumor of the kidney
SCA	Stem cell apheresis
SWI/SNF	SWItch/Sucrose Non-Fermentable
TEM	Temozolomide
TIGIT	T-cell immunoreceptor with immunoglobulin and ITIM domains
TKI	Tyrosine kinase inhibitor
TROFO	Trofosfamide
TT	Thiotepa
VCA	Combination of vincristine, cyclophosphamide, actinomycin-D
VCR	Vincristine
VEGF	Vascular endothelial growth factor
XPO1	Exportin 1
Y	Years

II List of figures

III List of tables

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V Own publications and congress contributions

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Decitabine in der Therapie rezidivierte und refraktärer Rhabdoider Tumoren – Erfahrungen mit 22 Patienten aus dem EU-RHAB- Register

Embryonale Tumortagung Herbst 2021, 1.-2. November 2021, Munich:
Rezidivstrategien in Rhabdoiden Tumoren – Decitabine und anderes.

20th International Symposium on Pediatric Neuro-Oncology (ISPNO 2022), 12.-15th June 2022, Hamburg:
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