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Review

Prioritization of Novel Agents for Patients with Rhabdomyosarcoma: A Report from the Children's Oncology Group (COG) New Agents for Rhabdomyosarcoma Task Force

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Abstract: Rhabdomyosarcoma is the most common soft tissue sarcoma diagnosed in children and adolescents. Patients that are diagnosed with advanced or relapsed disease have exceptionally poor outcomes. The Children's Oncology Group (COG) convened a rhabdomyosarcoma new agent task force in 2020 to systematically evaluate novel agents for inclusion in phase 2 or phase 3 clinical trials for patients diagnosed with rhabdomyosarcoma, following a similar effort for Ewing sarcoma. The task force was comprised of clinicians and basic scientists who collectively identified new agents for evaluation and prioritization in clinical trial testing. Here, we report the work of the task force

including the framework upon which the decisions were rendered and review the top classes of agents that were discussed. Representative agents include poly-ADP-ribose polymerase (PARP) inhibitors in combination with cytotoxic agents, mitogen-activated protein kinase (MEK) inhibitors in combination with type 1 insulin-like growth factor receptor (IGFR1) inhibitors, histone deacetylase (HDAC) inhibitors, and novel cytotoxic agents.

Keywords: rhabdomyosarcoma; clinical trials; metastasis; relapse; new agents

1. Introduction

Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma in children and adolescents, with approximately 350 children diagnosed annually in the United States [1]. Historically, the two main subtypes of RMS were classified by histologic characteristics and designated as alveolar RMS (ARMS) and embryonal RMS (ERMS) [2,3]. More contemporary classification utilizes the presence of molecular translocations to classify RMS into fusion-positive RMS (FP-RMS) and fusion-negative RMS (FN-RMS). This designation is based on the presence of a *PAX3/7-FOXO1* fusion gene in most cases [4,5]. Approximately 80% of the cases previously categorized as ARMS harbor one of these translocations and are classified as FP-RMS, whereas ERMS and fusion negative ARMS are classified as FN-RMS [2,6,7]. Hereafter, FP-RMS or FN-RMS will be used, except when referencing previously conducted trials or studies.

The five-year overall survival of pediatric RMS is approximately 70%, although for high-risk patient groups the outcomes are poor [3,8]. Patients with stage 4 disease, other than those who are less than 10 years of age with stage 4 ERMS, have a 3-year event free survival (EFS) of less than 25% [3,9–11]. Recent studies for patients with metastatic RMS have attempted to intensify chemotherapy and incorporate new agents such as cixutumumab, irinotecan, and temozolomide, but those studies have not improved cure rates for this group of patients [12–14]. These inferior outcomes highlight the need to evaluate novel therapeutics for RMS. However, the inherent difficulties of designing and executing clinical trials to evaluate new agents in a rare disease such as RMS make it essential to conduct a comprehensive and critical evaluation of the appropriateness of testing each new agent in this population. Prior working groups for osteosarcoma and Ewing sarcoma within the Children's Oncology Group (COG) have been useful for disease-specific drug identification, evaluation, and prioritization of agents for evaluation in clinical trials [15,16]. To this end, the COG Soft Tissue Sarcoma Committee established the New Agents for RMS Task Force.

The goal of the RMS Task Force was to bring together laboratory scientists, clinical investigators, and clinicians to identify, evaluate, and prioritize new agents for the treatment of RMS. In this report, we summarize the framework that was utilized to prioritize potential agents for clinical investigation, and we review the group's analysis and discussion of the proposed agents.

2. Modified Framework for Assessing Novel Agents in Rhabdomyosarcoma

The RMS task force modified the framework created by the Ewing sarcoma task force to define the key criteria specific to RMS to systematically identify and evaluate new agents based on clinical and non-clinical criteria (Figure 1) [16].

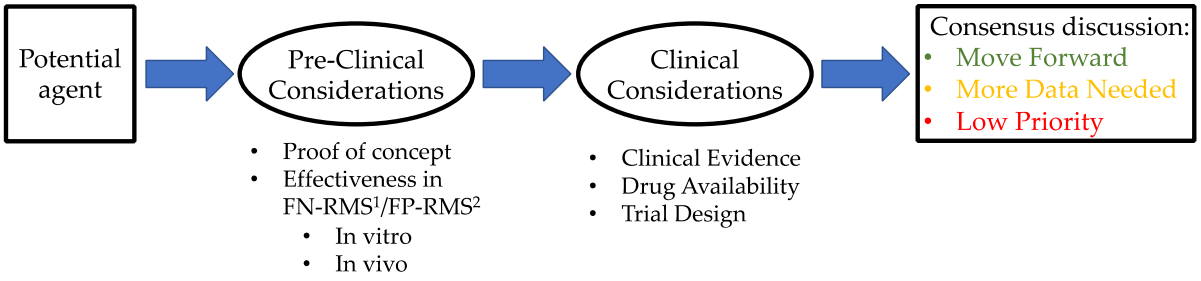

Figure 1. Framework used by the task force to evaluate new agents. [1] Fusion-positive rhabdomyosarcoma. [2] Fusion-negative rhabdomyosarcoma.

2.1. Non-Clinical Criteria

The non-clinical criteria utilized in the modified RMS framework that remained unchanged from the Ewing sarcoma framework were: (1) evidence that the target was critical to RMS tumorigenesis or had specific expression in RMS; and (2) proof of concept data that showed the drug is active against the intended target, including in vitro and in vivo activity in RMS cell lines and animal models. In contrast to Ewing sarcoma where most tumors exhibit a characteristic fusion of EWS-FLI1, the genetic makeup of RMS is more diverse. Hence, drug candidates were considered within the context of RMS subtypes: FN-RMS, FP-RMS, as well as drugs that are active in both subtypes. The goal was to conduct a broad search to identify novel agents that would be the most useful in treating this patient population.

2.2. Clinical Criteria

The clinical criteria utilized in the modified RMS framework that remained unchanged from the Ewing sarcoma framework were: (1) signal of activity in early phase testing; (2) drug availability—either US Food and Drug Administration (FDA) approved or included in the Cancer Therapy Evaluation Program (CTEP) portfolio, or collaboration with an industry partner; (3) availability of a recommended pediatric phase 2 dose (RP2D); and (4) feasibility in combination therapy with currently used therapeutics for RMS.

3. Using the Framework to Critically Evaluate a Model Agent: Temsirolimus

Temsirolimus, a prodrug of sirolimus (rapamycin), inhibits activation of the mammalian target of rapamycin (mTOR) pathway, which has been shown to be involved in both FP-RMS and FN-RMS oncogenesis. In preclinical studies, RMS cell lines are sensitive to sirolimus and temsirolimus, and both drugs have also shown activity in RMS xenografts by both inducing tumor regression and prolonging survival [17–19]. Sirolimus was also shown to enhance the activity of vincristine and cyclophosphamide in RMS xenografts [20]. Temsirolimus was evaluated in phase 1 trials in both adults and children, and a pediatric RP2D was identified [21,22]. Thus, temsirolimus met the criteria for an agent of high priority with (1) evidence that the target was important to RMS oncogenesis; (2) proof of concept in vitro and in vivo activity in RMS; (3) signal of activity in RMS; (4) recommended phase 2 dosing; and (5) proof of feasibility in combination [17–24]. Temsirolimus was subsequently studied in a phase 2 trial (NCT01222715) for patients with relapsed RMS that compared bevacizumab to temsirolimus, where both agents were administered in combination with cyclophosphamide and vinorelbine. The results of this trial show that patients treated on the temsirolimus arm had a superior 6-month EFS and higher response rates compared to those treated with bevacizumab [25]. Based on this evidence, temsirolimus was moved into phase 3 testing for intermediate risk RMS patients in 2014 on ARST1431 (NCT02567435). The success of the phase 2 testing of temsirolimus provides evidence that formal evaluation using the modified framework is beneficial in systematically prioritizing agents for future testing in cooperative group trials.

4. Using the Model to Evaluate Novel Agents for Use in Future Clinical Trials

Agents were nominated by committee members for evaluation and discussion by the group. The full list of the nominated agents is listed in Table 1 and the top five agents based on the task force rankings are designated in bold font. The data available for each agent were presented to the task force and evaluated using the above criteria. After all the potential agents were presented, task force members were asked to submit a prioritization list. Below, we review the available data and the group's discussion of the top five classes of agents based on the task force ranking, which are summarized in Table 2.

Table 1. List of agents nominated by the task force.

Class	Example Agents
Novel cytotoxic agents	**Microtubule inhibitors**: **Eribulin** **Topoisomerase I inhibitors**: **PLX038**, PEN-866
DNA damage/repair	**PARP [1] inhibitors/Cytotoxic agents**: **Olaparib/temozolomide**
Epigenetic targets	**HDAC [2] inhibitors**: **Entinostat** Bromodomain inhibitors
Immune Targets	B7-H3 inhibitors PD1 [3]/PD-L1 [4] inhibitors FGFR4 [5] CAR T-cell [6]
Tyrosine kinase inhibitors	Multi-targeted tyrosine kinase inhibitors: cabozantinib, regorafenib, pazopanib ALK [7] inhibitors: crizotinib Eph [8]/ephrin receptor inhibitors **MEK [9] inhibitors/IGF1R [10] inhibitors**: **trametinib/ganitumab**
Cell cycle inhibitors	CDK4/6 [11] inhibitor: palbociclib Wee1 inhibitor CHK1 [12] inhibitor

[1] Poly ADP-ribose polymerase. [2] Histone deacetylase. [3] Programmed cell death protein 1. [4] Programmed death-ligand 1. [5] Fibroblast growth factor receptor 4. [6] Chimeric antigen receptor T-cell. [7] Anaplastic lymphoma kinase. [8] Erythropoietin-producing hepatocellular. [9] Mitogen-activated protein kinase. [10] Insulin-like growth factor 1 receptor. [11] Cyclin-dependent kinase 4/6. [12] Checkpoint kinase 1.

Table 2. Summary of the available data for the top five agents identified by the task force.

Agent Rank	Agent	Preclinical Evidence	Adult Clinical Data	Pediatric Clinical Data	Drug Availability	Consensus Decision
In trial	temsirolimus	×	×	×	×	In trial
1	PARP [1] inhibitor/cytotoxic agent	×	×	Ongoing phase 1 of olaparib in combination with temozolomide for EWS [2] and RMS [3] (age $\geq$ 16 years)	×	Need more preclinical combination studies, need phase 1 combination data
2	MEK [4] inhibitor/IGF1R [5] inhibitor	×	×	×		Need more preclinical testing to determine if doses in in vitro studies are achievable in humans
3	PLX038	×	×			Need preclinical combination studies, need phase 1 pediatric dose
4	HDAC [6] inhibitor	×	×	×	×	Need more preclinical testing in vivo to mimic human PK [7] data

Table 2. *Cont.*

Agent Rank	Agent	Preclinical Evidence	Adult Clinical Data	Pediatric Clinical Data	Drug Availability	Consensus Decision
5	eribulin	×	×	Ongoing phase 2 in RMS [3], ongoing phase 1/2 in combination with irinotecan in R/R [8] solid tumors	×	Need phase 2 pediatric data

The top five agents are listed in order of their rank based on votes by the task force members. Areas where the task force felt there was sufficient evidence or data are noted with "×" and areas where there were no available data or insufficient evidence are blank. [1] Poly ADP-ribose polymerase. [2] Ewing sarcoma. [3] Rhabdomyosarcoma. [4] Mitogen-activated protein kinase. [5] Insulin-like growth factor 1 receptor. [6] Histone deacetylase. [7] pharmacokinetic. [8] Relapsed/refractory.

5. Poly-ADP-Ribose Polymerase Inhibitors in Combination with Cytotoxic Agents

Poly-ADP-ribose polymerases (PARP) are nuclear enzymes that are involved in repairing DNA damage. These enzymes (PARP1, PARP2, and PARP3) are overexpressed in both FP-RMS and FN-RMS tumor samples [26]. PARP1 is the primary enzyme of the PARP family and binds to DNA single-strand breaks to facilitate repair [27]. The inhibition of PARP leads to the accumulation of DNA strand breaks and subsequently apoptosis and cell death [27]. Olaparib, an inhibitor of PARP1 and PARP2, also leads to trapping of the PARP enzyme on damaged DNA and may result in improved cytotoxicity [28]. Due to efficacy in preclinical models and early phase studies in adult malignancies, PARP inhibition is a therapeutic target of interest in RMS that was ranked highly by the task force.

PARP inhibition was evaluated in vitro in FN-RMS and FP-RMS cell lines using multiple agents in this class. Olaparib (inhibitor of PARP 1/2) and AZD2461 (inhibitor of PARP 1/2/3) resulted in a reduction in growth at clinically achievable concentrations (1–5 μM olaparib and 5–10 μM AZD2461); however, talazoparib (inhibitor of PARP 1/2) was not effective against FP-RMS or FN-RMS [26,29,30]. Preclinical combination studies in RMS have shown that PARP inhibitors can enhance the efficacy of topoisomerase II inhibitors, ionizing radiation, and alkylating agents such as temozolomide [26,29,31,32]. The combination of olaparib with temozolomide was particularly effective when tested in mouse and zebrafish xenografts [33]. This combination led to a potent reduction in tumor size in both FN-RMS and FP-RMS in each of the tested models, whereas treatment with each drug individually had limited responses [33].

In the clinical setting, multiple PARP inhibitors, including olaparib, rucaparib, niraparib, and talazoparib, have been FDA-approved as single agents for use in adult cancers. PARP inhibitors have also been studied in clinical trials in combination with other cytotoxic agents, including temozolomide, topotecan, paclitaxel, and cyclophosphamide [34–40]. However, the combination of PARP inhibitors with cytotoxic chemotherapy led to increased toxicity, particularly myelosuppression, that required dose reductions in several studies [40–43]. PARP inhibition in RMS is currently being studied in an ongoing phase 1 clinical trial evaluating olaparib in combination with temozolomide in Ewing sarcoma and RMS in patients aged 16 years and older (NCT01858168).

The combination of a PARP inhibitor with cytotoxic agents, specifically temozolomide, was rated highly by the group due to their availability, as both agents are FDA-approved. Prior studies suggest that dose reductions are indicated given the increased risk of myelosuppression with the combination, and data from the ongoing phase 1 study in RMS is not yet available. In preclinical studies, the combination was effective in FN-RMS and FP-RMS xenograft models, but the group determined that additional preclinical studies evaluating PARP inhibitors in combination with other less myelotoxic agents, such as irinotecan, would be beneficial. Given the ongoing trial of the combination in adolescents and adults with RMS, the committee preferred to await the results of this study before proceeding with a new clinical trial for children with RMS utilizing this combination.

6. MEK Inhibitors in Combination with Type 1 Insulin-Like Growth Factor Receptor Inhibitors

Another combination of interest was the inhibition of mitogen-activated protein kinase (MEK) and type 1 insulin-like growth factor receptor (IGF1R). RAS pathway mutations are reported in approximately 50% of FN-RMS which leads to the activation of MEK1/2 [44,45]. Preclinical studies have demonstrated that RAS is an oncogenic driver in FN-RMS, suggesting that targeting this pathway may be effective treatment [46–50]. IGF1R is a receptor tyrosine kinase of interest in the treatment of RMS because it is overexpressed in both FN-RMS and FP-RMS and IGF1R inhibition was effective in preclinical studies of FN-RMS and FP-RMS [51–55].

In preclinical studies of RAS-mutated FN-RMS, treatment with trametinib, a selective inhibitor of MEK1/2, led to decreased cell viability when tested in vitro and also resulted in decreased tumor volume and prolonged survival in mouse xenografts [56]. However, the effect of trametinib was limited in xenograft models, with animals ultimately developing disease progression [56]. When trametinib was administered in combination with BMS-754807, a small molecule inhibitor of IGF1R and the insulin receptor, there was a more profound decrease in cell viability and delay in tumor growth [56].

Based on the promising preclinical data of the combination of MEK and IGF1R inhibition in RAS mutated FN-RMS, the task force discussed the possibility of evaluating this combination in a clinical trial for this patient population. There are four FDA-approved inhibitors of MEK1/2, including trametinib, binimetinib, selumetinib, and cobimetinib [57–60]. Selumetinib was recently approved for children with neurofibromatosis type 1 who have inoperable plexiform neurofibromas. Selumetinib was well tolerated in the clinical trials leading to its FDA approval in April 2020 for neurofibromatosis type 1 and has also demonstrated activity in children with low grade gliomas [61–64]. Trametinib has also been studied in pediatrics and was well tolerated in a phase 1 study in patients with a low-grade glioma or plexiform neurofibroma and is now being studied in an ongoing phase 2 study (NCT03363217) [65].

The main categories of IGF1R inhibitors include monoclonal antibodies that target IGF1R or its ligands (IGF-1 and IGF-2), or IGF1R tyrosine kinase inhibitors [66,67]. While there are several FDA-approved MEK inhibitors, there are no FDA-approved IGF1R inhibitors. Overall the efficacy of IGF1R inhibition in clinical trials has been limited, and the current focus is to identify new combinations of IGF1R inhibitors with novel agents that may lead to improved efficacy, as well as identify biomarkers to predict patient subgroups which will respond to IGF1R inhibition [66–68].

A number of clinical trials of IGF1R inhibitors for patients with RMS have been conducted, including a phase 2 study where R1507, a monoclonal antibody targeting IGF1, was studied in adults with relapsed or refractory sarcomas [69]. There were 36 individuals with RMS enrolled in this study (ARMS: 12, ERMS: 3, unknown type: 21) and one patient with ERMS experienced a confirmed partial response, while three patients with RMS experienced short-lived decreases in tumor size of greater than 50% [69]. In pediatric RMS, a recent COG trial evaluated the addition of cixutumumab (monoclonal antibody against IGF1R) or temozolomide to cytotoxic chemotherapy in unselected patients with metastatic RMS, and found that neither agent improved outcomes [12]. An ongoing phase 2 clinical trial based on preclinical data evaluating the combination of ganitumab (monoclonal antibody targeting IGF1R) with dasatinib (a multi-kinase inhibitor) in children and adults with RMS (NCT03041701) aims to determine whether this combination can produce more durable responses [70].

IGF1R inhibitors have not been evaluated in combination with MEK inhibitors in clinical trials for patients with RMS. However, the combination of selumetinib with cixutumumab was recently evaluated in adults with advanced malignancies, and an early report from the study noted one dose limiting toxicity of visual changes, and one partial response [71]. Based on the preclinical data of MEK inhibition and IGF1R inhibition in RAS mutated FN-RMS, this may be a subset of patients who will respond to this combination of

agents. Although this combination was of interest to the task force, it was determined that additional preclinical data were needed to determine whether the exposures achievable in humans are effective in preclinical studies.

7. Histone Deacetylase Inhibitors

Epigenetic processes control gene expression through histone acetylation and deacetylation, and are important in oncogenesis. In certain cancers, there is dysregulation of these histone acetylation processes [72]. Histone deacetylase (HDAC) inhibitors are a class of medications that can reactivate proapoptotic genes that have been suppressed in tumor cells. Treatment with HDAC inhibitors in preclinical studies of FP-RMS and FN-RMS led to reduced cell growth, apoptosis, and induced differentiation of RMS cells [73–75]. HDAC inhibition is of interest particularly in FP-RMS because the fusion protein PAX3/7-FOXO1 is an oncogenic driver that is epigenetically regulated [76,77]. Specifically, an in vitro study demonstrated that treatment with entinostat, a selective class I and class IV HDAC inhibitor, led to HDAC inhibition and suppressed the expression of PAX3-FOXO1 and PAX7-FOXO1 or its downstream effects in FP-RMS cells [77–79].

The activity of entinostat in RMS was evaluated in two xenograft studies, and the researchers drew different conclusions. Keller et al. studied the effectiveness of entinostat in PDX models of FN-RMS and FP-RMS. In these studies, entinostat was administered daily at dose of 4 mg/kg for 21 days [78,80]. This dosing schedule led to decreased growth of FN-RMS and FP-RMS tumors in mice, and in FP-RMS PDX models the combination of entinostat with vincristine was more effective than either agent alone [78]. Preclinical work also demonstrated that HDAC inhibition leads to the disruption of the core regulatory circuitry of FP-RMS, and treatment with entinostat in FP-RMS led to a decrease in PAX3-FOXO1 protein levels via inhibition of HDAC3 [77–79]. However, in the experiments performed by Houghton et al. as part of the National Cancer Institute (NCI)-sponsored Pediatric Preclinical Testing Program, entinostat was administered twice daily at a dose of 2.5 mg/kg for 4 days over 3 consecutive weeks in xenograft models of both FN-RMS and FP-RMS. In this study, the effect of entinostat on tumor growth was not significant in the majority of FP-RMS xenograft models, and no effect was observed in mice bearing FN-RMS tumors [81]. Additionally, there was no increased activity when entinostat was administered in combination with other cytotoxic chemotherapy agents [81].

The results from these conflicting studies were discussed in detail by the task force, but it is difficult to compare the results, as different dosing strategies were used for the xenograft experiments. In humans, entinostat has a half-life of 50 h and is administered weekly, but in mice the half-life is much shorter and requires more frequent dosing [72,78,81]. The pharmacokinetic (PK) data from both studies were analyzed by two independent pharmacologists to assist in the data comparison given the different dosing schedules. However, it was concluded that the PK data in mice were difficult to extrapolate to humans. Mice can tolerate higher dose levels without toxicity compared to humans, and when tested in humans, it is possible that the maximum tolerated dose may not reach the point of clinical efficacy.

There are multiple FDA-approved HDAC inhibitors for hematologic malignancies in adults including vorinostat, belinostat, and romidepsin, which are approved for T-cell lymphoma; and panobinostat which is approved for multiple myeloma [82]. Entinostat is not FDA-approved, but has been evaluated in clinical trials for patients with breast cancer [83]. HDAC inhibitors have been evaluated in clinical trials for adults with sarcomas and have demonstrated limited success as monotherapy [82]. In pediatrics, HDAC inhibitors have been evaluated as single agents in phase 1 clinical trials which included patients with RMS and while these agents were well tolerated, there were no objective responses reported [84–88]. These agents may be more effective in combination with chemotherapy or other targeted agents, and there is an ongoing clinical trial for individuals with RMS aged 16 years and older studying the HDAC inhibitor mocetinostat in combination with vinorelbine (NCT04299113).

Based on the difficulties extrapolating the mouse xenograft responses and PK data to humans, along with the conflicting results, and limited efficacy of HDAC inhibitors in pediatric phase 1 trials, the task force determined that more preclinical testing is needed to further investigate the effectiveness of entinostat in RMS before proceeding with a new prospective clinical trial for patients with RMS.

8. Novel Cytotoxic Agents: PLX038

PLX038 is earlier in development compared to many of the other agents discussed, but was highly rated amongst the group. PLX038 is a pegylated prodrug of anticancer agent SN-38, which is the active metabolite of irinotecan. Irinotecan is a topoisomerase I inhibitor that inhibits the repair of single strand DNA breaks and has demonstrated activity in RMS [13,89,90]. In comparison to irinotecan, PLX038 leads to the sustained release of SN-38, which may result in improved efficacy over conventional topoisomerase I inhibitors due to the accumulation of the drug within tumors [91]. This agent was evaluated in 32 xenograft models of pediatric cancers, including RMS [92]. PLX038 was highly active, with 78% of the xenografts experiencing more than a 50% reduction in tumor size after one dose [92]. Additionally, PLX038 showed equal or slightly improved responses in the same study when compared to irinotecan. In the clinical setting, PLX038 is under investigation in two ongoing clinical trials. The first is a phase 1 study of single agent PLX038 in adults with solid tumors (NCT04209595). The second is a phase 1 study at the NCI evaluating the combination of PLX038 and a PARP inhibitor rucaparib (NCT04209595), which recently enrolled the first patient [93].

PLX038 warrants further preclinical study in RMS and in combination with other agents such as vincristine and temozolomide, as these have demonstrated improved outcomes with limited overlapping toxicities in pediatric sarcomas when used in combination with irinotecan. Additionally, the results from the ongoing phase 1 studies in adults will be beneficial in identifying a dose for use in a future pediatric study.

9. Novel Cytotoxic Agents: Eribulin

Microtubule inhibitors such as vincristine are a mainstay in the treatment of RMS, but can cause peripheral neuropathy, which limits their use [94]. Other microtubule inhibitors include taxanes, which are microtubule stabilizing agents, but these agents have had limited success in pediatric malignancies [95,96]. Eribulin is a novel microtubule inhibitor which is a synthetic analogue of the natural product halichondrin B, derived from the marine sponge *Halichondria okadai* [97,98]. The mechanism of eribulin is unique in that it leads to apoptosis by inhibiting the polymerization of tubulin subunits, but it does not affect microtubule shortening [99].

Based on the widespread use of microtubule inhibitors in pediatrics, eribulin was studied in vitro and in vivo in the Pediatric Preclinical Testing Program [100]. Eribulin demonstrated activity in RMS and 6/7 RMS xenografts (5 FP-RMS and 1 FN-RMS) achieved a complete remission (CR) or maintained a CR in response to treatment [100]. Furthermore, there was increased activity of eribulin in the RMS xenograft models compared to vincristine. The combination of eribulin with irinotecan was synergistic in several FP-RMS xenografts, and more efficacious than the combination of vincristine and irinotecan in models with wild type TP53 [101].

In the clinical setting, eribulin is FDA-approved for adults with breast cancer and liposarcoma. In pediatric patients eribulin was evaluated in a phase 1 study of 23 pediatric patients with relapsed or refractory solid tumors [102]. While there were no patients with RMS enrolled on this study, there was one patient with Ewing sarcoma who experienced a partial response and three patients had stable disease [102]. The RP2D of eribulin was determined to be 1.4 mg/m^2, which is the same as the adult dose [102]. There is an ongoing phase 2 study evaluating eribulin which includes pediatric patients with relapsed or refractory RMS (NCT03441360). Eribulin was also studied in combination with irinotecan in a phase 1/2 trial of children with relapsed or refractory solid tumors [103]. The results

from the phase 1 portion of the study were recently reported [103]. There were 13 patients enrolled including four individuals with RMS. There were no dose limiting toxicities reported and at the time of data cut off there were four patients who continued to receive treatment with irinotecan and eribulin, including one individual with RMS [103]. The phase 2 portion of the study is ongoing (NCT03245450).

Eribulin is an agent of interest in RMS as it was effective in preclinical studies and there is an identified RP2D in pediatrics. However, microtubule inhibitors such as eribulin may overperform in mouse models as compared to humans. This may be due to their steep dose response curve suggesting that activity may drop significantly below the threshold concentration/exposure. This was seen in osteosarcoma whereby significant responses were seen in PDX models but none were observed in the phase 2 trial in patients with recurrent osteosarcoma [100,104,105]. Ultimately the recommendation of the task force was to await the results from the ongoing trials prior to designing a new clinical trial for RMS.

10. Other Targeted Agents

In addition to the top-rated agents reviewed above, other potential targets/agents of interest with promising early pre-clinical data are emerging. We discuss three such agents: fibroblast growth receptor 4 (FGFR4) targeted chimeric antigen receptor (CAR) T-cells, bromodomain and extraterminal domain (BET) protein inhibitors, and ephrin receptor inhibitors. While these agents are early in development, preclinical data for new promising agents are constantly being generated, and a brief summary of the available data for the three agents of interest is described below.

FGFR4 is a receptor tyrosine kinase that is expressed in FN-RMS and FP-RMS [44,106–108]. FGFR4 inhibition was effective in preclinical studies of FN-RMS and FP-RMS and FGFR4 is differentially expressed in RMS compared to mature skeletal muscle, therefore it may be a beneficial target for immunotherapy [109,110]. FGFR4 targeted chimeric antigen receptor (CAR) T-cells are currently in pre-clinical development and were effective against RMS in both in vitro and in vivo studies [111–113].

BET protein inhibitors are another promising class of agents for the treatment of FP-RMS. BET proteins are highly expressed in FP-RMS and the BET protein BRD4 is required for the PAX3-FOXO1 fusion protein to exhibit its oncogenic effects [114,115]. Preclinical studies demonstrated that BET inhibition disrupts the interaction between BRD4 and PAX3-FOXO1 leading to a reduced half-life of the fusion oncogene, and FP-RMS cell lines were susceptible to BET inhibition [114,115]. BET inhibitors also demonstrated antiangiogenic effects in FP-RMS xenograft models [116]. In adult clinical trials BET inhibitors have had limited efficacy and are not yet FDA-approved [117]. There is an ongoing trial evaluating the BET inhibitor BMS-986158 in children but results are not yet available (NCT03936465).

Erythropoietin-producing hepatocellular (Eph) proteins are a family of receptor tyrosine kinases that include Eph-A and Eph-B receptors that bind the ligands ephrin-A and ephrin-B, respectively [118]. Eph-A/ephrin-A signaling is important in myogenic differentiation and upregulation of Eph/ephrin proteins was identified in both FN and FP-RMS [119–121]. A preclinical study found that GLPG1790, a pan-Eph inhibitor, led to growth inhibition and promoted myogenic differentiation in FN-RMS when evaluated in vitro [120]. In contrast, inhibition of EphB4 signaling was not effective in FN-RMS, but demonstrated a decrease in tumor progression in a murine model of FP-RMS, suggesting that inhibition of EphB4 may be more effective when evaluated in combination with other agents [122]. Inhibitors of Eph/ephrin have been studied in clinical trials in adults with solid tumors, but these agents have not been studied in patients with RMS [118,123,124].

11. Discussion

There is a critical need for novel therapies in the treatment of RMS, particularly in patients with metastatic disease, as their outcomes remain poor. Disease-specific working groups within the COG have been useful in facilitating discussions to analyze and prioritize drugs using a multidisciplinary team of basic scientists, clinical investigators, and clinicians.

Our group utilized a modified framework to identify agents for consideration in the next COG clinical trial for patients with RMS. Unique to RMS was the consideration of agents in the context of disease subtypes. Oncogenic drivers and molecular aberrations are known to differ between FP-RMS and FN-RMS, and this is important to consider when evaluating the potential efficacy of targeted therapies for RMS. Novel treatments for RMS may require investigation within a clinical trial that is subtype-specific in order to advance outcomes in this disease.

Of the agents that were considered, the top candidates included novel cytotoxic agents and targeted therapies. The overarching conclusion of the RMS new agent task force was that despite a rigorous review of several promising agents, none of the agents are currently ready for clinical trial testing by COG as more data are needed prior to evaluation in a large phase 2 or 3 clinical trial for patients with RMS. While microtubule inhibitors such as eribulin and the combination of a PARP inhibitor and temozolomide both met the criteria of the proposed framework, the group's recommendation was to await the data from ongoing clinical trials with these agents prior to proceeding with a COG clinical trial incorporating either of these agents. Other agents had preclinical data in RMS but lacked an identified RP2D in pediatrics, such as PLX038. It was also determined that additional preclinical data were needed for HDAC inhibitors such as entinostat, and MEK inhibitors such as trametinib, particularly in terms of pharmacokinetic studies to determine whether the doses needed to attain preclinical activity will be clinically achievable.

The incorporation of new agents into RMS treatment has the potential to modify the current treatment landscape to provide improved outcomes for these patients. Novel cytotoxic agents such as eribulin and PLX038 may provide enhanced activity and ultimately replace the use of the similar cytotoxic agents vincristine and irinotecan that are an established part of chemotherapy regimens for RMS. On the other hand, targeted therapies may be most beneficial when administered with chemotherapy or in combination with other targeted agents. Suggestions for incorporating these agents in future clinical trials for RMS include the addition of targeted therapies to cytotoxic chemotherapy in patients with metastatic disease in order to improve cure rates. In patients with less advanced disease, novel agents may be evaluated either in addition to, or in place of standard chemotherapy agents in order to both improve outcomes and/or decrease toxicity. Agents which are earlier in clinical development should continue to undergo initial evaluation in studies for relapsed patients.

Although the task force concluded that additional data were needed before proceeding with a clinical trial, this review process was a useful way for experts in the field to convene and thoroughly evaluate the available preclinical and clinical data for each of the potential agents. Through these discussions, we were able to prioritize agents and identify areas where additional preclinical or early phase clinical studies were needed, or where results from ongoing trials would inform the design and/or dosing of a pediatric RMS trial. We believe that these ongoing discussions will lead to subsequent collaborative efforts between basic science researchers and clinical investigators so that deficiencies in the available data can be further investigated. With continued poor outcomes in advanced stage RMS, as well as the overall rarity of this disease, it is imperative to acquire the necessary background data before moving forward with an RMS-specific clinical trial or phase 2/3 studies.

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