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Insights into pediatric rhabdomyosarcoma research: challenges and goals

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Synopsis

Overall survival rates for pediatric patients with high-risk or relapsed rhabdomyosarcoma (RMS) have not improved significantly since the 1980s. Recent studies have identified a number of targetable vulnerabilities in RMS, but these discoveries have infrequently translated into clinical trials. We propose streamlining the process by which agents are selected for clinical evaluation in RMS. We believe that strong consideration should be given to the development of combination therapies that add biologically targeted agents to conventional cytotoxic drugs. One example of this type of combination is the addition of the WEE1 inhibitor, AZD1775, to the conventional cytotoxic chemotherapeutics, vincristine and irinotecan.

Keywords

rhabdomyosarcoma; early phase clinical trials; cancer biology; genomics

Introduction

Rhabdomyosarcoma (RMS) is the third most common extracranial solid tumor of childhood, accounting for 3 percent of childhood cancers and comprising approximately 350 cases in the US annually. RMS is also found in adult patients, which accounts for approximately 100 additional cases annually[1]. RMS tumor cells morphologically resemble cells arrested in the early stages of skeletal muscle development [2]. However, a large percentage of RMS tumors occur in locations normally lacking skeletal muscle such as the head and neck, genitourinary tract, and retroperitoneum [3]. Childhood RMS is subdivided into two major subtypes, *PAX*-fusion negative (previously called embryonal RMS) and *PAX*-fusion positive (previously called alveolar RMS), which have distinct histological features and genetic alterations. Spindle cell/sclerosing RMS has recently emerged as a third pediatric RMS subtype (Figure 1), while a fourth RMS subtype, pleiomorphic RMS, is seen exclusively in adults. Current treatment regimens use *PAX*-fusion status for risk assignment as the *PAX*-fusion positive group denotes a high-risk subtype with a less favorable prognosis than those who are *PAX*-fusion negative [4,5]. Spindle cell/sclerosing RMS also has a poor prognosis [6].

Treatment for RMS is multidisciplinary, including chemotherapy plus local control via surgical resection and/or radiation therapy. Relapse-free survival rates with this aggressive treatment regimen approach 90% for patients with low-risk disease and 70–80% for patients with localized disease, with significant treatment-associated morbidity [7]. However, the 5-year event-free survival rate for patients with metastatic disease at diagnosis continues to be less than 30% [8], and patients with relapsed disease have a similarly dismal prognosis [9]. Neither the survival rates nor the side effects of treatment for high-risk RMS have changed

appreciably in the last 30 years. Improvement in these survival rates is dependent upon identification of clinically effective agents that target RMS-specific vulnerabilities.

In this consensus manuscript generated from input from clinicians, scientists, patients, and advocacy groups, we summarize the recent progress that has been made in the understanding of RMS biology and advances in RMS treatment. The genetically-engineered *Drosophila*, zebrafish, or mouse models; human cell lines; or patient-derived xenografts available for basic or translational research are reveiwed (Table 1 and 2). Recent preclinical successes, such as the combination of the WEE1 inhibitor, AZD1775, with irinotecan and vincristine[10], will be discussed. We also highlight other drugs and drug combinations that are currently under preclinical study in RMS, including MEK inhibitors in combination with PI3 kinase/mTOR, IGF-1R or CDK4/6 inhibitors [11–13], HDAC inhibitors [14,15], DNA methyltransferase inhibitors [16], PARP inhibitors in combination with temozolomide [17], SMO inhibitors [18], asparaginase [19], and Aurora kinase inhibitors (J Shipley and B Schäfer, personal communication) (Table 2). In addition, we highlight biological and clinical questions that remain unanswered for RMS, as well as new questions that have been identified. We conclude with our recommendations to improve the efficiency of translation of scientific findings into clinical trials.

Critical Biological Problems

Significant progress has been made in the last decade in the understanding of the molecular basis for RMS development. These advances in knowledge are the result of several large-scale next generation sequencing studies of primary RMS tumors and extensive mechanistic studies facilitated in part by a variety of animal and xenograft models of RMS (Table 1). Through these genomic and mechanistic studies, RMS biologists have comprehensively characterized the landscape of mutations, copy number changes, genomic rearrangements, DNA methylation and histone modification changes, and defined a number of molecular mechanisms that drive RMS subtypes. The major conclusion of these studies is that there are two molecularly distinct subtypes of childhood RMS, defined by the presence (*PAX*-fusion positive RMS) or absence (*PAX*-fusion negative RMS) of a *PAX* gene rearrangement. Genomic characterization has further revealed several targetable vulnerabilities in these tumor subtypes. Due to this finding, the presence or absence of a *PAX*-fusion has been incorporated as part of the diagnostic criteria for RMS [4,5].

PAX-fusion positive RMS is associated with one of several balanced chromosomal translocations resulting in the creation of an aberrant transcription factor. In most cases, the N-terminal DNA binding domain of PAX3 is fused to the C-terminal transactivation domain of FOXO1. Less commonly, the DNA binding domain of PAX7 is fused to FOXO1, or rarely, PAX3 is fused to the nuclear receptor coactivator, NCOA1, or the chromatin remodeler, INO80D. The gene expression patterns of tumors driven by any of these aberrant transcription factors are similar, although the PAX3-FOXO1 fusion carries a worse prognosis than other fusions [4,20,21]. *PAX*-fusion positive RMS tumors have a low mutation rate, but commonly exhibit whole genome duplication and focal amplification of *MYCN* or *CDK4* [22]. PAX3-FOXO1 binds to and seeds the formation of super enhancers found at the loci for *MYCN* and the myogenic master transcription factors [23]. There are areas of DNA

hyper-and hypomethylation that can distinguish between the *PAX*-fusion positive and negative subtypes[24]. These recurrent epigenetic changes suggest that the use of epigenetic modulators might have therapeutic benefit for patients with *PAX*-fusion positive RMS.

PAX-fusion negative RMS tumors, in contrast, have a higher rate of single nucleotide variations. Recurrent mutations in known cancer genes such as *HRAS, NRAS, KRAS, ALK, FGFR4, PIK3CA, FBXW7, NF1, TP53, CTNNB1*, or *BCOR* are found in this subtype, yet some sequenced tumors do not have an identifiable driver mutation [22,25,26]. Importantly, we now appreciate that the majority of fusion negative RMS tumors are driven by RAS pathway activation. However, the prognostic implications of the mutations in these known cancer genes have yet to be defined. Loss of imprinting at chromosome 11p15.5, leading to paternal isodisomy and resulting overexpression of *IGF2* is nearly universal among fusion negative RMS tumors. These tumors also have complex karyotypes owing to chromosome and chromosome-arm level gains and losses [22]. In addition to loss of function *TP53* mutations, *PAX*-fusion negative tumors have focal amplification of the TP53 negative regulator, *MDM2* [27], such that altered TP53-dependent transcription is a common feature of these tumors. The DNA methylation pattern in *PAX*-fusion negative tumors is similar to that of normal tissues [24], but super enhancers are observed in these tumors at *MYC*, negative regulators of MAP kinase signaling, and the myogenic master transcription factors [13]. While *PAX*-fusion negative RMS is associated with several familial cancer predisposition syndromes, including Li-Fraumeni syndrome and the RASopathies, particularly neurofibromatosis type 1 and Costello syndrome, most children diagnosed with *PAX*-fusion negative RMS do not have a family history of cancer[28]. A better understanding of the risk factors for RMS development in these patients is needed.

Sclerosing/spindle cell RMS has recently been characterized as a separate RMS subtype. This aggressive sarcoma is frequently driven by mutations in the myogenic master transcription factor, *MYOD1* [29]. Mutations in the RAS or PI3 kinase pathways frequently co-occur with the *MYOD1* mutations [6]. In addition, a majority of sclerosing/spindle cell RMS tumors also harbor *VGLL2*-related fusions, with a subset harboring *NCOA2* rearrangements [30]. Epigenetic and transcriptomic characterization, functional studies, and animal model development is needed to better understand the biology of this RMS subtype.

Ongoing biological questions

Despite the progress made with genomic characterization of *PAX*-fusion positive and *PAX*-fusion negative RMS, many important biological questions remain including:

1. *PAX*-fusion positive RMS tumors show a higher propensity to metastasize compared to *PAX*-fusion negative tumors, and loss of TP53 increases the invasive potential of *PAX*-fusion negative RMS tumors in a zebrafish model [31], but what are the mechanisms that govern invasion and metastasis in *PAX*-fusion positive and negative RMS?
2. The YAP/TAZ [32,33], RAS/RAF/MEK/ERK [13,34], PI3 kinase/mTOR [35], MYOD/MYF5 [36,37], Notch [38–40], WNT [34,41], Hedgehog [42], and EZH2 [43] pathways have

been implicated in *PAX*-fusion negative RMS as blocking muscle differentiation; can this knowledge be leveraged diagnostically or therapeutically?

3. Animal modeling studies have shown that *PAX*-fusion negative RMS can be initiated from myogenic and non-myogenic (endothelial) precursors, while differentiating fetal myoblasts are most poised to develop *PAX*-fusion positive RMS [14,44–51]. Since the RMS cell of origin influences not only histological identity but also site of disease and response to therapy [14], what are the RMS cell(s) of origin in human disease?
4. What is the role of immune and other cells in the tumor microenvironment in driving RMS progression, metastasis, and therapy resistance?
5. What are the risk factors and germline mutations associated with an increased risk of RMS development?
6. What are the most predictive preclinical models for RMS and can these be exploited for rapid and better prioritization of pre-clinical therapy testing?
7. How can we best leverage new technologies, such as CRISPR-Cas9 screening of protein domains [52], to identify new drug targets for RMS?
8. What are the mechanisms by which *MYOD1*^{L122R} drives spindle cell/sclerosing RMS tumorigenesis?

Critical Clinical Problems

Despite the surge in our understanding of the molecular mechanisms underlying RMS in recent years, the clinical translation of such discoveries has lagged behind. Since 2014, there have been only two interventional trials opened specifically for patients with RMS: one for the upfront treatment of a subgroup of newly diagnosed patients (NCT02567435) and one for the treatment of patients with relapsed or refractory disease (NCT03041701). The problem with the lack of newly opened trials is two-fold; questions about which new scientific findings may have clinical benefit or applicability are left unanswered, and patients have limited access to experimental treatment options, which they are in dire need of after standard therapies have been exhausted.

Notably, the rationale for each of the two aforementioned trials is based on substantial preclinical data implicating the importance of the relevant pathways in RMS. The upfront trial is a Children's Oncology Group (COG) study for patients with newly diagnosed intermediate risk RMS comparing vincristine, actinomycin D and cyclophosphamide alternating with vincristine and irinotecan (VAC/VI) with VAC/VI plus temsirolimus for this subgroup (NCT02567435). This study was initiated following the outcome of a prior COG study showing superiority of a temsirolimus containing regimen for RMS patients at relapse [53], as well as abundant preclinical data showing the importance of the mTOR pathway for RMS survival and growth [54,55]. The trial for patients with relapsed or refractory RMS is a phase I/II study investigating the safety and efficacy of the combination of the IGF-1R monoclonal antibody, ganitumab with the SRC family kinase inhibitor, dasatinib (NCT03041701). Earlier preclinical work described the efficacy of small molecule and

antibody-based inhbitors of IGF-1R in RMS [56], and previous early phase clinical studies demonstrated that IGF-1R antibodies yielded meaningful but short-lived responses in patients with relapsed RMS [57]. However, the addition of an IGF-1R antibody to upfront intensive multiagent chemotherapy did not improve outcomes for unselected patients with metastatic disease [58]. The current trial is based on further preclinical work showing that inhibition of IGF-1R activates a SRC family kinase bypass resistance pathway. Cotargeting IGF-1R with a monoclonal antibody such as ganitumab and SRC family kinases with dasatinib provided therapeutic enhancement in animal models [59], which supported clinical translation of this combination.

Despite the lack of RMS-specific clinical trials that have been initiated in recent years, several early phase clinical trials have been initiated for patients with solid tumors or sarcomas that include patients with RMS among the eligible participants. These include studies of new cytotoxic agents or new cytotoxic combinations; targeted agents; immunotherapeutic agents or modalities and allogeneic cellular transplants; or new applications of local control methods such as hyperthermic intraperitoneal chemotherapy[60]. Additional details for these trials can be found in Table 3. For several of these trials, promising preclinical data exist to support pursuit of these therapeutic targets and agents in RMS [11,14,34,35,54,61–65]. However, for many, minimal or no published preclinical data exist, and there is a limitation with currently available RMS models to adequately evaluate some of these therapies (*e.g.* the need to evaluate immunotherapeutics in immune competent animal models). In addition, since these types of clinical studies typically enroll a small number of patients with each tumor subtype, they rarely provide sufficient information about activity in a given tumor type. Furthermore, patients treated on these smaller early phase studies typically are heavily pre-treated with a high burden of disease, which may make interpretation of outcomes difficult.

Ongoing clinical questions

Given that there have been so few RMS-specific clinical trials despite the advances that have been made in understanding the biology of this disease, a number of important questions remain regarding how best to move agents from the bench to the bedside and design informative trials. These include:

1. What is the threshold for preclinical data that is sufficient to initiate a clinical trial?
2. Which new drugs/pathways should be prioritized?
3. How should we address the disease-free period that high-risk patients experience between end of therapy and relapse? Should we be giving maintenance therapy [66], and if so, with what?
4. How can we better provide local control in sites such as the abdomen and pelvis where high local failure rates continue?
5. Since RMS has a relatively low mutational burden and is unlikely to be immunogenic, how can we best leverage immunotherapeutic treatment options

against RMS? Can we improve our modeling of these agents with development of humanized animal models?

6. How can we best design rational combinations of targeted agents, conventional chemotherapeutics, and/or immunotherapeutics?

7. How can we better engage the adult sarcoma centers to participate in RMS-specific trials?

8. Can we specifically target the clinically aggressive, *MYOD1*-mutant, spindle cell/sclerosing RMS subtype [67]?

Future Directions and Consensus Goals

The primary concerns of both investigators and patient advocates centers on eliminating the deleterious side effects of the available treatment options and optimizing translation of preclinical findings into clinical trials. To best address this, the criteria by which drugs are selected for inclusion in clinical trials must be standardized. In addition, clinical trials must be designed such that our ability to build upon our knowledge of RMS biology to inform future trials is maximized. Finally, improved access to information about clinical trials should be provided to patients with known poor prognosis and for patients who are expected to achieve remission with severe long-term sequelae. Specific recommendations to achieve these goals are outlined below.

Initiate RMS-specific clinical trials based upon robust preclinical work

Our consensus opinion, based on the currently available preclinical data, is that the combination of the WEE1 inhibitor, AZD1775, with the chemotherapeutic agents vincristine and irinotecan should be prioritized for evaluation in a clinical trial for patients with RMS. WEE1 is a tyrosine kinase that is activated in response to DNA damage. WEE1 phosphorylates and inactivates CDK1, which halts progression through the G2/M checkpoint and allows for DNA repair prior to initiation of mitosis. WEE1 inhibition in the setting of chemotherapy-induced DNA damage leads to mitotic catastrophe. AZD1775 has been studied preclinically in RMS [64] as well as in a COG Phase I trial in combination with irinotecan (NCT02095132), but the number of patients with RMS enrolled on that study was small, and the results have not yet been reported.

We as a community feel comfortable proposing AZD1775 in combination with irinotecan and vincristine as the next clinical trial for patients with initially metastatic or relapsed/refractory RMS. However, we encourage investigators to establish pharmacodynamic markers, such as assays for DNA damage, in animal models of RMS treated with this combination in preparation for potential use as early response markers for patients receiving AZD1775/vincristine/irinotecan on study. As outlined above, additional therapies that warrant further preclinical testing include bromodomain inhibitors [23] and HDAC inhibitors in *PAX*-fusion positive RMS and MEK inhibitors in RAS-driven *PAX*-fusion negative RMS. We would encourage investigators that are engaged in preclinical research to be mindful of the criteria needed to support the clinical translation of novel drugs (see Table 4) and to design experiments that attempt to address these questions. Finally, we encourage

investigators to make use of novel clinical trial designs, such as basket trials, so that the clinical efficacy of new drugs and combinations can be assessed rapidly with a minimum number of enrolled patients.

Maximize information learned from each patient diagnosed with RMS

We recognize that our best resource for understanding RMS biology and for developing therapies that improve survival while minimizing side effects of treatment are the patients with RMS themselves. Since RMS is a rare disease, each patient who is diagnosed with RMS in North America be offered enrollment on the COG study, Project:EveryChild (NCT02402244). This project aims to create both a database of clinical data as well as a biorepository of disease-specific specimens. In addition, for patients with relapsed RMS who have somatic mutational analysis indicating an actionable finding, clinicians should offer enrollment on a clinical trial, such as the Pediatric MATCH in North America (NCT03155620) or ESMART (NCT02813135) in Europe. In this way, RMS-specific responses to these agents can be prospectively evaluated. Furthermore, efforts should be made to incorporate on-treatment tumor and liquid biopsies into treatment trials for newly diagnosed and relapsed patients. Tumor tissue and circulating tumor cells can be used to evaluate target-specific pharmacodynamic markers, while circulating tumor DNA and exosomes can be used as early markers of response to therapy [68]. These studies are essential for determining that the intended target is engaged by the drug administered, as well as for improving our understanding of intrinsic and acquired resistance to therapies. This knowledge will, in turn, inform future clinical trials. As well, establishing liquid biopsies and newer nuclear medicine imaging techniques such as FLT-PET as early markers of disease response will facilitate completion of trials in a timely manner, such that follow-up trials that build upon knowledge gained from our current trials can begin.

Establish international, multi-disciplinary research teams to facilitate discovery

Several of the critical biological problems described above are currently being investigated by more than one of the members of the RMS community. For example, many investigators are interested in targeting the myogenic transcription factor, *MYOD1*, while several investigators are interested in targeting oncogenic RAS in RMS. We suggest that investigators continue to assemble into collaborative groups aimed at efficiently translating understanding of these sub-topics of RMS biology into clinical trials and encourage these groups to collectively pursue funding opportunities to support this type of research. To facilitate data sharing, the COG is working to establish a centralized database to provide all investigators access to the genomic and clinical outcome data that has already been generated.

In conclusion, we predict that the research described above, conducted by our collaborative community of investigators, has the potential to produce additional RMS-specific clinical trials in the near future. In the next phase of RMS research, we aim to improve upon those trials with the ultimate goal of understanding RMS biology and identifying treatments for RMS that provide meaningful clinical benefit and minimize toxicity.

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Abbreviations:

COG	Children's Oncology Group
HDAC	histone deacetylase
PARP	poly (ADP-ribose) polymerase
RMS	rhabdomyosarcoma
VAC	vincristine, actinomycin D, cyclophosphamide
VI	vincristine, irinotecan

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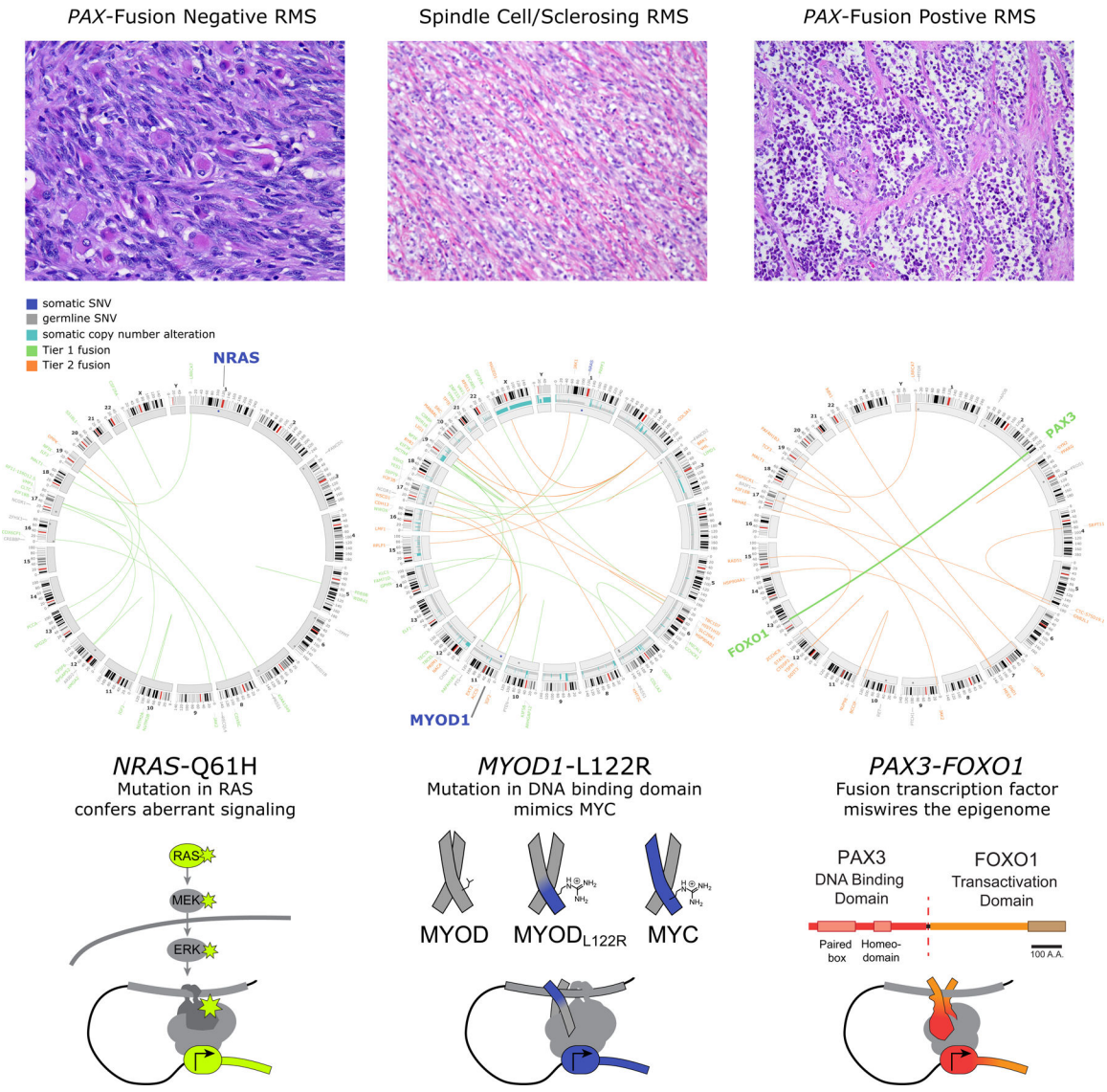

Figure 1.

Histopathology and Genomics of the RMS subtypes

Representative H&E staining (top), Circos plots (middle), and schematics of the genomic drivers (bottom) of each of the three RMS subtypes, *PAX*-fusion negative RMS, spindle cell/sclerosing RMS and *PAX*-fusion positive RMS. Histology courtesy of C.R. Antonescu. Circos plots adapted from [22,25]. In these plots, chromosomes 1–22 as well as the sex chromosomes are arrayed in a circle and arranged clockwise. The length of the chromosome in Mb is depicted outside each representation. Mutations for each tumor are indicated outside the chromosome number. Somatic single nucleotide variants (SNVs, blue), germline SNVs (gray), Tier 1 chromosomal translocations (translocations with strong clinical significance, green) and Tier 2 chromosomal translocations (translocations with potential clinical significance, orange) are shown. The outermost track of the Circos plot is a representation of the cytogenetic banding pattern of each chromosome, with the centromere colored red. For the spindle cell/sclerosing RMS tumor, somatic copy number alterations

(aqua) are shown in the center track. In the innermost track, dots represent a somatic (blue) or germline (gray) SNV. The position of the dot within the track is representative of the variant allele frequency (VAF) for that SNV: SNVs with a higher VAF are positioned closer to the center of the Circos plot. In the center of the plot, lines link genes that are partners in Tier 1 (green) or Tier 2 (orange) translocations. In this figure, driver mutations for each RMS subtype are bolded (*NRAS* mutation, *MYOD1* mutation and *PAX3-FOXO1* translocation) are highlighted.

Table 1:

Available animal models for pediatric RMS. Models highlighted here were either discussed at the Summer's Way RMS workshop or identified through a comprehensive review of the literature.

***PAX*-fusion negative:**			
Organism	**Type**	**Model**	**Reference**
Mouse	Genetically engineered	HGF/SF;Ink4a/Arf$^{-/-}$ M-Cre-Trp53$^{-/-}$ M-Cre-Trp53$^{-/-}$;Ptch1$^{+/-}$ Myf5-Cre-Trp53$^{-/-}$;Ptch1$^{+/-}$ Pax7-CreER-Trp53$^{-/-}$;Ptch1$^{+/-}$ Pax7$^{CE/+}$, LSL-Kras$^{G12D/+}$;Trp53$^{Fl/Fl}$ aP2-Cre;Smo^{M2} aP2-Cre;Smo^{M2};Cdkn2a$^{Fl/Fl}$	70 44,48,71 45,47
	Syngeneic	Myoblast Trp53$^{-/-}$;KRAS^{G12D} Myoblast Trp53 $^{-/-}$;FGFR4^{V550E}	35,72
	Xenograft	SkMC/HSMM + T/t-Ag + hTERT + HRAS^{G12V} Human cell lines Patient-derived xenografts	10,50,73
Zebrafish	Genetically engineered	rag2-KRAS^{G12D} cdh15-KRAS^{G12D} mylz2-KRAS^{G12D}	74–76
	Xenograft	Human cell lines Patient-derived xenografts	
***PAX*-fusion positive:**			
Organism	Type	Model	Reference
Mouse	Genetically engineered	M-Cre-Pax3-Foxo1;Trp53$^{-/-}$ Myf6-Cre-Pax3-Foxo1;Trp53$^{-/-}$ Stk3$^{F/F}$;Stk4$^{F/F}$;Pax3$^{PF/PF}$;Cdkn2a$^{F/F}$;Myf6$^{ICN/+}$	49,77–79
	Xenograft	Dbt myoblast + Pax3-Foxo1 + MYCN HSMM + PAX3-FOXO1 + hTERT + MYCN Human cell lines Patient-derived xenografts	79 51 10,73
Zebrafish	Genetically engineered	CMV-GFP2A-PAX3FOXO1;tp53$^{M214K/M214K}$	74,80
	Xenograft	Human cell lines Patient-derived xenografts	
Drosophila	Genetically engineered	Mhc-Gal4;UAS-Pax7-Foxo1	81

Table 2:

Preclinical targets in pediatric RMS

Target	Potential Agent
Asparagine metabolism	PEG-asparaginase
Aurora kinases	alisertib
BRD4 inhibitor	OTX015
CDK4/6	palbociclib
DNA methyltransferases	5-azacytidine
Histone deacetylases	entinostat
IGF-1R	ganitumab
MEK 1/2	trametinib
NOTCH	RO4929097
PARP	olaparib
PI3 kinase/ mTOR	buparlisib
SMO	vismodegib
VANGL	N/A
WEE1	AZD1775

Table 3:

Early Phase Clinical Trials for which Patients with RMS are Eligible

Agent	Class/ Molecular Target	NCT Number	Date of Initiation
eribulin	Cytotoxic	NCT03441360 NCT03245450	2/21/18 8/10/17
Nab-paclitaxel	Cytotoxic	NCT02945800 NCT03507491	10/26/16 4/25/18
PEN-866	Cytotoxic	NCT03221400	7/8/17
afatinib	Targeted - ErbB	NCT02372006	2/26/15
cabozantinib	Targeted/ multi-kinase	NCT02867592	8/16/16
erlotinib	Targeted - EGFR	NCT02689336	2/23/16
copanlisib	Targeted - PI3 kinase	NCT03458728	3/8/18
lenvatinib	Targeted - multi-kinase	NCT03245151	8/10/17
entinostat	Targeted - HDAC	NCT02780804	5/24/18
Pediatric MATCH	Targeted – multiple	NCT03155620	5/16/17
HER2 CAR T cell	Immunotherapy - HER2	NCT00902044	5/14/09
nivolumab/ipilimumab	Immunotherapy	NCT02304458	12/2/14
enoblituzumab	Immunotherapy	NCT02982941	12/6/16
Allogeneic HSCT	Transplant	NCT02890758 NCT02508038	9/7/16 3/24/15
High intensity focus ultrasound	Local control	NCT02557854 NCT02536183	9/23/15 8/31/15
Stereotactic body radiation therapy	Local control	NCT02581384	10/21/15
Hyperfractionated radiation therapy	Local control	NCT03651375	8/29/18

Table 4:

Criteria for Prioritization of Drugs for Translation to Clinical Trials in Pediatric RMS. We propose that at least 6 of these criteria be met for an agent to be prioritized for clinical translation, and meeting criteria 4 and 5 should be required.

1.	Is there a biomarker identified in human subjects that predicts sensitivity to targeting the pathway (i.e. SNV, amplification, deletion, etc)?
2.	Does the tumor depend on this target *in vitro*?
3.	Does the tumor depend on this target *in vivo*?
4.	Is the proposed drug efficacious *in vitro* (5 – 8 independent cell lines, if available)?
5.	Is the proposed drug efficacious *in vivo* (at least 3 independent models, including genetically engineered, cell line xenograft and patient derived xenograft models)?
6.	Does the presence of the biomarker from question 1 predict response to the proposed drug *in vitro* and *in vivo*?
7.	Are the concentrations of proposed drug required for efficacy *in vitro* achieved *in vivo*? Achievable in patients?
8.	Are resistance mechanisms to the proposed drug known?
9.	Are there drugs with which the proposed drug synergizes *in vitro* and *in vivo*?
10.	Is the proposed drug formulated in such a way that it can be delivered to pediatric patients?