Extremity Rhabdomyosarcoma—An Integrated Clinicopathologic and Genomic Study to Improve Risk Stratification

Henry de Traux de Wardin, MD[1]; Bin Xu, MD, PhD[1]; Josephine K. Dermawan, MD, PhD[1]; Mariel H. Smith, MD[2]; Suzanne L. Wolden, MD[3]; Cristina R. Antonescu, MD[1]; and Leonard H. Wexler, MD[4]

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ABSTRACT

PURPOSE Extremity rhabdomyosarcoma (RMS) is associated with a very poor outcome compared with other sites, mainly because of its high incidence of alveolar histology and regional lymph node involvement. To better define prognostic markers in this clinical subset, we investigated our experience of 61 patients with extremity RMS treated at our tertiary cancer center for the past 2 decades.

PATIENTS AND METHODS The patients had a median age of 8 years at diagnosis, equal gender distribution, and two-thirds occurred in the lower extremity. Most (85%) patients had *FOXO1* fusion–positive alveolar RMS (ARMS), with 70% having a *PAX3::FOXO1* transcript. Remaining were seven patients with fusion-negative embryonal RMS (ERMS) and two with *MYOD1*-mutant spindle cell/sclerosing RMS (SRMS). In 40% of the patients, material was available for DNA-based targeted sequencing using MSK-IMPACT cancer gene panel.

RESULTS One-third of patients presented with localized disease at diagnosis while the remaining had regional nodal (18%) or distant metastases (51%). Metastatic disease, high-risk group, and age 10 years or older significantly affected the overall survival (OS; hazard ratio [HR], 2.68 [$P = .004$], 2.78 [$P = .010$] and 2.26 [$P = .034$], respectively). Although the presence of metastatic disease had a dismal impact on 5-year EFS and OS (19% and 29%, respectively), nodal involvement had a comparatively lower impact on 5-year EFS and 5-year OS (43% and 66%, respectively). *PAX3::FOXO1* ARMS had worse prognosis and afflicted older children compared with *PAX7::FOXO1* (HR = 3.45, $P = .016$). The most common events in the ARMS group included *MED12* alterations, *CDK4* amplifications, and *CDKN2A* deletions (8%-17%). The latter two abnormalities were mutually exclusive, enriched for acral and high-risk lesions, and correlated with poor outcome on OS ($P = .02$).

CONCLUSION Our data provide rationale for considering the integration of molecular abnormalities to refine risk stratification in extremity RMS.

ACCOMPANYING CONTENT

Appendix

Data Supplement

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INTRODUCTION

Rhabdomyosarcoma (RMS) is the most frequent pediatric soft-tissue sarcoma with a median age at diagnosis of 5 years. In the past decade, the fine-tuning of multimodal chemotherapy regimens has greatly improved the relapse-free survival for patients with localized diseases.[1-3] Currently, 70% of the patients survive with no relapse, however, often developing one or more clinically significant treatment-related toxicities.[4]

RMS of the extremities account for 15% of cases, occur more often in older children, and have an overall poor outcome.[5,6] The known prognostic factors for RMS, such as older age and alveolar subtype, also apply to extremity RMS. Moreover, nodal involvement has a significant impact on overall survival (OS), being detected in 33%-43% of patients with extremity RMS and being associated with poor survival rates (9%-32%).[7,8] Recent publications have highlighted the importance of accurate staging for nodal involvement and adjusted radiotherapy regimens.[1,9] However, the role of molecular markers in outcome prediction for this clinical subset has yet to be investigated. In this study, we investigate a large cohort of patients with extremity RMS treated at our tertiary cancer center for the past 20 years aiming to better define clinical, pathologic, and genomic factors that correlate with outcome.

CONTEXT

Key Objective

To better define clinical and genomic prognostic markers of extremity rhabdomyosarcoma (RMS) using the experience gathered from a single tertiary center during the past two decades.

Knowledge Generated

The adverse outcome was related to the higher incidence of alveolar subtype and regional lymph node involvement in the extremity RMS. Although the presence of metastatic disease had a dismal impact on 5-year overall survival, nodal involvement had a comparatively lower survival impact. In addition, we found that *CDK4* amplifications and *CDKN2A* deletions correlate with survival while *PAX3::FOXO1*–positive alveolar RMS had a worse prognosis and afflicted older children compared with *PAX7::FOXO1*.

Relevance

Our data provide rationale for integrating molecular alterations to refine risk stratification, including *CDK4* amplifications and *CDKN2A* deletions.

PATIENTS AND METHODS

Patient Selection

The Department of Pediatrics files were searched for patients with extremity rhabdomyosarcoma (RMS) treated at our institution between January 2000 and December 2021. The study was approved by the IRB committee, and signed patient informed consent was obtained. A total of 61 patients were identified in which the pathologic diagnosis was confirmed, and follow-up information was available (Appendix Table A1, Data Supplement).

Diagnosis and Staging

Primary histology was confirmed either by pathologic assessment of the core biopsy or open surgical excision. Molecular analysis in alveolar RMS (ARMS) was performed by either Archer FusionPlex[10] or fluorescence in situ hybridization using custom bacterial artificial chromosomes probes flanking *FOXO1*, *PAX3*, and *PAX7* genes, as previously described.[11,12] MSK-IMPACT, a targeted DNA-based sequencing panel (410-505 genes),[13] was used in 40% of the patients with available material to assess mutational landscape and copy number alterations. Risk group assignment for the purpose of this study was based on current guidelines, although clinical decision making in real time was made on the basis of then-contemporaneous risk-stratification criteria[14,15] (Appendix Fig A1, Data Supplement).

Therapeutic Modalities

The initial treatment included multidrug chemotherapy regimens and radiotherapy (see Appendix).

Chemotherapy

All patients received chemotherapy according to their risk group and either on or as-per MSKCC institutional pilot trial IRB 03-099 or comparable Children's Oncology Group (COG) clinical trials includingD9602, D9802, D9803, ARST0331, ARST0431, and ARST0531 (Appendix Fig A1). Chemotherapy regimens contained at least one alkylating agent (generally cyclophosphamide) and a minimum of two other drugs. Most high-risk patients received maintenance chemotherapy with either six cycles of single-agent irinotecan (on IRB 03-099) or up to 24 months of daily oral cyclophosphamide in combination with vinorelbine and bevacizumab or temsirolimus. Postrelapse therapy was individualized on the basis of previous therapy and tolerance.

Radiotherapy

Radiation therapy was recommended for all patients with ARMS, including patients who achieved up-front total gross surgical resection. Patients with embryonal RMS (ERMS) and sclerosing RMS (SRMS) starting chemotherapy before gross total resection or with R1 or R2 resection received radiation as well (see Appendix).

Surgery

For every patient with ERMS, an individualized determination was carried to assess whether more aggressive surgery either up-front or after chemotherapy could result in a meaningful reduction in the intensity or duration of chemotherapy or in the dose of postoperative radiation while still preserving function. For patients with ARMS, up-front surgery was never recommended (see Appendix).

Follow-Up Assessment

After treatment completion, all patients were assessed clinically and with relevant imaging to monitor for local, regional, and/or metastatic recurrence.

Statistical Analysis

Data from all patients were retrospectively evaluated. Statistical calculations were performed using SAS statistical package (release 9.4, SAS Institute Inc, Cary, NC), see Appendix.

RESULTS

Patient Population and Staging

A total of 61 patients were identified, with a median age of 8 years (range, 8 months-27 years, mean 10 years) and a 1:1 male-to-female ratio. Clinical and tumor characteristics are summarized in Table 1. In brief, 42 (69%) tumors were located in the lower extremity (foot, n = 10; calf, n = 15; thigh, n = 12; buttock, n = 5) and 19 in the upper extremity (hand, n = 11; forearm, n = 8). Tumor size was > 5 cm in 37 (60%) cases. Histologic type included 52 (85%) ARMS, seven ERMS, and two SRMS. A *FOXO1* gene rearrangement and its partner were confirmed in all patients with ARMS, including 36 *PAX3::FOXO1* and 16 *PAX7::FOXO1* cases. The risk groups were evenly distributed in our cohort, with half of the patients having high-risk disease (30 of 61) while the other half had low or intermediate risk (31 of 61). Within the ARMS subgroup, *PAX3::FOXO1* were more likely to be high risk (24 of 36, 67%) compared with *PAX7::FOXO1* counterpart (4 of 16, 25%).

Regarding stage at presentation, 20 patients had localized disease (N0, M0), 11 patients had regional nodal involvement (N1, M0), and 31 patients had metastatic disease (N0/1, M1). As most patients in this specific cohort had ARMS disease, risk stratification was predominantly dependent on the presence or absence of distant metastatic spread. Metastatic sites included bone marrow (17 of 31), bone (16 of 31) lung (3 of 31), in-transit (1 of 31), breast (2 of 31), distant nodes only (3 of 31), and other thoracoabdominal organs (7 of 31). Bone metastasis was most frequently found in extremity, iliac, vertebrae, skull base, and orbit. Nodal involvement alone was found in 27% (8 of 30) of high-risk patients. One patient with ERMS (<1 year) considered at his local institution to have metastatic disease (suspicious lung nodule) was subsequently determined to be eligible for enrollment on the then-current COG intermediate risk trial, ARST-1431. Local control was achieved by radical limb-preservation surgery without radiation therapy, and no whole-lung radiation was delivered. Disease relapses in the intermediate-risk group occurred in 45% (13 of 29) of patients and were more likely to be regional (46%—6 of 13) than metastatic (38%—5 of 13). Two patients experienced local relapse. One of them did not receive primary local control treatment because of parental refusal.

Lymphoscintigraphy-guided sentinel node mapping and sampling was done for 72% of patients (44 of 61). Cases that did not benefit from it (n = 17) were either largely metastatic (n = 10), had N1 status confirmed by imaging or clinical examination (n = 4), or because of practician preference (n = 3). Four patients experienced in-transit relapse after negative lymph node sampling. One patient had eight inguinal lymph nodes sampled, all negative for tumor; however, imaging showed iliac and pelvic adenopathy. Two patients with negative baseline ^{18}F-labeled fluorodeoxyglucose positron emission tomography/computed tomography (^{18}F-FDG-PET/CT) were found to have nodal involvement on sentinel node sampling. Microscopically, in one patient, the lymph node showed a 1.2 mm focus of tumor while in the other, scattered tumor cells were observed highlighted only by desmin and myogenin immunostaining.

Treatment

All patients received chemotherapy. 36 patients were treated as per MSK 03-099 protocol;[16] 15 as per ARST-0331, -0431,-0531[17-19]; 5 as per COG-D9803;[20] and five were treated by modified NCI PB 93-C-125.[21]

Radiotherapy was given to all patients at primary and metastatic site, except for eight patients, including three ERMS with satisfactory local control achieved by surgery alone, one ARMS for which radiotherapy was deemed unnecessary at the outside institution (tumor <5 cm, R0 surgery), two ARMS because of parental refusal or physician decision, and two SRMS, one for whom RT was deemed unnecessary after up-front total resection and another who progressed through therapy. Additional information is provided in the Appendix.

Targeted DNA-Based Sequencing

Twenty-four (40%) patients from our cohort had their tumor sequenced using MSK-IMPACT. A total of 50 genes had alterations of interest and were plotted as having a potential oncogenic role (Appendix Fig A2) and are available at cBioPortal.[22] Among these, 16 genes were deemed the most relevant on the basis of known pathogenic impact or their recurrent incidence (Fig 1). ARMS harbored gene amplifications or deletions more frequently compared with the higher incidence of single-nucleotide variations (SNVs) detected in ERMS (Appendix Table A2). ARMS harbored an average of 3.5 gene alterations per tumor (range, 1-9) while ERMS harbored an average of 3.7 gene alterations per tumor (range, 1-8). The most common molecular abnormalities in ARMS were *MED12* alterations, *CDK4* amplifications, and *CDKN2A* deletions (8%-17%). The latter two abnormalities occurred in 28% of ARMS, being mutually exclusive, correlating with acral sites, and detected mostly in high-risk

TABLE 1. Patient and Tumor Characteristics and Correlation With OS

Variable	No. (%)	Univariate Analysis HR (OS)	*P*
Total	61 (100)		
Sex			
Male	30 (49)		
Female	31 (51)		
Age at diagnosis, years			**.034**
<10	35 (57)	1	
≥10	26 (43)	2.26	
Primary tumor site			.095
Upper extremity	19 (31)	1	
Lower extremity	42 (69)	0.52	
Primary tumor site			
Foot	10 (16)		
Calf	15 (25)		
Thigh	12 (20)		
Gluteus	5 (8)		
Forearm	8 (13)		
Hand	11 (18)		
Tumor size, cm			.127
<5	20 (33)	1	
≥5	37 (61)	0.54	
Unknown	4 (9)		
Histology			
Alveolar (fusion-positive)	52 (85)	1	
Embryonal (fusion-negative)	7 (11)	0.35	.388
Spindle/sclerosing	2 (4)	0.59	.602
Lymph node involvement			**.008**
N0	25 (41)	1	
N1	35 (57)	3.02	
NX	1 (2)		
Metastatic status			**.001**
M0	30 (49)	1	
M1	31 (51)	3.62	
Risk group			**.010**
Low	3 (5)		
Intermediate	28 (46)	1	
High	30 (49)	2,78	
Fusion type			**.016**
PAX7::FOXO1	16 (31)	1	
PAX3::FOXO1	36 (69)	3.45	
Radiation to primary site			
No	8 (13)		
Yes	53 (87)		
Relapse			
Yes	27 (44)		
No	34 (56)		
Type of first relapse/progression			
Local	2 (7)		
Locoregional	8 (30)		
Metastatic	17 (63)		

NOTE. Bold font indicate statistically significant factors.
Abbreviations: HR, hazard ratio; OS, overall survival; M, metastasis; N, node.

patients (5 of 6). *CDK4* was the most prevalent amplified gene within the 12q13-15 locus, which also involved *MDM2* and *GLI1* coamplifications in a smaller subset of cases. Three patients with ARMS revealed *MED12* gene alterations including two missense mutations and one deletion. In fact, *MED12* mutations [c.4523A>T (p.H1508L), c.5420G>T (p.G1807V)] were the only recurrent SNVs detected in the ARMS cohort. *FOXO1* amplification was detected in one case each of *PAX3* and *PAX7* fusion–positive ARMS.

Interestingly, among the three patients with ERMS with IMPACT results, the putative drivers were all different: one case showed *HRAS* hot spot Q61L mutation (co-occurring with a *MED12* nonsense mutation [c.4399C>T (p.R1467*)]); the second case showed deletions in *TP53*, *RB*, and *FGFR4*; while the third case showed only an *NF1* frameshift deletion. The two SRMS cases showed the hot spot *MYOD1 L122R* exon 1 mutation and in one case co-occurring with a PIK3CA insdel.

Statistical Analysis

Clinical Data

The median follow-up time for the entire cohort was 49 (range, 12-273) months. Univariate analyses of survival variables revealed that metastatic disease at diagnosis was the most impactful factor on EFS (hazard ratio [HR] = 2.7, P = .004) and OS (HR = 3.6, P = .001; Table 1). This was confirmed to be significant on OS by multivariate analysis (HR = 2.8, P = .01). Nodal involvement was found to be impactful on EFS (HR = 1.9, P = .06) and OS (HR = 3.0, P = .008) on univariate survival analysis. Risk group affected EFS (HR = 2.6, P = .005) and OS (HR = 2.8, P = .001) on univariate analysis and was confirmed by multivariate analysis on OS (HR = 2.7, P = .02; Fig 2A). Age older than 10 years was only significant for OS calculations (HR = 2.26, P = .03; Fig 2B) on univariate survival analysis (see Appendix). Both cases of *MYOD1*-mutant SRMS were not included in any further statistical analysis; moreover, the dismal prognosis of this disease has already been well established.[23]

Moreover, EFS and OS by histologic subtype (ARMS or ERMS) were not statistically significantly different on univariate survival analysis likely because of the low number of ERMS in our cohort (7 of 61).

The 5-year overall EFS for the entire cohort was of 36%, and the 5-year OS was 59% (Table 2). In the ARMS subset, the 5-year EFS was 30% while the 5-year OS was 56%. Although limited by the very low number, both the 5-year EFS and OS in ERMS was 82% (Table 2).

Five-year EFS was only 20% for high-risk patients, with many developing off-treatment relapse (14 of 30) while the remaining had progressive disease during primary treatment (10 of 30). Among these 10 patients who progressed while on therapy, two had an initial treatment response.

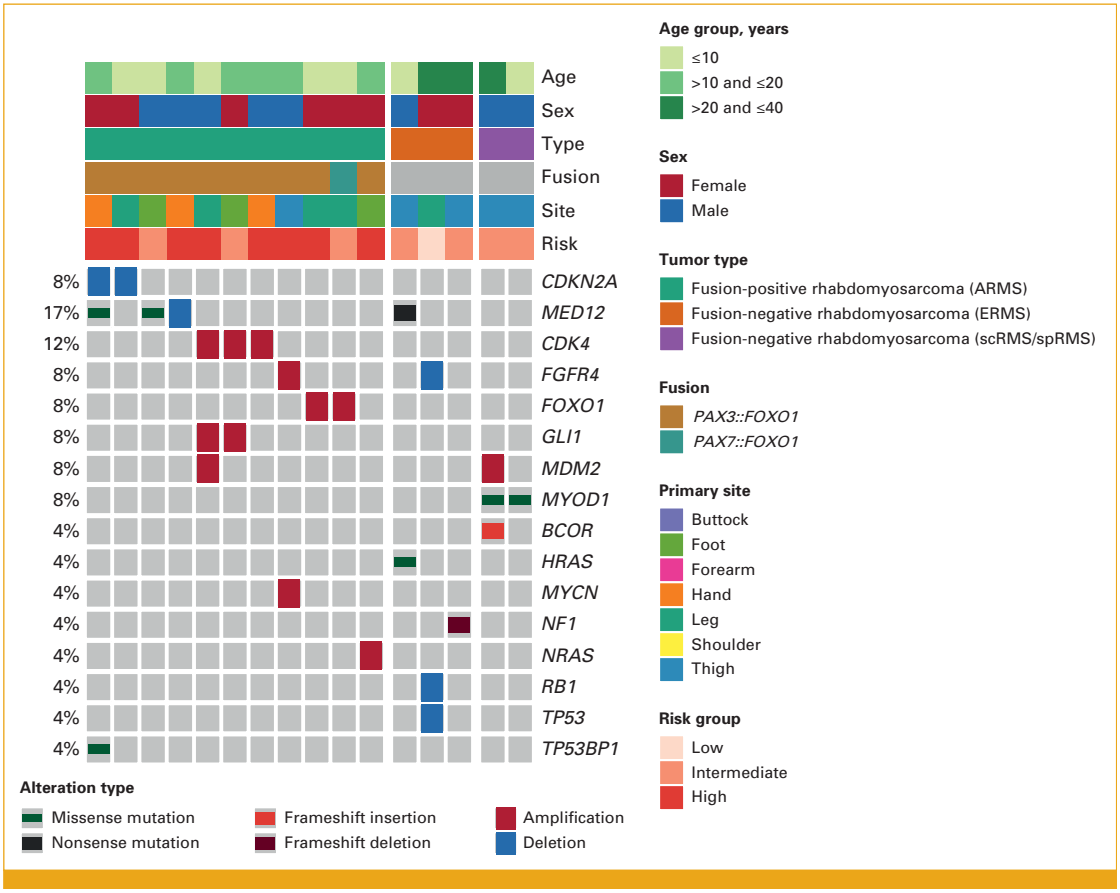

FIG 1. Oncoprint summary of molecular alterations for tumors with detectable oncogenic mutations. Data are shown for 11 ARMS, three ERMS, and two SRMS. The remaining eight patients' tumors (seven ARMS and one ERMS) lacked key molecular alterations (other than the fusion driver in ARMS) and are not illustrated here (see in addition Appendix Fig A2). Mutation detection frequency (left column, %) is applied to the whole cohort tested by NGS (n = 24 patients). Each patient represents a column, and each gene query is listed in a row. Age, sex, histotype, fusion type, tumor site, and initial risk group have been color-coded. ARMS, alveolar rhabdomyosarcoma; ERMS, embryonal rhabdomyosarcoma; scRMS, sclerosing RMS; spRMS, spindle cell RMS.

The 5-year EFS and OS were calculated for patients with localized disease (N0M0), nodal involvement (N1M0), and metastatic (M1; Table 3). Localized disease (N0, M0) was present in 20 patients, for whom 5-year EFS and OS were 58% and 84%, respectively. Nodal involvement (N1, M0) was present in 11 patients, with a 5-year EFS and OS of 43% and 66%, respectively. In total, 6 of 11 relapsed. Finally, 31 patients had metastatic disease (N0/1, M1), 17 (55%) of them had on-treatment progression and seven had off-treatment relapse (23%). The 5-year EFS was 19%, and the 5-year OS was 29%.

Statistical analysis was also done on the ARMS cohort (n = 52), showing a correlation of patients ≥10 years with shorter OS (HR = 2.28, *P* value = .040) by univariate but not by multivariate analysis. Similarly, risk group significantly affected OS and EFS on univariate analysis (HR = 2.69, *P* = .018; and HR = 2.30 *P* = .020, respectively); however, it was nonsignificant on multivariate analysis.

Various correlations with *PAX3::FOXO1* (n = 36) and *PAX7::FOXO1* (n = 16) fusion types were also analyzed within the ARMS group. *PAX7::FOXO1* fusion occurred in tumors from younger patients (median, 3.5 years; range, 0.7-16.2 years) compared with *PAX3* fusion (median, 11 years; range, 0.8-27 years). Compared with *PAX7*, *PAX3::FOXO1* fusion variant constituted a statistically significant adverse factor for OS in both univariate analysis (HR = 3.45, *P* = .016) and by multivariate analysis adjusted for risk group and age (HR, 2.86; 95% CI, 1.013 to 8.044; *P* = .004; Figs 2C and 2D). There was no significant difference of EFS between the two groups on univariate analysis. The gap between 5-year OS for *PAX7*-ARMS versus *PAX3*-ARMS (86% and 36% respectively)

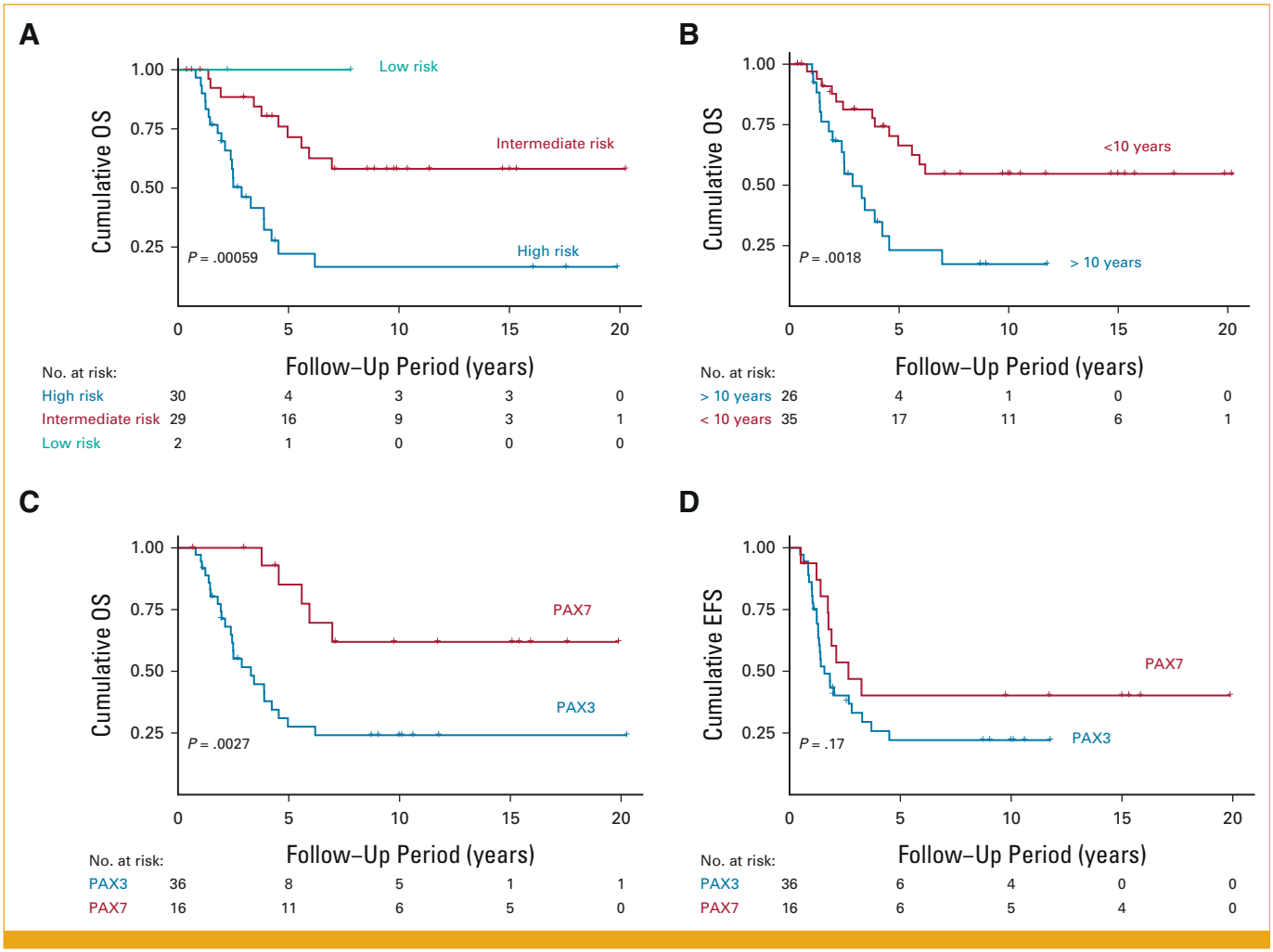

FIG 2. Log-rank test for univariate analysis showing OS curves for all patients. (A) Various risk groups (P = .007) and (B) age category (P = .029). Univariate log-rank test for (C) OS (P = .004) and (D) EFS (P = .251) in relationship to fusion type in ARMS cohort. ARMS, alveolar rhabdomyosarcoma; EFS, event-free survival; OS, overall survival.

was more striking compared with EFS (40% and 26%). This highlights the impact of fusion type in postrelapse survival rather than on the relapse risk itself. Among the 8 of 16 patients with *PAX7*-ARMS who relapsed, four survived (50%) while only one patient was considered cured (6%) among the 18 of 36 patients with *PAX3*-ARMS who relapsed (Appendix Table A3, Data Supplement).

We also compared hand and foot sites with the other locations as a previous study suggested that they might have an adverse effect on OS[5]; however, there was no statistical difference noted on univariate survival analysis (HR = 1.93, P = .097) between RMS arising in hand or foot sites versus those from other locations.

Gene Sequencing Data Survival Analysis

A survival analysis was only performed on the 18 ARMS subset as there were only four ERMS and two SRMS with targeted sequencing data available. OS and EFS analysis were performed for *CDKN2A* and *CDK4* alteration status on the basis of MSK-IMPACT for *CDKN2A* alone (n = 2), *CDK4* alone (n = 3), or *CDK4* and *CDKN2A* combined (n = 5). The presence of *CDK4* amplification and *CDKN2A* deletions combined was found to correlate significantly with worse OS (P = .029; Fig 3A), but not for EFS (P = .21). All except one of the five patients harboring one of these two molecular alterations had high-risk disease. The median OS for the entire ARMS group was 5 years and dropped to 3.9 years in the subset of ARMS harboring *CDK4* or *CDKN2A* abnormalities. The median EFS also decreased from 1.7 years in the entire ARMS group to 1.0 year in the CDK4/CDKN2A altered subset. A subanalysis targeted on the high-risk ARMS subset (n = 13) showed similar results on OS (P = .04; Fig 3B) and EFS (P = .08).

DISCUSSION

Despite in-depth disease staging initiatives, local control improvement, and multimodality chemotherapy tuning, extremity RMS maintains a poor outcome compared with

TABLE 2. EFS and OS for the Entire Cohort Related to Histology-Defined Subsets

	EFS			OS		
% survival	All Patients	ARMS	ERMS	All Patients	FP	FN
1 year, %	90	88	100	98	98	100
3 year, %	44	39	82	70	69	82
5 year, %	36	30	82	59	56	82
10 year, %	36	30	82	48	44	82
Mean survival, years	9.1			12		
Median survival, years	2.3	5.9	Not reached	Not reached	2.0	Not reached

Abbreviations: ARMS, alveolar rhabdomyosarcoma; EFS, event-free survival; ERMS, embryonal rhabdomyosarcoma; FN, fusion negative; FP, fusion positive; OS, overall survival.

other RMS locations. This aggressive biology is likely driven by two main factors: a high incidence of ARMS, which in our series accounted for 85% of extremity tumors (70% being PAX3-positive), and a high incidence of metastatic disease at presentation, which represented the majority of our cohort, with either isolated regional nodal (16%) or distant metastatic (51%) disease. For the group as a whole, the successful local control management of the primary tumor with first-line radiotherapy approach was seen in more than 90% of patients, and for those with localized tumors, local control was achieved in 95% of patients. The by-far greatest risk of treatment failure in this group of patients, then, is the inability of multimodal therapy to control occult or established regional nodal and/or distant metastatic disease.

Regarding local control, results from the IRS-IV trial describe 7% local failure rates, 20% regional, and 28% distant control failure in localized extremity RMS.[24] The D9803 trial showed 15% local failure rates for similar patient population, further highlighting the unfavorable impact of extremity location on RMS relapse.[25] Moreover, this study reveals that patients with ARMS and clinical group I/II tumors are associated with excellent local control rates but experience increased distant failures. This underscores that more aggressive up-front surgery is unlikely to improve the outcome in this patients' subset, whose leading risk of treatment failure is regional nodes and/or distant metastatic recurrence. In contrast, a recent French study focusing on localized extremity RMS reported that most relapses were locoregional and describing a local control failure rate of 27%.[6] This study included patients treated in the MMT-95 protocol in which routine systematic irradiation was not provided in patients showing good disease response to chemotherapy and surgery. This could explain the discrepancy between European and North American local control rates. The results of our localized subset show very similar findings to those of the Donaldson and Wolden publications, having a 5-year EFS of 58%, a 5-year OS of 84%, and a majority of metastatic relapses (63%). Our local control failure rate was low, with 7% (4 of 61) for our whole cohort and 5% (1 of 21) for the localized disease subset (see Appendix).

TABLE 3. EFS and OS for the Entire Cohort Breakdown on the Basis of Localized Disease (N0M0), Nodal Involvement Only (N1M0), and Metastatic Disease (M1)

	EFS			OS		
% survival	N0M0	N1M0	M1	N0M0	N1M0	M1
1 year, %	100	100	80	100	100	97
3 year, %	64	56	27	95	78	45
5 year, %	58	43	19	84	66	29
10 year, %	58	43	19	73	39	24

Abbreviations: EFS, event-free survival; OS, overall survival.

The presence of nodal involvement, surprisingly, had a lower impact on the 5-year EFS and 5-year OS (43% and 66%, respectively) comparatively to the dismal impact of metastatic disease (19% and 29%, respectively). Previous data suggested that patients with N1 RMS should be managed similarly as M1 patients, as they have shown comparable survival rates.[26] Although nodal involvement alone did not qualify for high-risk classification and therapy in our data set, the difference in survival may be related to the aggressive treatment of nodal disease by systematic radiotherapy and surgery. An accurate diagnosis of locoregional spread seems, therefore, essential to increase survival. Indeed, 56% of our cohort had lymph node involvement at diagnosis, further highlighting the importance of an in-depth attempt to identify disseminated disease in extremity RMS. We recommend that ^{18}F-FDG-PET/CT and sentinel node mapping be used as standard of care for risk stratification. Systematic biopsy of regional lymphatic drainage basins and/or suspicious (enlarged or hypermetabolic) nodes has also been proven of high added value. Our series confirm the joint efficiency achieved by concomitant techniques as some patients showed nodal involvement on imaging only while others could solely be seen by sentinel nodal sampling. The combined use of these three techniques could greatly increase the average sensitivity (61%) of PET/CT when used for positive lymph node detection,[8] especially in extremity RMS. Nodal involvement has been reported in 5%-10% of all pediatric and adolescent RMS.[27]

The high incidence of ARMS subtype (>80%) in the extremity RMS may explain the disproportionally high rate of disseminated disease at diagnosis in our study (41 of 61, 67%) and thus the unfavorable outcome detected with extremity location. The experience provided by prophylactic nodal irradiation (PNI) seemed encouraging as all patients (n = 4) who benefited from it did not relapse compared with a 50% 5-year EFS of comparable cases. Although based on very small numbers, these data are encouraging and, to our knowledge, represent the first report of PNI-positive impact on survival in

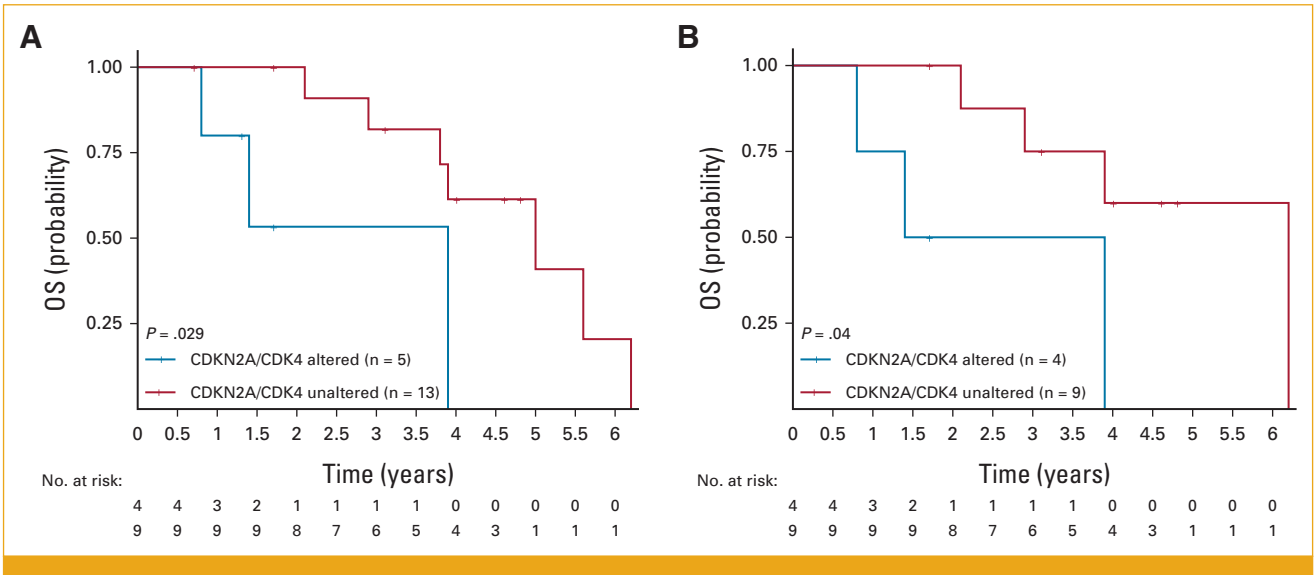

FIG 3. (A) Univariate log-rank analysis of 18 patients with ARMS with targeted DNA-based sequencing showing that *CDKN2A* deletions or *CDK4* amplifications had an impact on OS ($P = .029$). (B) Univariate log-rank analysis of 13 patients with high-risk ARMS with targeted sequencing showing that *CDKN2A* deletions or *CDK4* amplifications had an impact on OS ($P = .04$). ARMS, alveolar rhabdomyosarcoma; OS, overall survival.

the extremity RMS subset. These results reinforce previous case studies showing similar benefit on outcome, using PNI to cervical lymph nodes in parameningeal region RMS[28] and inguinal PNI for perianal or perineal RMS.[29]

Our focused statistical analysis on the ARMS cohort was able to confirm that *PAX3::FOXO1* fusion was associated with poor outcome, both on OS and EFS by univariate and multivariate analyses, as previously demonstrated.[30] This finding is even more striking as other well-known adverse factors, such as age older than 10 years and tumor size, were not found to be statistically significant in our ARMS cohort, likely because of the low number of cases.

To our knowledge, this study also reports on the largest cohort of *PAX7::FOXO1* extremity ARMS to date (n = 16), highlighting their predilection for younger age at diagnosis and being associated with a better OS. The correlation of this molecular subset with younger age has been previously documented.[31] In fact, patients with *PAX7*-ARMS showed a striking difference in postrelapse survival as 50% of them are considered cured today while only 6% of patients with *PAX3::FOXO1* ARMS are alive long-term after relapse. When comparing the clinical outcome of the two fusion type cohorts, the main difference relied on OS rather than EFS, with *PAX7* patients with a 5-year OS of 86% versus *PAX3* patients with a 5-year OS of 36%, in keeping with a favorable outcome of *PAX7* patients postrelapse (See Appendix).

In the ARMS cohort, the presence of *CDK4* gene amplifications and *CDKN2A* deletions was associated with poor outcome and higher risk group at diagnosis. *CDKN2A* loss of function has been shown to considerably increase tumor susceptibility in a knock-in *Pax3-Foxo1* mouse model.[32] Moreover, in a stepwise in vitro study, p16INK4A/p14ARF loss of function was a necessary partner to *PAX3::FOXO1* gain of function leading to a synergetic action of early cell proliferation while *MYCN* amplification and hTERT stabilization were late events leading to tumors in vivo.[33] More recently and similar to our findings, the enrichment of *CDK4* and *CDKN2A* copy number aberrations has been described in patients with ARMS, with data suggesting a mutually exclusive pattern[34,35] and an adverse impact of *CDKN2A* loss on survival.[36] Moreover, our results showed that the presence of either *CDK4* amplification or *CDKN2A* deletion correlated with high-risk patients and had an adverse impact on OS. Of interest only one ERMS in our cohort tested by targeted NGS showed the presence of *TP53* deletion, which co-occurred with *RB* and *FGRF4* deletions. None of the extremity ARMS tested showed the presence of *TP53* mutations. Overall, the number of secondary genetic events outside the *FOXO1* fusion-driver was relatively low (range of 1-9 gene alterations, average 3.5-3.7), thus highlighting relatively few potential therapeutic targets for a more personalized treatment (see Appendix).

In summary, we report our clinical and genomic experience on one of the largest cohort of extremity RMS treated at a single tertiary cancer center. Our study was driven by unanswered questions regarding the disproportionally poor outcome of this clinical subset compared with other RMS locations and hypothesized that genomic landscape analyses may provide additional insights into their distinct pathogenesis. In keeping with previous data, we confirm that metastatic spread at diagnosis, risk group, and *FOXO1* fusion subtype remain the most critical and significant actors of poor survival rates.

Moreover, the targeted DNA-based sequencing revealed that copy number alterations in *CDKN2A* and *CDK4* genes occur in 28% of extremity ARMS, being associated with poor OS and correlating with acral location and high risk. Although this finding only partially explains the dismal outcome of patients with extremity RMS, it appears to be the first step in dissecting the additive effect of molecular alterations in establishing risk stratification and prognosis in this disease. Although half of our cohort presented with metastatic disease, the 5-year OS for patients with N1 was 66% compared with 29% for M1 patients, suggesting that an accurate diagnosis of locoregional spread and the systematic treatment by radiotherapy and primary resection coupled to lymphadenectomy may have improved prognosis. On the other hand, the alarmingly high rate of metastatic disease in extremity RMS and the consequence this has on prognosis impel us to undertake concrete efforts toward understanding the dynamics of disease dissemination in RMS.

AFFILIATIONS

[1]Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, NY
[2]Pediatric Hematology/Oncology, Massachusetts General Hospital for Children, Boston, MA
[3]Department of Radiation Oncology, Memorial Sloan Kettering Cancer Center, New York, NY
[4]Department of Pediatrics, Memorial Sloan Kettering Cancer Center, New York, NY

CORRESPONDING AUTHOR

Leonard H. Wexler, MD, Department of Pediatrics, Memorial Sloan Kettering Cancer Center, 1275 York Ave, New York, NY 10065; Twitter: @mskcancercenter; e-mail: wexlerl@mskcc.org.

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DATA SHARING STATEMENT

Genomic data sharing is made publicly available at: https://cbioportal.mskcc.org/study/summary?id=rms_msk_2023

AUTHOR CONTRIBUTIONS

Conception and design: Henry de Traux de Wardin, Josephine K. Dermawan, Suzanne L. Wolden, Cristina R. Antonescu, Leonard H. Wexler
Financial support: Leonard H. Wexler
Provision of study materials or patients: Suzanne L. Wolden, Leonard H. Wexler
Collection and assembly of data: Henry de Traux de Wardin, Josephine K. Dermawan, Mariel H. Smith, Suzanne L. Wolden, Cristina R. Antonescu, Leonard H. Wexler
Data analysis and interpretation: Henry de Traux de Wardin, Bin Xu, Josephine K. Dermawan, Mariel H. Smith, Cristina R. Antonescu, Leonard H. Wexler
Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated unless otherwise noted. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO's conflict of interest policy, please refer to www.asco.org/rwc or ascopubs.org/po/author-center.
Open Payments is a public database containing information reported by companies about payments made to US-licensed physicians (Open Payments).

Leonard H. Wexler
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APPENDIX

Risk group assignment	Criteria per COG trial	
	D-series (1997-2004)	**ARST-series (2004-current)**
Low	D9602 (NCT00002995): (ERMS only) **Subset A** Favorable site, any size, stage I, group I and II, N_0; Favorable site, any size, stage I, group III, N_0, (orbit only); Unfavorable site, ≤5cm, stage II, group I, N_0, N_x **Subset B** Favorable site, any size, stage I, group II, N_1; Favorable site, any size, stage I, group III, N_1 (orbit only); Favorable site (except orbit), any size, stage I, group III, N_0, N_1; Unfavorable site, ≤5 cm, stage II, group II, N_0, N_x; Unfavorable site, ≤5 cm with N_1 or >5 cm any size, stage III, group I/II, N_0, N_x, N_1	ARST0331 (NCT00075582): (ERMS only) **Subset 1** Stage I, group I and II, N_0; Stage I, group III, N_0, N_x, (orbit only); Stage II, group I, N_0, N_x, and Group II **Subset 2** Stage I, group III, N_0, N_x, (non-orbital); Stage III, group I/II
Intermediate	D9803 (NCT00003958) Stage I-III, group I-III, ARMS; stage II/III, group III, ERMS; stage IV, group IV, ERMS, <10 years	ARST0531 (NCT00354835) Stage II/III, group III, ERMS; stage I-III, group I-III, ARMS
High	D9802 (NCT00003955) Stage IV, group IV, except ERMS <10 years	ARST0431 (NCT00354744) Stage IV, group IV

FIG A1. Current and previous COG-STS risk stratification criteria. ARMS, alveolar rhabdomyosarcoma; ERMS, embryonal rhabdomyosarcoma; COG, Children's Oncology Group; N_0, no regional nodal involvement; N_1, regional nodal involvement; N_x, nodal involvement unknown.

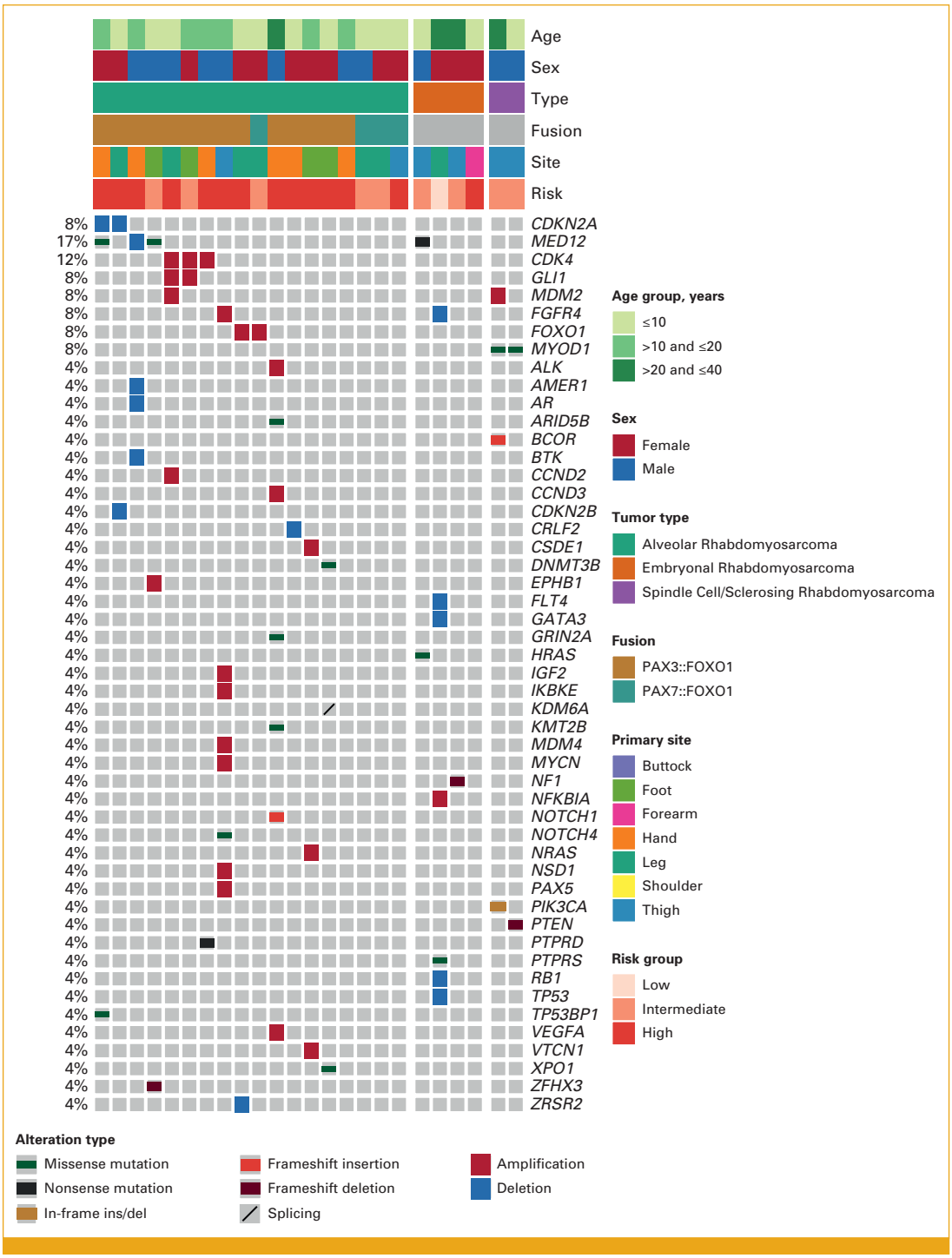

FIG A2. Oncoprint summary of molecular alterations detected for the entire RMS extremity cohort (18 ARMS, 4 ERMS, and 2 SRMS) with detectable oncogenic SNV or CNV mutations. Each patient represents a column, and each gene query is listed in a row. Age, sex, histotype, fusion type, tumor site and initial risk group have been color-coded. Mutation detection frequency (left column, %) is applied to the whole cohort (n = 24 patients).

TABLE A1. Detailed Clinicopathological Data for Each Patient (n = 61) Included in the Collection

Patient Code	Impact	Vital Status	Sex	Age at Diagnosis, Years	Diagnostic	Fusion Type	Site	Laterality	T	Size, cm	Stage	Group	Risk Group	N status	M Status	Initial RT?	Initial Surgery	First Relapse? (off treatment)	First Relapse Local, Regional, or Metastatic?	Progression of Disease While on Treatment?	Time From Diagnosis to Death
2	No	Alive	Male	4.1	ARMS	PAX3	Foot	Left	T2a	<5	2	III	Int	0	0	Yes	No	Yes	Regional	No	NA
3	No	Deceased	Female	16.4	ARMS	PAX3	Hand	Right	T2a	>5	3	III	Int	1	0	Yes	No	Yes	Metastatic	Yes	3.4
5	No	Alive	Female	15.9	ARMS	PAX3	Hand	Right	T2b	>5	3	III	Int	1	0	Yes	Delayed primary excision	No	NA	No	NA
9	No	Deceased	Male	20.9	ARMS	PAX3	Hand	Left	T2a	<5	4	IV	High	1	1	Yes	No	No	NA	Yes	1.1
12	No	Deceased	Male	17.5	ARMS	PAX3	Forearm	Left	T2b	>5	4	IV	High	0	1	Yes	No	Yes	Metastatic	No	3.3
13	No	Alive	Female	15.3	ARMS	PAX3	Foot	Right	T2b	>5	3	II	Int	0	0	Yes	Up-front surgery	No	NA	No	NA
16	No	Deceased	Male	14.6	ARMS	PAX3	Hand	Left	T2a	<5	4	IV	High	1	1	Yes	No	No	NA	Yes	1.4
17	No	Deceased	Female	23.0	ARMS	PAX3	Foot	Right	T2a	<5	4	IV	High	1	1	Yes	No	Yes	Metastatic	Yes	2.5
18	No	Deceased	Female	15.2	ARMS	PAX3	Foot	Left	T2b	>5	4	IV	High	1	1	Yes	No	Yes	Metastatic	Yes	2.5
19	No	Deceased	Male	27.5	ARMS	PAX3	Forearm	Right	Unknown	Unknown	4	IV	High	1	1	Yes	Up-front surgery	No	NA	Yes	1.8
22	No	Deceased	Male	22.6	ARMS	PAX3	Forearm	Right	T2b	>5	4	IV	High	1	1	Yes	No	No	NA	Yes	1.2
23	No	Deceased	Male	0.8	ARMS	PAX3	Buttock	Right	T2a	<5	3	Iic	Int	1	0	Yes	Up-front surgery	Yes	Metastatic	Yes	1.9
24	No	Deceased	Male	9.0	ARMS	PAX3	Hand	Right	T2a	<5	4	IV	High	1	1	Yes	No	Yes	Metastatic	Yes	2.4
25	No	Deceased	Female	1.9	ARMS	PAX3	Thigh	Right	T2b	>5	3	Iii	Int	1	0	Yes	Delayed primary excision	Yes	Metastatic	Yes	1.5
26	No	Alive	Female	8.8	ARMS	PAX3	Forearm	Left	T2b	>5	3	III	Int	0	0	Yes	Delayed primary excision	No	NA	No	NA
28	No	Deceased	Female	10.4	ARMS	PAX3	Foot	Left	T2a	<5	4	IV	High	0	1	Yes	Salvage surgery	Yes	Metastatic	Yes	2.4
29	No	Alive	Female	5.8	ARMS	PAX3	Foot	Right	T2a	<5	4	IV	High	1	1	Yes	No	No	NA	No	NA
32	No	Deceased	Female	17.8	ARMS	PAX3	Calf	Right	T2b	>5	4	IV	High	0	1	Yes	No	Yes	Metastatic	Yes	2.0
33	No	Alive	Male	9.4	ARMS	PAX3	Thigh	Right	T2b	>5	3	Iia	Int	0	0	Yes	Up-front surgery	No	NA	No	NA
35	No	Alive	Male	6.2	ARMS	PAX3	Calf	Right	T2b	>5	3	III	Int	1	0	Yes	No	No	NA	No	NA
36	Yes	Deceased	Female	4.8	ARMS	PAX3	Hand	Left	T2a	<5	4	IV	High	0	1	Yes	No	Yes	Metastatic	No	6.2
37	Yes	Deceased	Male	5.0	ARMS	PAX3	Foot	Left	T2b	>5	3	III	Int	1	0	NO	No	Yes	Local	Yes	5.0
38	No	Alive	Female	10.2	ARMS	PAX3	Calf	Left	T2b	>5	3	III	Int	0	0	Yes	No	No	NA	No	NA
41	Yes	Deceased	Female	8.1	ARMS	PAX3	Foot	Right	Unknown	Unknown	4	IV	High	1	1	Yes	Salvage surgery	Yes	Local	Yes	3.9
43	No	Alive	Male	17.5	ARMS	PAX3	Forearm	Right	T2b	>5	4	IV	High	1	1	Yes	No	No	NA	No	NA
44	Yes	Deceased	Female	10.1	ARMS	PAX3	Foot	Left	T2a	<5	4	IV	High	1	1	Yes	No	No	NA	Yes	2.9
46	Yes	Deceased	Male	18.4	ARMS	PAX3	Hand	Left	T2a	<5	4	IV	High	1	1	Yes	No	Yes	Metastatic	Yes	3.9
47	Yes	Deceased	Male	6.7	ARMS	PAX3	Calf	Left	T2a	<5	4	IV	High	1	1	Yes	No	No	NA	Yes	0.8
50	Yes	Deceased	Female	14.4	ARMS	PAX3	Hand	Right	T2b	>5	4	IV	High	1	1	Yes	No	No	NA	Yes	1.4

(continued on following page)

TABLE A1. Detailed Clinicopathological Data for Each Patient (n = 61) Included in the Collection (continued)

Patient Code	Impact	Vital Status	Sex	Age at Diagnosis, Years	Diagnostic	Fusion Type	Site	Laterality	T	Size, cm	Stage	Group	Risk Group	N status	M Status	Initial RT?	Initial Surgery	First Relapse? (off treatment)	First Relapse Local, Regional, or Metastatic?	Progression of Disease While on Treatment?	Time From Diagnosis to Death
51	Yes	Deceased	Female	5.1	ARMS	PAX3	Calf	Left	T2b	>5	4	IV	High	1	1	Yes	No	Yes	Metastatic	No	2.1
52	Yes	Pal	Male	23.3	ARMS	PAX3	Hand	Right	T2a	<5	4	IV	High	1	1	Yes	No	Yes	Metastatic	No	2.4
54	Yes	Alive	Male	13.6	ARMS	PAX3	Hand	Left	T2a	<5	4	IV	High	1	1	Yes - proton	No	No	NA	No	NA
56	Yes	Alive	Female	5.8	ARMS	PAX3	Calf	Left	T2b	>5	4	IV	High	1	1	Yes	No	No		Yes	NA
57	Yes	Deceased	Male	20.2	ARMS	PAX3	Hand	Right	T2a	<5	4	IV	High	1	1	Yes	No	Yes	Metastatic	No	1.0
59	Yes	Alive	Female	11.0	ARMS	PAX3	Foot	Right	T2a	<5	2	III	Int	0	0	Yes	Delayed primary excision	No	NA	No	NA
60	Yes	Deceased	Male	17.6	ARMS	PAX3	Thigh	Left	T2b	>5	4	IV	High	1	1	Yes	No	Yes	Local, regional	No	4.5
1	No	Deceased	Male	16.2	ARMS	PAX7	Calf	Left	T2a	<5	3	III	Int	0	0	No	Delayed primary excision	Yes	Regional	No	7.0
4	No	Alive	Male	5.7	ARMS	PAX7	Buttock	Right	T2b	>5	4	IV	High	1	1	Yes	Delayed primary excision	No	NA	No	NA
6	No	Alive	Male	2.1	ARMS	PAX7	Thigh	Left	T2b	>5	4	IV	Int	1	0	Yes	Delayed primary excision	No	NA	No	NA
7	No	Alive	Male	5.5	ARMS	PAX7	Calf	Left	T2b	>5	4	IV	High	1	1	Yes	Salvage surgery	No	NA	No	NA
8	No	Deceased	Female	1.4	ARMS	PAX7	Buttock	Right	T2b	>5	3	III	Int	1	0	Yes	Delayed primary excision	Yes	Regional	Yes	5.9
10	No	Alive	Male	3.0	ARMS	PAX7	Buttock	Left	T2b	>5	4	IV	High	1	1	Yes	Delayed primary excision	Yes	Metastatic	No	NA
11	No	Alive	Female	2.6	ARMS	PAX7	Calf	Left	Unknown	Unknown	3	III	Int	0	0	Yes	Delayed primary excision	Yes	Regional	No	NA
14	No	Alive	Female	3.3	ARMS	PAX7	Calf	Left	T2b	>5	3	III	Int	0	0	Yes	Delayed primary excision	No	NA	No	NA
20	No	Alive	Male	5.8	ARMS	PAX7	Thigh	Left	T2a	<5	3	III	Int	1	0	Yes	No	No	NA	No	NA
21	No	Alive	Female	5.0	ARMS	PAX7	Thigh	Right	T2b	>5	3	i	Int	0	0	Yes	Up-front surgery	Yes	Regional	No	NA
27	No	Alive	Male	1.3	ARMS	PAX7	Forearm	Right	T2b	>5	3	III	Int	0	0	Yes	Delayed primary excision	No	NA	No	NA
30	No	Deceased	Male	3.6	ARMS	PAX7	Calf	Right	T1b	>5	3	I	Int	0	0	Yes	Up-front surgery	Yes	Regional	Yes	4.5
45	Yes	Deceased	Male	0.7	ARMS	PAX7	Calf	Right	Unknown	Unknown	2	IIa	Int	0	0	No	Up-front surgery	Yes	Regional	Yes	5.6
48	Yes	Deceased	Female	7.0	ARMS	PAX7	Calf	Left	T2b	>5	3	III	Int	0	0	Yes	Delayed primary excision	Yes	Matastatic	No	3.8

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TABLE A1. Detailed Clinicopathological Data for Each Patient (n = 61) Included in the Collection (continued)

Patient Code	Impact	Vital Status	Sex	Age at Diagnosis, Years	Diagnostic	Fusion Type	Site	Laterality	T	Size, cm	Stage	Group	Risk Group	N status	M Status	Initial RT?	Initial Surgery	First Relapse? (off treatment)	First Relapse Local, Regional, or Metastatic?	Progression of Disease While on Treatment?	Time From Diagnosis to Death
49	Yes	Alive	Female	0.8	ARMS	PAX7	Thigh	Right	T2b	>5	4	IV	High	0	1	Yes	Salvage surgery	No		Yes	NA
61	Yes	Alive	Female	4.1	ARMS	PAX7	Calf	Left	T2b	>5	3	III	Int	1	0	Yes	No	No		No	NA
15	No	Alive	Female	2.1	ERMS	NA	Calf	Left	T2b	>5	3	III	Int	0	0	Yes	Delayed primary excision	No	NA	No	NA
31	No	Alive	Female	3.1	ERMS	NA	Thigh	Right	T1b	>5	3	I	Low	0	0	No	Up-front surgery	No	NA	No	NA
34	No	Deceased	Female	1.5	ERMS	NA	Buttock	Left	T2b	>5	4	IV	High	1	1	Yes	Delayed primary excision	No	NA	Yes	1.2
42	Yes	Alive	Female	37.0	ERMS	NA	Thigh	Left	T1a	<5	2	III	Low	0	0	No	Up-front surgery	No	NA	No	NA
53	Yes	Alive	Female	3.3	ERMS	NA	Forearm	Left	T2b	>5	4	IV	High	1	1	Yes	No	No	NA	No	NA
55	Yes	Alive	Female	31.7	ERMS	NA	Thigh	Left	T1a	<5	2	I	Low	0	0	No	Up-front surgery	No	NA	No	NA
58	Yes	Alive	Male	0.7	ERMS	NA	Thigh	Right	T2b	>5	3	III	Int	0	1	No	Delayed primary excision	No	NA	No	NA
39	Yes	Deceased	Male	35.5	ScRMS	NA	Forearm	Right	T2b	>5	3	III	Int	x	0	Unknown	Salvage surgery	Yes	Metastatic	No	1.4
40	Yes	Alive	Male	8.2	ScRMS	NA	Thigh	Right	T2b	>5	3	III	Int	0	0	Yes	Delayed primary excision	No	NA	No	NA

NOTE. Each case has a unique case number. Ordered by tumor type.
Abbreviations: ARMS, alveolar rhabdomyosarcoma; ERMS, embryonal rhabdomyosarcoma; INT, intermediate; NA, not available; RT, radiotherapy.

TABLE A2. Copy Number Variation $\log_2$ Ratios for Each Gene per Patient (patient code referenced in Appendix Table A1) and Tumor Type

Patient Code	Tumor Type	CDKN2A	MED12	CDK4	FGFR4	FOXO1	GLI1	MDM2	MYCN	RB1	TP53	NRAS
36	ARMS	0	0	0	0	0	0	0	0	0	0	0
37	ARMS	0	0	0	0	0	0	0	0	0	0	0
44	ARMS	0	0	0	0	0	0	0	0	0	0	1.608
46	ARMS	0	0	4.64217	0	0	0	0	0	0	0	0
47	ARMS	0	0	9.38853	0	0	9.38853	8.05453	0	0	0	0
50	ARMS	−2.39778	0	0	0	0	0	0	0	0	0	0
51	ARMS	0	0	0	0	2.00708	0	0	0	0	0	0
52	ARMS	0	0	0	0	0	0	0	0	0	0	0
54	ARMS	0	−1.88688	0	0	0	0	0	0	0	0	0
56	ARMS	−3.40204	0	0	0	0	0	0	0	0	0	0
59	ARMS	0	0	2.00416	0	0	2.00416	0	0	0	0	0
60	ARMS	0	0	0	2.62661	0	0	0	8.36404	0	0	0
61	ARMS	0	0	0	0	3.1145	0	0	0	0	0	0
55	ERMS	0	0	0	−2.09144	0	0	0	0	NA	−4.0505	0
39	SRMS	0	0	0	0	0	0	4.43244	0	0	0	0
40	SRMS	0	0	0	0	0	0	0	0	0	0	0

Abbreviations: ARMS, alveolar rhabdomyosarcoma; ERMS, embryonal rhabdomyosarcoma; SRMS, sclerosing rhabdomyosarcoma.

TABLE A3. Detailed Description of Patients With ARMS Who Had Disease Progression While on Primary Therapy or Disease Relapse Once Off Treatment

PAX7::FOXO1 ARMS	n = 16, No. (%)
Progression	1 (6)
Relapse	8 (50)
Local	1 (12.5)
Regional	6 (75)
Metastatic	1 (12.5)

PAX3::FOXO1 ARMS	n = 36, No. (%)
Progression	8 (22)
Relapse	18 (50)
Local	2 (11)
Regional	2 (11)
Metastatic	14 (78)

NOTE. Patients are separated by the fusion type of their tumors.
Abbreviation: ARMS, alveolar rhabdomyosarcoma.