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The prognostic significance of anaplasia in childhood rhabdomyosarcoma: A report from the Children's Oncology Group

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Abstract

Background: Established prognostic indicators in rhabdomyosarcoma (RMS), the most common childhood soft tissue sarcoma, include several clinicopathologic features. Among pathologic

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Archana Shenoy performed data curation, investigation and composed the original draft.

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Yueh-Yun Chi and Minjie Li performed formal analysis and reviewed/edited the draft.

Jack F. Shern, Javed Khan, Susan M. Hiniker and Candace F. Granberg reviewed results and edited the draft.

Douglas S Hawkins coordinated funding reviewed results and edited the draft.

David M Parham and Lisa A Teot performed the data collection, investigation and reviewed/edited the draft.

Erin R Rudzinski conceptualized the project, performed data curation, investigation and contributed to the original draft.

Declaration of Interest statement:

Douglas S Hawkins has the following disclosures: Loxo Oncology, Bayer, Bristol Myers Squibb, Lilly: Clinical trial fees paid to Seattle Children's to offset costs of study conduct; reimbursed for or provided travel, housing, and food to attend medial advisory board meetings; Celgene: Reimbursed for or provided travel, housing, and food to attend medial advisory board meetings; Eisai, Glaxo Smith Kline, Sanofi, Novartis, Amgen, Seattle Genetics, Jazz Pharmaceuticals, Incyte: Clinical trial fees paid to Seattle Children's to offset costs of study conduct.

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features, anaplasia has been suggested as a potential prognostic indicator, but the clinical significance of anaplasia remains unclear.

Methods: Patients enrolled on one of five recent Children's Oncology Group clinical trials for RMS (D9602, n=357; D9802, n=80; D9803, n=462; ARST0331, n=335; and ARST0531, n = 414) with prospective central pathology review were included in this study. Clinicopathologic variables including demographic information, risk group, histologic subtype, and anaplasia were recorded along with overall survival (OS) and failure-free survival (FFS) with failure defined by recurrence, progression or death. The log-rank test was used to compare OS and FFS.

Results: Anaplasia was more common in embryonal RMS (27% of all embryonal RMS) than other subtypes of RMS (11% for alveolar RMS, 7% for botryoid RMS, 11% for spindle cell RMS). On multivariate analyses, anaplasia was not an independent prognostic factor in RMS (OS: Hazard ratio (HR)=1.12, p=0.43; FFS: HR=1.07, p=0.56) across all subtypes or within embryonal RMS only (OS: HR=1.41, p=0.078; FFS: HR=1.25, p=0.16). Among tumors with *TP53* mutations, 69% had anaplasia, while only 24% of tumors with anaplasia had a tumoral *TP53* mutation.

Conclusions: Anaplasia is not an independent indicator of adverse outcomes in RMS. Emerging information on the prognostic significance of *TP53* mutations raises the possibility that anaplasia may be a surrogate marker of *TP53* mutations in some cases. Tumoral *TP53* mutation status may be investigated as a prognostic indicator in future studies.

Keywords

Rhabdomyosarcoma; anaplasia; TP53 Genes

Introduction:

Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma in childhood[1]. In addition to clinical features, including clinical group, stage, and age, certain histologic features have been important prognostic markers for RMS over the last decades[2,3]. It is now recognized, however, that histologic features may act as a surrogate for underlying biologic features. For instance, alveolar histology represents an approximate surrogate marker of *FOXO1* fusion positivity, the latter of which is one of the most important negative prognostic factors in RMS, but roughly 20% of alveolar RMS are fusion-negative and have a better outcome[4,5]. Although histologic subtype is no longer part of risk stratification for patients with RMS, the significance of anaplastic histology has remained unclear.

Palmer et al first noted the presence of "Wilms tumor-like" anaplasia (characterized by pleomorphic cells *and* large atypical mitotic figures) in RMS, and in his series anaplasia was associated with a worse prognosis[6,7]. Subsequent small studies demonstrated in univariate analysis that anaplasia was associated with a worse prognosis in embryonal RMS (ERMS)[8]. The first large retrospective review of anaplasia[9] demonstrated histologic anaplasia (with or without atypical mitotic figures) in 3% of RMS, but noted that anaplasia was more commonly seen in ERMS. This report distinguished focal (rare scattered cells) and diffuse anaplasia (cohesive clusters), with diffuse anaplasia being associated with worse outcome compared to no anaplasia. Qualman et al applied the same definition of anaplasia to a prospective series of 655 patients enrolled on International Rhabdomyosarcoma Study

Group (IRSG) therapeutic trials between 1995 and 1998. This analysis demonstrated a higher rate of anaplasia (13%; 7% focal, 6% diffuse) with inferior outcome seen on univariate analysis for intermediate risk patients with ERMS, but not when controlled for other prognostic factors by multivariate analysis[10].

We present a large analysis of 1648 patients with RMS enrolled on one of five Children's Oncology Group (COG) RMS clinical trials studies between 1997 and 2013, and for whom central pathology assessment of anaplasia was performed prospectively, to determine whether anaplasia is an independent prognostic factor in RMS.

METHODS:

Clinicopathologic variables:

Patients diagnosed with RMS between 1997–2013 and enrolled on one of five COG clinical RMS studies (D9602, n=357[11]; D9802, n=80[12]; D9803, n=462[13]; ARST0331, n=335[14]; or ARST0531, n = 414[15]) with central pathology review were included in this analysis. Central pathology review was performed prospectively within 21 days of trial enrollment. One hundred sixty-seven (167) cases were excluded due to lack of central review data. Anaplasia was defined as the presence of large hyperchromatic nuclei (3x size of other tumor nuclei) and/or the presence of atypical mitoses[16]. Focal anaplasia was defined as scattered anaplastic cells and diffuse anaplasia was defined by the presence of "foci or large sheets" of anaplastic cells[9]. Clinicopathologic variables including age, race, sex, primary site, tumor invasiveness, regional lymph node involvement, tumor size IRSG stage, clinical group, and risk stratification as defined in the prior analysis by Qualman et al and used in COG trials as of 2013 (Supplemental Table 1)[17] were analyzed with clinical outcome data and presence or absence of anaplasia[18,19]. For a subset of patients including in this analysis, data regarding tumor *TP53* mutational status (based upon targeted panel sequencing) was available for correlation with the presence of anaplasia[20].

Statistical methods:

Failure free survival (FFS) was defined as time from study entry to disease recurrence, progression, or death (from any cause) as a first event. Overall survival (OS) was defined as time from study entry to death from any cause or censored at the time of the last follow-up. Follow-up is current as of December 31, 2018. The log-rank test and Cox proportional hazards model were used to compare survival data. The chi-square test was performed to assess association between clinicopathologic variables. Software programs SAS and R were used for the analysis.

RESULTS:

A total of 1648 patients diagnosed with RMS were included in the study (Table 1). Seven hundred ninety-two patients had typical ERMS, with an additional 195 patients with botryoid RMS and 168 with spindle cell RMS. Four hundred thirty-three patients (26%) had alveolar RMS (ARMS). A *FOXO1* fusion was present in 75% of ARMS (258 of 346 patients) for whom *FOXO1* fusion status was available) (Table 2).

Anaplasia was seen across all age groups with no statistically significant difference between groups (Table 1). Anaplasia was more common in clinical groups I and II, favorable primary sites, Stage 1 and T1 (Table 1). Overall, there was no significant difference in FFS (5-year EFS of 68%, 69% and 76% for no, focal and diffuse anaplasia, respectively; p=0.22) or OS (5-year OS of 79%, 80% and 85% for no, focal and diffuse anaplasia, respectively; p=0.15) between non anaplastic RMS and anaplastic RMS (Figure 1 and 2). In multivariate analysis controlling for risk group and age, anaplastic RMS was not a significant indicator of clinical outcome (Supplemental Table 2). The median follow-up duration for surviving patients was 7 years (Range: 1 day – 14.8 years)

Alveolar RMS:

Anaplasia was less commonly documented in ARMS in comparison to ERMS (Table 2). Forty-seven patients with ARMS (of 433; 11%) had anaplasia, more frequently documented in FOXO1 fusion negative ARMS (p = 0.0003). On univariate analysis, OS and FFS was not significantly different between ARMS with or without anaplasia (Supplemental table 3).

Botryoid and Spindle cell RMS:

A majority of Botryoid RMS (93%) and Spindle cell RMS (79%) were not anaplastic. The presence of anaplasia did not significantly alter the OS and EFS for either histologic subtype.

Embryonal RMS:

The majority of RMS with histologic anaplasia were ERMS (n = 211 of 309 cases; 68%). Both by univariate and multivariate analysis, anaplastic morphology showed no statistically significant association with OS (hazard ratio (HR)=1.26, p=0.23 and HR=1.41, p=0.078) or FFS (HR=1.16, p = 0.34 and HR=1.25, p=0.16) (Table 3). In a sub-group analysis of the intermediate risk ERMS, anaplasia showed no significant association with OS (HR=1.58, p = 0.08) or EFS (HR=1.13, p=0.61) (Supplemental table 4).

TP53 mutation:

Tumor *TP53* mutation analysis was available for 146 patients, of which thirteen (9%) had a *TP53* pathogenic mutation (Table 4). Thirty-eight of tumors with known TP53 status demonstrated anaplasia. Nine of thirty-eight (24%) of the tumors with anaplasia harbored a *TP53* mutation. In contrast, a vast majority of tumors with *TP53* mutations demonstrated histologic anaplasia (9/13; 69%). In tumors with no anaplasia, *TP53* mutations were rarely observed (4/108, 3.7%).

DISCUSSION:

This study represents the largest prospective analysis of anaplasia in pediatric RMS. Focal or diffuse anaplasia was present in 19% of patients with RMS, with no statistically significant differences across age groups (Table 1). Anaplasia was observed more commonly in RMS occurring within favorable sites and tumors that are completely resected at diagnosis (Group I/II). In contrast to prior reports, anaplasia was not an independent prognostic indicator in RMS in either univariate or multivariate analyses.

Anaplasia was more common in ERMS, similar to what has been demonstrated in previous studies[8–10]. Although several previous studies have suggested differences in clinical outcome for patients with anaplastic ERMS on univariate analysis, no association with outcome was confirmed on multivariate analysis. Although we showed no significant difference in outcome by univariate or multivariate analyses, our results (Hazard ratio 1.25 for FFS and 1.41 for OS) are similar to those published by Qualman et al. (Hazard ratio 1.6 for FFS and 1.7 for OS).

Anaplasia was more frequently seen in IRS Clinical Group 1, Stage 1, favorable site, small tumors and overall low-risk tumors. It is possible that a subset of tumors in our study population (IRS Group 1) were completely resected at diagnosis. We acknowledge that a larger sample thus available for evaluation may represent a potential bias affecting our observations.

There are a few differences between our study population and those described in prior reports. Qualman et al, had a higher percentage of ARMS (30%) than in our study; however, the diagnosis of ARMS in the Qualman et al study was made based on International Classification of Rhabdomyosarcoma (ICR) criteria using histology alone, and the percentage of ARMS diagnoses was likely overestimated[10]. Our study integrated translocation information for ARMS and used re-review histologic diagnoses which likely explains the increased numbers of ERMS[21].

There was an increased prevalence of anaplasia (19%; Focal- 8% and Diffuse – 11%) in our study, in contrast to the observations of Qualman et al (13%; Focal- 6% and Diffuse – 7%) or Kodet (~3%, 110/approximately 3000 cases, 58 focal and 52 diffuse). The reason for this is unclear. The definition of anaplasia did subtly shift between 1983 and 1993 from requiring the presence of atypical mitoses to allowing for nuclear enlargement with or without atypical mitotic figures although the definition has been standard since 1993[7,9]. This is unlike the definition of anaplasia in Wilms tumor where presence of atypical mitoses is a requirement. Also, unlike anaplasia in Wilms tumor (assessed on resection specimen), anaplasia in RMS was mostly assessed on pre-treatment biopsies and assigned into focal and diffuse groups as defined above, upon central review.

There is limited literature on cytogenetic/molecular aberrations in anaplastic RMS. Earlier studies investigated chromosomal abnormalities in RMS, demonstrating genomic imbalance including chromosomal gains and losses across the different subtypes of RMS[22,23]. Subsequently, it has been shown that genomic amplification is more frequent in anaplastic ERMS and fusion positive ARMS[23]. Recently, in a small series of 87 cases of RMS, Casey et al reported that a subset of RMS harbors a high tumor mutation burden (TMB) and such tumors correlate with poor overall survival; they proposed that high TMB is an independent risk factor in the prognostication of RMS[24]. This observation has not yet been confirmed in a larger series of RMS.

Studies have also suggested that anaplasia is related to TP53 mutational status. Hettmer et al observed a high rate of germline *TP53* pathogenic variants (73%; 11/15 patients) in anaplastic RMS[25] and suggested anaplasia may be more common in patients with Li

Fraumeni syndrome. In a series of 631 RMS with somatic mutational analysis, Shern et al demonstrated tumoral *TP53* mutations in 12% of RMS, which was also associated with inferior outcome[20]. We were able to combine this tumor mutational data and central pathology review data for 146 patients in this study. In this subset of patients, tumor *TP53* mutations were present in 9% (13/146). Tumor *TP53* mutations were identified in only 24% (9/38) of all anaplastic RMS in our study; however, most tumors with *TP53* mutations had anaplastic morphology (9/13; 69%). Casey et al identified a *TP53* mutation in a subset of their patients with high TMB, and showed a significant correlation with poor OS[24]. Further analysis of TMB and *TP53* mutational status may be useful in future prospective studies of RMS.

Conclusions:

We demonstrate that anaplasia is not an independent adverse prognostic factor in RMS but that the prevalence of the diagnosis has climbed in recent years, suggesting a shift in criteria. If on future investigation, tumor *TP53* mutation is confirmed as an independent adverse prognostic factor, anaplasia could be used as to identify tumors with a higher probability of harboring a *TP53* mutation. Future studies including both germline and somatic sequencing are needed to confirm the role of *TP53* mutations in the risk stratification of RMS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights:

- Anaplasia is commonly seen in embryonal rhabdomyosarcoma compared to other subtypes
- Anaplasia is not an independent indicator of adverse prognosis in rhabdomyosarcoma
- TP53 mutation status & association with adverse prognosis needs more investigation

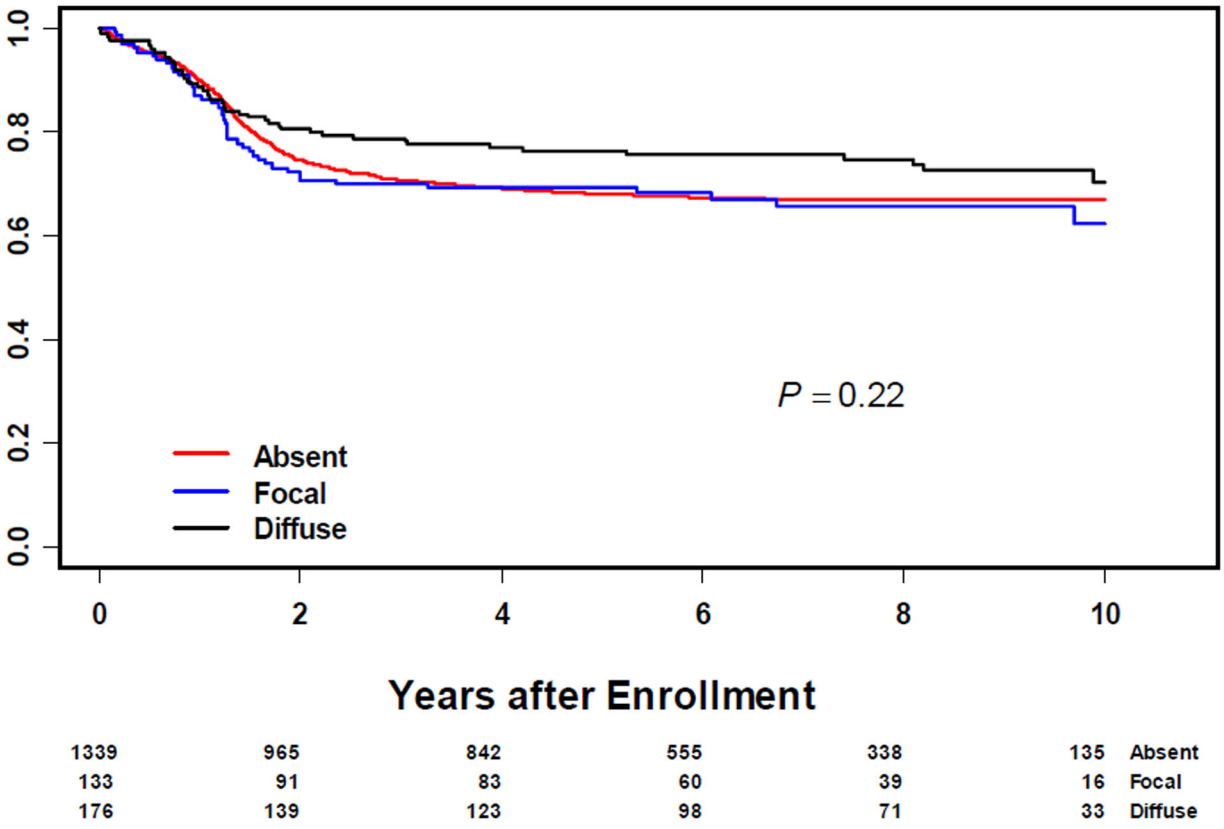

Figure 1:
Failure Free Survival in All Patients with Rhabdomyosarcoma by Anaplasia Status

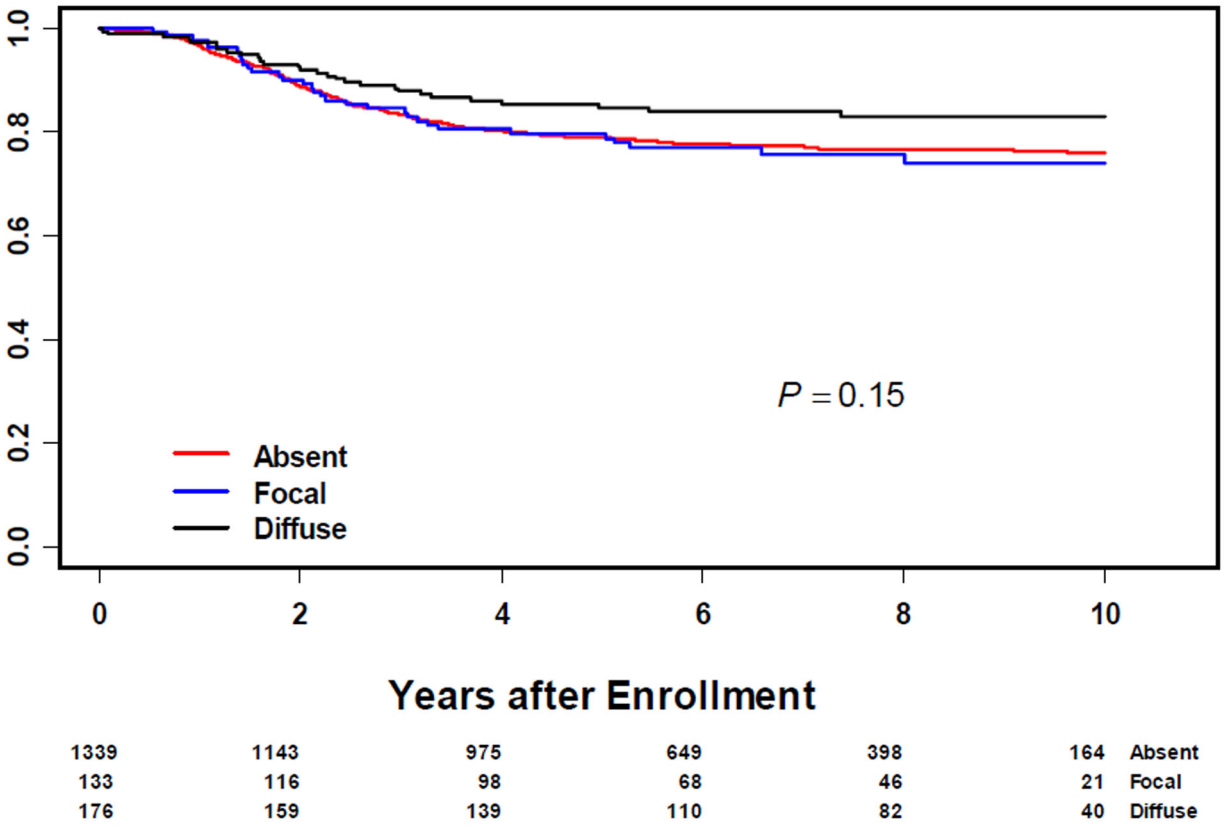

Figure 2:
Overall Survival in All Patients with Rhabdomyosarcoma by Anaplasia Status

Table 1:

Clinicopathologic characteristics of patients with Rhabdomyoscarcoma with and without anaplasia (Children's Oncology Group studies, 1997–2013)

Characteristic	Anaplasia			p-value[1]
	None (n=1339)	Focal (n=133)	Diffuse (n=176)	
Age, years				0.084
<1	67 (86%)	4 (5%)	7 (9%)	
1–9	821 (80%)	92 (9%)	118 (11%)	
≥10	451 (84%)	37 (7%)	51 (9%)	
Race				0.15
White	979 (81%)	103 (8%)	130 (11%)	
Non-white	235 (85%)	18 (6%)	25 (9%)	
Unknown	125 (79%)	2 (8%)	21 (13%)	
Sex				0.097
Male	807 (80%)	86 (9%)	116 (11%)	
Female	532 (83%)	47 (7%)	60 (9%)	
Clinical Group				<0.0001
I	210 (71%)	38 (13%)	46 (16%)	
II	229 (75%)	22 (7%)	56 (18%)	
III	801 (86%)	63 (7%)	63 (7%)	
IV	98 (84%)	10 (8%)	9 (8%)	
Unknown	1	-	-	
Stage				<0.0001
1	550 (76%)	63 (9%)	108 (15%)	
2	247 (86%)	17 (6%)	22 (8%)	
3	443 (85%)	43 (8%)	37 (7%)	
4	98 (84%)	10 (8%)	9 (8%)	
Unknown	1	-	-	
Primary Site				<0.0001[2]
All favorable sites	550 (76%)	65 (9%)	109 (15%)	
Orbit	169 (82%)	12 (6%)	25 (12%)	
Head and neck/non-PM	127 (84%)	8 (5%)	16 (11%)	
GU, nonbladder/prostate	254 (69%)	45 (12%)	68 (19%)	
All unfavorable sites	789 (86%)	68 (7%)	67 (7%)	
Parameningeal	332 (89%)	21 (6%)	22 (6%)	
Bladder/prostate	126 (88%)	10 (7%)	8 (6%)	
Extremity	128 (81%)	15 (9%)	16 (10%)	
Other	203 (83%)	22 (9%)	21 (8%)	
Tumor invasiveness				0.0002

Characteristic	Anaplasia			p-value[1]
	None (n=1339)	Focal (n=133)	Diffuse (n=176)	
T1	790 (78%)	87 (9%)	131 (13%)	
T2	546 (86%)	46 (7%)	45 (7%)	
Unknown	3	-	-	
Lymph node involvement				0.059
N0	1091 (80%)	119 (9%)	147 (11%)	
N1	237 (85%)	14 (5%)	27 (10%)	
Unknown	11	-	2	
Tumor size, cm				
≤5	763 (82%)	68 (7%)	104 (11%)	0.64
>5	547 (81%)	61 (9%)	70 (10%)	
Unknown	29	4	2	
Risk Group				
High Risk	98 (83%)	10 (9%)	9 (8%)	<0.0001
Intermediate Risk	573 (87%)	50 (7%)	37 (6%)	
Low Risk	667 (77%)	73 (8%)	130 (15%)	
Unknown	1	-		
Study				
D9602	278 (78%)	41 (11%)	38 (11%)	<0.0001
D9802	71 (89%)	5 (6%)	4 (5%)	
D9803	366 (79%)	52 (11%)	44 (10%)	
ARST0331	252 (75%)	18 (5%)	65 (19%)	
ARST0531	372 (90%)	17 (4%)	25 (6%)	

[1]Chi-square test assessing association with anaplasia status (present or absent), after excluding “Unknown” if applicable.

[2]Chi-square test assessing favorable vs unfavorable site with anaplasia status

Table 2:

Prevalence of Anaplasia by Histology[1]

	Anaplasia			p-value[2]
	None (n=1339)	Focal (n=133)	Diffuse (n=176)	
Histology				<0.0001
Embryonal	581 (73%)	88 (11%)	123 (16%)	
Botryoid	181 (93%)	5 (2%)	9 (5%)	
Spindle cell	133 (79%)	20 (12%)	15 (9%)	
Alveolar	386 (89%)	19 (4%)	28 (7%)	
FOXO1 +	247 (96%)	6 (2%)	5 (2%)	0.0003[3]
FOXO1 −	73 (83%)	6 (7%)	9 (10%)	
FOXO1 unknown	66 (76%)	7 (8%)	14 (16%)	
NOS[4]	58 (96%)	1 (2%)	1 (2%)	

[1] Determined by central histologic review

[2] Chi-square test assessing association with anaplasia status (present or absent)

[3] Chi-square test assessing association with anaplasia status (present or absent) with FOXO1 status in ARMS

[4] NOS (Not otherwise specified) = insufficient sample to determine status

Table 3:

Univariate and Multivariate Analysis of Prognostic Factors in all Patients with Embryonal Rhabdomyosarcoma

	Univariate Analysis		Multivariate Analysis	
	HR[1] (95% CI)	**P value**	**HR (95% CI)**	**p-value**
Failure Free Survival				
Intermediate risk	1.72 (1.30–3.27)	0.0001	2.37 (1.74–3.24)	<0.0001
High risk	3.55 (2.38–5.30)	<0.0001	5.35 (3.45–8.29)	<0.0001
Age ≥ 10 years	1.07 (0.79–1.46)	0.66	1.30 (0.95–1.78)	0.11
Anaplastic Morphology	1.16 (0.86–1.58)	0.34	1.25 (0.92–1.70)	0.16
Overall Survival				
Intermediate risk	2.28 (1.60–3.26)	<0.0001	4.33 (2.81–6.67)	<0.0001
High risk	5.57 (3.58–8.68)	<0.0001	12.05 (7.07–20.54)	<0.0001
Age ≥ 10 years	1.21 (0.82–1.76)	0.34	1.64 (1.11–2.42)	0.013
Anaplastic Morphology	1.26 (0.86–1.83)	0.23	1.41 (0.96–2.05)	0.078

[1]Hazard ratio

Table 4:

Subset of Rhabdomyosarcoma Patients with known TP53 Mutation Status

TP53 mutation	No anaplasia (n=108)	Focal anaplasia (n=16)	Diffuse anaplasia (n=22)
Absent in tumor	104	11	18
Present in tumor	4	5	4