Comparison of Clinical Features and Outcomes in Patients with Extraskeletal Versus Skeletal Localized Ewing Sarcoma: A Report from the Children's Oncology Group

Thomas Cash[1], Elizabeth McIlvaine[2], Mark D. Krailo[2], Stephen L. Lessnick[3], Elizabeth R. Lawlor[4], Nadia Laack[5], Joel Sorger[6], Neyssa Marina[7], Holcombe E. Grier[8], Linda Granowetter[9], Richard B. Womer[10], Steven G. DuBois[11]

Author Justification: All authors included on this manuscript have made significant contributions and warrant inclusion as co-authors. Thomas Cash is the lead author and investigator on this manuscript. Elizabeth McIlvaine and Mark Krailo performed the statistical analysis and both put in significant time to this study. Stephen Lessnick provided content expertise for our biologic and genomic comparisons and interpretation of this data. Elizabeth Lawlor provided our gene expression data and performed some of this analysis. Nadia Laack and Joel Sorger provided expertise in Radiation Oncology and Orthopedic Oncology, respectively, and provided insight into the approach to local control for these patients with extraskeletal tumors; this was critical to designing our analysis and interpreting the results. Neyssa Marina provided clinical expertise which shaped our understanding of our data analysis and interpretation. Holcombe Grier has been a leader in the field of Ewing sarcoma for the past two decades, played a role in the clinical trials included in this study, and helped in the design, interpretation and writing of this study. Linda Granowetter (INT-0154) and Richard Womer (AEWS0031) served as the principal investigators for the clinical trials included in this trial, and as such provided invaluable insight at every step. Steven DuBois served as senior author and oversaw all components of this study.

Institutional Affiliations and Addresses:

[1]Department of Pediatrics, Emory University, Children's Healthcare of Atlanta, Health Sciences Research Building, Brumley Bridge, 3rd Floor, W-350, 1760 Haygood Drive, Atlanta, GA 30322;

[2]Department of Preventive Medicine, Keck School of Medicine, University of Southern California, 2001 N Soto Street, SSB 210C, MC 9234, Los Angeles, CA 90032; [3]Center for Childhood Cancer and Blood Disorders at Nationwide Children's Hospital and the Division of Hematology, Oncology and BMT at The Ohio State University, 700 Children's Drive, WA5011, Columbus, OH 43205; [4]Department of Pediatrics, University of Michigan, D4204 Med Prof Building Box 5718, 1500 East Med Center Drive, Ann Arbor, MI 48109-5718; [5]Department of Radiation Oncology, Mayo Clinic, 200 First St SW, Rochester, MN 55905; [6]Department of Orthopedics, Cincinnati Children's Hospital Medical Center, 3333 Burnet Ave, ML 2017, Cincinnati, OH 45229; [7]Department of Pediatrics, Stanford University School of Medicine and Lucille Packard Children's Hospital at Stanford, 1000 Welch Rd., Suite 300, Mail Code 5798, Palo Alto, CA 94304-1812; [8]Department of Pediatrics, Children's Hospital Boston / Dana-Farber Cancer Institute and Harvard Medical School, 44 Binney Street, Boston, MA 02115; [9]Department of Pediatrics, NYU School of Medicine and NYU Langone Medical Center, 160 East 32nd Street, 2nd Floor, New York, NY 10016; [10]Department of Pediatrics, University of Pennsylvania Perelman School of Medicine and Children's Hospital of Philadelphia, 324 South 34th St, Philadelphia, PA 19104; [11]Department of Pediatrics, UCSF School of Medicine and UCSF Benioff Children's Hospital, 550 16th Street, 4th Floor, San Francisco, CA 94158.

Corresponding Author:

Thomas Cash, M.D., M.Sc.

Health Sciences Research Building

Brumley Bridge, 3rd Floor, W-350

1760 Haygood Drive

Atlanta, GA 30322

Tel: 404-785-0910

Fax: 404-727-4455

E-mail: Thomas.Cash@choa.org

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Abbreviations	
ES	Ewing sarcoma
EFS	Event-free survival
EES	Extraskeletal Ewing sarcoma
ESR	Erythrocyte sedimentation rate
PNET	Primitive neuroectodermal tumor
COG	Children's Oncology Group
IRB	Institutional Review Board
LDH	Lactate dehydrogenase
DAVID	Database for annotation, visualization and integrated discovery
OS	Overall survival
HR	Hazard ratio
CI	Confidence interval

Abstract

BACKGROUND

The prognostic significance of having extraskeletal vs. skeletal Ewing sarcoma in the setting

of modern chemotherapy protocols is unknown. The purpose of this study was to compare the

clinical characteristics, biologic features, and outcomes for patients with extraskeletal and skeletal Ewing sarcoma.

METHODS

Patients had localized Ewing sarcoma (ES) and were treated on two consecutive protocols using 5-drug chemotherapy (INT-0154 and AEWS0031). Patients were analyzed based on having an extraskeletal (n=213) or skeletal (n=826) site of tumor origin. Event-free survival (EFS) was estimated using the Kaplan-Meier method, compared using the log-rank test, and modeled using Cox multivariate regression.

RESULTS

Patients with extraskeletal Ewing Sarcoma (EES) were more likely to have axial tumors (72% vs. 55%; $P < 0.001$), less likely to have tumors > 8 cm (9% vs. 17%; $P < 0.01$), and less likely to be white (81% vs. 87%; $P < 0.001$) compared to patients with skeletal ES. There was no difference in key genomic features (type of *EWSR1* translocation, *TP53* mutation, *CDKN2A* mutation/loss) between groups. After controlling for age, race, and primary site, EES was associated with superior EFS [hazard ratio = 0.69; 95% CI: 0.50-0.95; $P = 0.02$]. Among patients with EES, age ≥ 18 years, non-white race, and elevated baseline erythrocyte sedimentation rate (ESR) were independently associated with inferior EFS.

CONCLUSION

Clinical characteristics, but not key tumor genomic features, differ between EES and skeletal ES. Extraskeletal origin is a favorable prognostic factor, independent of age, race, and primary site. **INTRODUCTION**

Ewing sarcoma (ES) is the second most common malignant bone tumor of childhood. It most commonly arises from bone but can develop in extraskeletal sites. Based upon data from clinical trials and from large registry data, extraskeletal Ewing sarcoma (EES) accounts for approximately 20% to 30% of cases.[1,2] Historically these patients were treated on rhabdomyosarcoma protocols, however it is now recognized that patients with EES benefit from treatment protocols for patients with Ewing sarcoma of the bone.[2-5]

Previous reports have suggested that there may be clinical differences between patients with extraskeletal and skeletal Ewing sarcoma.[1,3,4,6,7] For example, patients with extraskeletal disease have been reported to be older and to have a propensity for axial tumor origin. The prognostic significance of having an EES using contemporary treatment protocols remains unclear, though two more recent reports have suggested superior outcomes for these patients.[1,7] Likewise, it is unknown if the approach to local control of the primary tumor differs based upon tissue of origin. Moreover, potential biologic differences between skeletal and extraskeletal tumors remain largely unexplored.

In order to address these gaps in our knowledge, we used a large cohort of patients treated on two consecutive cooperative group clinical trials to compare the clinical features, approach to local control, and outcomes in patients with extraskeletal versus skeletal localized Ewing sarcoma. Additionally, we explored potential biologic differences between these two groups, providing a comprehensive evaluation of differences in somatic mutations and gene expression.

METHODS

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Patients

The cohort included eligible patients treated on all arms of the cooperative group trials INT-0154 and AEWS0031.[2,8] Eligible patients were those with newly diagnosed, localized Ewing sarcoma or primitive neuroectodermal tumor (PNET) of the bone or soft tissue who were diagnosed based on having histological and/or immunohistochemical findings consistent with Ewing sarcoma or PNET. Information regarding translocation status was used as supportive data but was not required for study eligibility. Patients were aged ≤ 30 years for INT-0154 and ≤ 50 years for AEWS0031. Patients with unknown skeletal vs. extraskeletal status were excluded from this analysis. There were no other exclusion criteria. Details of the treatment on these two trials have previously been published, but all patients received alternating cycles of vincristine-doxorubicin-cyclophosphamide and ifosfamide-etoposide given on an every two- or three-week basis.

Patients in this analysis were treated primarily at Children's Oncology Group (COG) centers located in the United States and Canada. Institutional review board (IRB) approval was obtained locally by each participating center, with written informed consent obtained for all patients prior to enrollment. This secondary analysis utilized de-identified data and was exempt from separate IRB review.

Primary Predictor Variable

Patients were categorized for analysis as having extraskeletal or skeletal Ewing sarcoma. For both INT-0154 and AEWS0031, any tumor with any degree of bone involvement was considered a primary bone tumor. Initial designation of extraskeletal origin was based upon site report and confirmed by study chair review of baseline imaging reports, when available. For AEWS0031, records of this review were maintained and there were originally

136 patients designated as extraskeletal by the treating facility. Baseline imaging reports were available for the study chair to review in 118 of these 136 patients. For the 18 patients that did not have baseline imaging reports available for review, the original designation of extraskeletal from the treating facility was maintained. Of the 118 patients with available imaging reports, the study chair determined on his review that 17 patients had skeletal tumors, and so these 17 patients were reclassified, resulting in a final cohort of 119 patients with extraskeletal ES from AEWS0031.

Outcome Variables

Primary sites were categorized into groups based on the involved body compartment. To account for the various extraskeletal locations within a given body compartment, all structures including skin, subcutaneous or connective tissue, lymph nodes, muscles, and organs were included in the given specific site designation. The one exception was sites designated as paraspinal that included primary sites involving or adjacent to the cervical, thoracic, and lumbar spine. The sites were classified as follows: head and neck; thorax and abdomen; pelvis; proximal extremity (proximal humerus through elbow, proximal femur through knee); distal extremity (below elbow through wrist and hand, below knee through ankle and foot); paraspinal; and "other". Primary sites were further categorized for analysis as either axial or non-axial and pelvic or non-pelvic.

In addition to primary site, the following clinical variables were analyzed: age; sex; race; tumor size; baseline lactate dehydrogenase (LDH); baseline erythrocyte sedimentation rate (ESR); and type of local control. Age was further categorized as either < 18 or ≥ 18 years of age [9], and tumor size further categorized as ≤ 8 or >8 cm in maximum dimension. Tumor size data were not collected on AEWS0031. For LDH, the institutional upper limit of normal

was used to divide patients into those with elevated LDH values and those with normal values. For ESR, the 75th percentile of the entire cohort was used as a cut point to divide patients into those at or above the 75th percentile and those below. Clinical outcomes of interest included death, second malignancy, relapse/progression, and type of first relapse/progression (local failure; distant failure; or combined failure).

Information regarding tumor biology was also obtained where available and included type of *EWSR1* translocation as determined in a prior study.[10] Presence or absence of a *TP53* mutation or *CDKN2A* mutation/loss were assessed in a subset of patients included in a retrospective analysis.[11] To investigate differences in gene expression between EES and skeletal ES, we interrogated Affymetrix expression array data from a cohort of 46 patients (GEO: GSE63157).[12]

Statistical Methods

Categorical variables were compared between patients with extraskeletal and skeletal Ewing sarcoma using two-sided Fisher exact or chi-squared tests. Continuous variables were compared between groups using the Wilcoxon rank sum test. Patients who had missing data for a given characteristic were not included in the statistical tests for differences between groups.

The primary outcome of interest was event-free survival (EFS) which was defined as the time elapsed between study entry and either the occurrence of an analytic event or the date of the last patient contact, whichever came first. Disease progression, diagnosis of any second malignant neoplasm, and death were considered analytic events. Patients who had not experienced an event as of their last contact were considered censored. Overall survival was a secondary outcome and was defined as time from study entry to death or last follow-up for

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surviving patients. Event-free and overall survival distributions were estimated by the

Kaplan-Meier method. Confidence intervals were calculated using the complementary log-

log distribution of the Kaplan-Meier estimate. Differences in event risk between groups in the

univariate setting were evaluated by the log-rank test. Those baseline variables with a

statistically significant effect on EFS based on the log-rank test were considered candidate

variables for regression models, though variables with extensive missing data, such as tumor

size, were removed from potential consideration. Backwards selection was used to generate a

Cox regression model with a threshold p-value of 0.05 to be retained in the model. For the

regression model containing all patients, the variable defining EES vs. skeletal ES was

retained throughout all models.

Differences in pattern of relapse between patients with EES and skeletal ES were examined

by way of cumulative incidence analysis. The relapse types of interest were local-only

progression, distant-only progression, and local plus distant progression. For each relapse

type of interest, the cumulative incidence distributions were estimated by the method of

Marubini and Valsecchi.[13] Tests for differences between groups in the incidence of the

relapse type of interest were conducted by way of competing risks regression using the

method of Fine and Gray.[14]

For gene expression studies, differentially expressed genes were identified as described

previously.[12] Briefly, non-annotated transcripts and transcripts with low expression in at

least 25% of samples (log2 signal < 2.6) and with low variability across all samples

(interquartile range of log2 signals < 0.5) were excluded. A moderated t-test was used to

identify differential expression between the remaining 4,615 annotated genes, as described

previously. [12] Genes with a fold change of at least 1.5 and a p-value of <0.05 were deemed

to be differentially expressed. The Database for Annotation, Visualization and Integrated

Discovery (DAVID) v6.7 was used for the functional enrichment analysis of the differentially expressed.[15]

All statistical analyses were performed using SAS version 9.2 (SAS Institute, Inc., Cary, NC) and STATA version 12 (StataCorp, College Station, TX).

RESULTS

Clinical Features Differ Between Extraskeletal and Skeletal Ewing Sarcoma

Of the 1039 localized Ewing sarcoma patients included in our analysis, 213 (20.5%) had EES while 826 (79.5%) had skeletal tumors. A comparison of the clinical features by site is shown in Table I. There was no difference in age at diagnosis between the two groups. Patients with EES were more likely to be non-white compared to patients with skeletal ES (14% vs. 6%; P < 0.001). The distribution of primary sites differed between the two groups. Patients with EES were more likely to have tumors arising in axial locations (72% vs. 55%; P < 0.001), but showed a trend towards having fewer pelvic primary sites (14% vs. 19%; P = 0.07). Patients with EES were more likely to have tumors < 8 cm (21% vs. 16%; P < 0.01). There was no significant difference in baseline LDH and ESR values.

Local control strategies differed between groups. Compared to patients with skeletal ES, those with EES were more likely to receive a combined modality approach (surgery plus radiation) for local control (30% vs. 17%; P < 0.01). We evaluated this pattern more closely using data available from AEWS0031. The majority of patients who received a combined modality approach received post-operative radiotherapy, with similar rates between EES and skeletal ES (91% and 83%, respectively). Margin status was available for 65 of the patients

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from AEWS0031 included in this analysis who received post-operative radiotherapy. Review of these data suggested that a greater proportion of patients with EES treated with post-operative radiotherapy had positive surgical margins compared to skeletal ES (66% vs. 48%).

Clinical Outcomes Differ Between Localized Extraskeletal and Skeletal Ewing Sarcoma

Event-free survival (EFS) and overall survival (OS) estimates are shown in Figure 1. The 5-year unadjusted EFS for patients with EES was 76% (95% confidence interval (CI): 69-81%) compared to 69% (95% CI: 65-72%) for patients with skeletal ES (P = 0.05; Figure 1A). The 5-year unadjusted OS for patients with EES was 85% (95% CI: 80-90%) compared to 78% (95% CI: 75-81%) for patients with skeletal ES (P = 0.11; Figure 1B). We also performed a sensitivity analysis of EFS focused exclusively on patients treated on AEWS0031 and observed a significantly decreased risk for event for patients with EES compared to skeletal ES [hazard ratio (HR) = 0.52; 95% CI 0.33-0.82; P = 0.005].

We constructed a Cox proportional hazards model in the full analytic cohort to assess the prognostic impact of extraskeletal origin while controlling for other known risk factors. After controlling for age, race, and primary site, patients with EES were at reduced risk for an event compared to patients with skeletal tumors (HR = 0.69; 95% CI: 0.50-0.95; P = 0.02).

We next evaluated whether the type of relapse differed between EES and skeletal ES patients. There were no statistically significant differences in the cumulative incidence of local, distant, or combined relapses between the two groups, though there was a trend to suggest higher incidence of isolated distant failure in patients with skeletal ES (data not shown).

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We next focused exclusively on patients with EES. We first performed a univariate analysis of EFS and found that age, race, and ESR were significantly associated with risk of an event (Table II). These variables were also significant predictors of OS, though we also observed a trend in which patients with pelvic EES had superior OS (HR=0.18; 95% CI: 0.02-1.29; P=0.09; Table II).   We then constructed a Cox proportional hazards model to identify independent prognostic factors for EFS just in the EES group. Age $\geq$ 18 years (HR 2.87; 95% CI 1.28-6.45; P = 0.01), non-white race (HR 2.86; 95% CI 1.31-6.25; P < 0.01), and elevated baseline ESR (HR 2.68; 95% CI 1.29-5.54; P < 0.01) were each independently associated with inferior outcome. Due to the extensive amount of missing data regarding tumor size in EES patients, we were not able to include this variable in our multivariate model.

Biologic Features in Extraskeletal and Skeletal Ewing Sarcoma

Data on *EWSR1* translocation status, *TP53* mutation, and *CDKN2A* status were available for 112, 93, and 107 patients, respectively, whose tumors have previously been profiled and results published (Table III).[10,11] There were no differences in the frequency of harboring any *EWSR1* translocation (P = 0.84) or, among those with *EWSR1/FLI1* translocations, in the subtype of *EWSR1/FLI1* translocation (P = 0.33) between EES and skeletal Ewing sarcoma. Likewise, the frequency of *TP53* mutation or *CDKN2A* mutation/loss did not differ between EES and skeletal Ewing sarcoma.

We next compared gene expression profiles between EES and skeletal ES in 46 tumors previously profiled as part of an earlier study.[12] As previously reported, unsupervised hierarchical clustering of these 46 tumors with available data failed to discriminate EES and skeletal ES. Similarly, supervised hierarchical clustering of these tumors did not yield a clear

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differential pattern of gene expression between these two clinical groups at a whole genome level (Figure 2A). To determine if significant differences in expression exist at the level of individual genes or gene families, we performed transcript-level analyses, as described in the Methods. These analyses identified 119 genes as being differentially expressed between EES and skeletal tumors. Forty-six genes were more highly expressed in EES tumors while 73 were relatively up regulated in bone tumors (Figure 2B). Gene ontology analysis revealed a significant enrichment of skeletal system development and extracellular matrix genes among the 73 bone tumor-associated genes (Figure 2C). No specific biologic processes were enriched among EES tumor-associated genes.

DISCUSSION

In this large cohort of patients with localized Ewing sarcoma treated with modern chemotherapy protocols, we confirmed that there are important clinical differences between EES and skeletal ES patients. We also found that extraskeletal tumor origin is a favorable prognostic factor, independent of age, race, and tumor site. We determined that older age, non-white race, and elevated baseline ESR are independent adverse prognostic factors in patients with EES. We found that the pattern of relapse does not differ between EES and skeletal ES patients. We did not find significant differences in key genomic features between extraskeletal or skeletal tumors, a null finding that nevertheless improves our understanding of potential biological differences between groups. We also highlight differences in gene expression between these two groups.

Consistent with previous reports, we found that EES accounts for 20.5% of cases of Ewing sarcoma.[1] We confirmed the findings of previous studies that showed that compared to

patients with skeletal ES, those with EES are more likely to be non-white with a non-pelvic, axial primary site. Data comparing tumor size between EES and skeletal ES are conflicting. Some studies have shown that EES tumors are smaller at diagnosis, while others have shown no difference.[1,3,7,16] We observed that EES patients were more likely to have smaller tumors (< 8 cm) compared to patients with skeletal ES. Interestingly EES patients with a pelvic primary site showed a trend towards improved overall survival, suggesting that this known adverse prognostic factor in skeletal ES may have the opposite effect in EES. Patients with EES in our study were more likely to receive a combined modality approach (surgery plus radiation) for local control. This is potentially due to the higher proportion of axial tumors seen in EES, a site which may be less amenable to complete resection with negative margins. Alternatively, it is possible that these patients were more likely to undergo upfront excisional biopsy without adequate margins, necessitating subsequent radiotherapy. Our results support a higher rate of positive surgical margins in this group of patients.

Prior studies have suggested that the outcomes for EES and skeletal ES are similar when treated with Ewing sarcoma protocols.[3-5,6,17,18] However, two more recently published studies reported improved survival for EES in comparison to skeletal ES.[1,7] Our findings are consistent with these more recent observations and increase the evidence base supporting a more favorable outcome for EES. The etiology for this pattern is not clear. While patients with EES were more likely to receive combined modality local control, previous analyses have not demonstrated improved outcomes with this approach, making this an unlikely explanation for the observed differences.[16,19] Differences in key genomic features in this disease are not a likely explanation as we did not observe such differences in our analysis. However, differential distribution of chemotherapy to bone vs. soft tissue sites and other

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differences in tumor microenvironment and angiogenesis identified in our gene expression analysis may provide clues for future investigation.

Prognostic factors identified specifically in patients with EES were similar to those reported for ES in general.[20-22] Elevated baseline ESR was significantly associated with inferior EFS among EES patients. Elevated ESR has been reported as an adverse prognostic factor in a variety of adult and pediatric cancers, including Ewing sarcoma in some studies.[23-31] We report for the first time the significance of ESR in patients with localized EES. ESR is known to be a marker of systemic inflammation, but the exact explanation for its prognostic implications in cancer remains unclear. There is a growing body of evidence that shows the importance of inflammation in all stages of tumor development, from initiation, to malignant transformation, to local invasion and establishment of metastatic niche.[32-35] Additionally, inflammatory cells have been shown to contribute to angiogenesis, immune suppression, and the establishment of the tumor microenvironment, which has been proven to be indispensable to the malignant conversion of cells and to the survival of the tumor.[32-35] Alternatively, increased ESR could be a marker of greater systemic burden of disease not appreciable on imaging (i.e. micrometastases), though we did not find that baseline LDH was prognostic in EES.

Recent literature has described a rare subset of highly aggressive *EWSR1* translocation-negative sarcomas that have distinct genetic signatures and have been referred to as undifferentiated small round cell sarcomas or Ewing-like sarcomas.[36-41] Of the patients with available translocation status available, our study included 13 translocation-negative tumors. While it is possible that our cohort included tumors harboring one of the mutations commonly seen in Ewing-like sarcomas such as *CIC-DUX4* or *BCOR-CCNB3*, the potential

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impact on our study is minimal given the small proportion of translocation-negative tumors and the fact that these tumors were balanced between groups.

One of the main strengths of our study was our ability to analyze a large cohort of EES patients treated with modern chemotherapy protocols and compare them to skeletal ES patients. Being able to confirm the diagnosis of extraskeletal ES for EES patients by review of the institutional baseline imaging reports increased the reliability of this designation in our study. We provide a description of the biologic features and genetic profiling in the largest cohort of EES patients. However, the conclusions that can be drawn from these comparisons are limited due to the relatively small sample size with available biological parameters. An additional limitation was that we lacked tumor size data on over half of the entire cohort, which limited our ability to control for this well-established prognostic factor in our multivariate models. Since patients with EES tended to have smaller tumors, differences in tumor size could potentially confound our observed relationship between tissue origin and prognosis. It is also possible that specific rare subgroups such as cutaneous ES may behave differently than the entire subgroup of EES.[42] We also acknowledge that the group of patients enrolled to COG studies is enriched for younger patients. This may account for the fact that we did not note a difference in age distribution between EES and skeletal ES, as has been reported by others.[1] In order to evaluate a more homogeneous patient population treated with similar chemotherapy, we focused our analysis on patients with localized disease. The extent to which our findings will generalize to patients with metastatic disease is unknown. We were also unable to confirm extraskeletal origin for all patients, particularly for patients on trial INT-0154. However, a sensitivity analysis focused exclusively on patients from AEWS0031 confirmed that patients with EES have superior EFS compared to patients with skeletal ES. Finally, while all patients received 5-drug therapy typical for this

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disease in North America, our sample size precluded sub-analyses based upon randomized arm of each of the included trials.

In conclusion, we demonstrate that clinical characteristics, but not key tumor genomic features, differ between EES and skeletal ES patients. We report for the first time differences in gene expression between these two groups. Prognostic factors in EES are similar to those established in patients with skeletal disease and ESR provides additional prognostic information. Additional efforts should be made to obtain specimens for biologic testing and gene profiling so that more robust comparisons of the genomic features analyzed in our study as well as newer mutations such as *STAG2* can be made between these two groups of patients. While outcomes are statistically superior for EES, the clinical difference is relatively modest. This finding together with the rarity of Ewing sarcoma supports the current practice of treating these two subgroups with similar approaches.

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Conflict of Interest Statement

None of the authors have any conflicts of interest related to this work or its publication. Author S.L.L. has a leadership role and stock/ownership interest in Salarius Pharmaceuticals, however, again, this is not a conflict for this study.

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LEGENDS

Figure 1. Kaplan-Meier estimates comparing 5-year **A**) event-free survival (EFS) and **B**) overall survival (OS) between patients with extraskeletal vs. skeletal localized Ewing sarcoma.
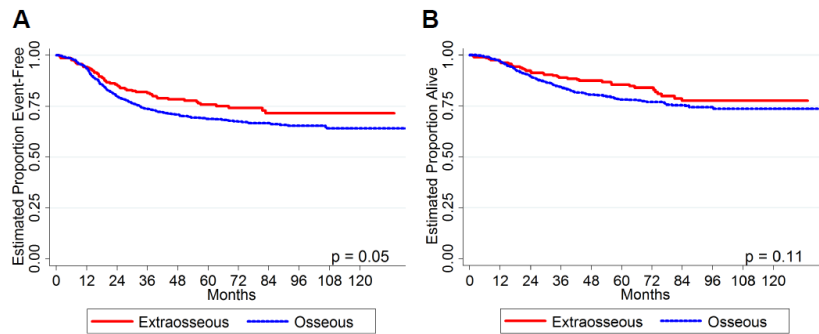
Figure 2. (A) Supervised hierarchical clustering reveals that genome wide expression profiles of extraskeletal and skeletal Ewing sarcoma tumors are largely equivalent. Clustering performed using 4,615 annotated and expressed genes. **(B)** Supervised clustering of differentially expressed genes (N=119 genes) between skeletal and extraskeletal Ewing sarcoma tumors (N= 46 tumors). Clustering limited to genes with fold changes of >1.5 and p<0.05. **(C)** Top 10 most enriched biologic processes among genes that are up regulated in skeletal compared to extraskeletal tumors.

TABLE I. Comparison of Clinical Features between Patients with Extraskeletal Versus Skeletal Localized Ewing Sarcoma

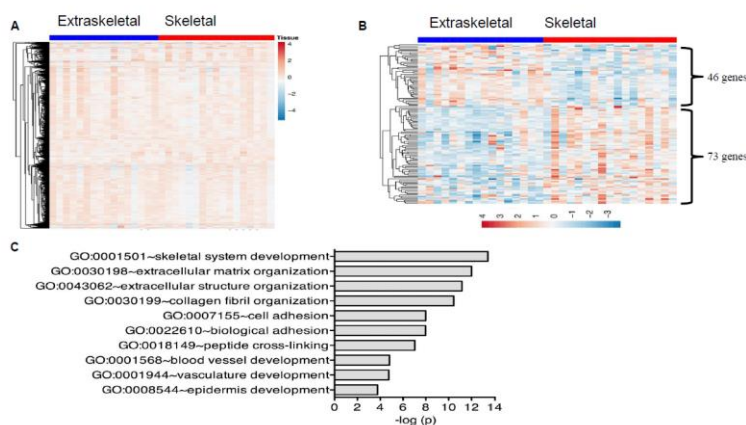| Characteristic	Extraskeletal[#] (n=213)	Skeletal[#] (n=826)	P-value
Median age (range), y	12 (0 – 30)	12 (0 – 45)	0.46[1]
Age			0.91[2]
< 18 years	187 (88%)	721 (87%)	
≥ 18 years	26 (12%)	105 (13%)	
Sex			1.0[2]
Male	116 (55%)	449 (54%)	
Female	97 (45%)	377 (46%)	
Race			< 0.001[2]
White	172 (81%)	721 (87%)	
Non-white	29 (14%)	51 (6%)	
Unknown	12 (6%)	54 (7%)	
Primary site			< 0.001[3]
Distal extremity	22 (10%)	191 (23%)	
Proximal extremity	34 (16%)	179 (22%)	
Pelvis	29 (14%)	160 (19%)	
Spinal/Paraspinal	15 (7%)	76 (9%)	
Thorax/Abdomen	92 (43%)*	164 (20%)	
Head and Neck	17 (8%)	56 (7%)	
Unknown	4 (2%)	0 (0%)	
Primary site			< 0.001[2]
Axial	153 (72%)	456 (55%)	
Non-axial	56 (26%)	370 (45%)	
Unknown	4 (2%)	0 (0%)	
Primary site			0.07[2]
Pelvic	29 (14%)	160 (19%)	
Non-pelvic	180 (85%)	666 (81%)	
Unknown	4 (2%)	0 (0)%	
Median tumor size (interquartile range), cm	6 (4-9)	9 (6-12)	<0.001[1]
Tumor size, cm			<0.01[2]
≤ 8	44 (21%)	136 (16%)	
> 8	18 (9%)	137 (17%)	
Unknown	151 (71%)	553 (67%)	
Lactate Dehydrogenase (LDH)			0.44[2]
Normal	135 (63%)	478 (58%)	
Elevated	61(29%)	249 (30%)	
Unknown	17 (8%)	99 (12%)	
Erythrocyte Sedimentation Rate (ESR)			0.51[2]
≤ 46 mm/hr	106 (50%)	397 (48%)	
> 46 mm/hr	32 (15%)	140 (17%)	
Unknown	75 (35%)	289 (35%)	
Local control			< 0.01[3]
Surgery	99 (46%)	435 (53%)	
Radiation	34 (16%)	166 (20%)	
Surgery + radiation	63 (30%)	138 (17%)	
Unknown	17 (8%)	87 (11%)	
Study			0.70[2]
INT-0154	94 (44%)	377 (46%)	
AEWS0031	119 (56%)	449 (54%)	

[1] Two-sided Wilcoxon test; [2] Two-sided Fisher Exact test; [3] Likelihood Ratio Chi-Squared test;

*Includes 13 cases of primary renal Ewing sarcoma; #Totals do not sum to 100% due to rounding.

TABLE II. Results of Univariate Analysis of Event-Free And Overall Survival for Patients with

Localized Extraskeletal Ewing Sarcoma

Characteristic	HR for Event (95% CI)	P-value[1]	HR for Death (95% CI)	P-value[1]
Age < 18 years ≥ 18 years	 Ref 2.63 (1.35-5.12)	0.005	 Ref 3.14 (1.47-6.69)	0.003

Sex
  Female
  Male
	
	Ref
	0.91 (0.53-1.56)
		0.74	
			Ref
			1.07 (0.56-2.04)
				0.84

Race
  White
  Non-white
	
	Ref
	2.62 (1.39-4.95)
		0.003	
			Ref
			3.51 (1.71-7.22)
				0.001

Primary Site
  Thorax/Abdomen
  Distal extremity
  Proximal extremity
  Pelvis
  Spinal/Paraspinal
  Head and Neck
	
	Ref
	0.48 (0.14-1.60)
	1.25 (0.61-2.55)
	0.63 (0.24-1.66)
	1.31 (0.50-3.44)
	0.81 (0.28-2.33)
		0.53	
			Ref
			0.69 (0.20-2.35)
			1.36 (0.60-3.05)
			0.18 (0.02-1.36)
			1.44 (0.48-4.28)
			0.57 (0.13-2.46)
				0.17

Primary Site
  Non-axial
  Axial
	
	Ref
	1.01 (0.54-1.86)
		0.99	
			Ref
			0.76 (0.38-1.52)
				0.44

Primary Site
  Non-pelvic
  Pelvic
	
	Ref
	0.64 (0.26-1.62)
		0.35	
			Ref
			0.18 (0.02-1.29)
				0.09

Tumor size, cm
  ≤ 6
  > 6
	
	Ref
	0.85 (0.34-2.12)
		0.73	
			Ref
			1.14 (0.40-3.25)
				0.81

Lactate Dehydrogenase (LDH)
  Normal
  Elevated
	
	Ref
	1.03 (0.56-1.88)
		0.93	
			Ref
			1.49 (0.75-2.95)
				0.26

Erythrocyte Sedimentation Rate (ESR)
  ≤ 46 mm/hr
  > 46 mm/hr
	
	Ref
	2.49 (1.25-4.96)
		0.01	
			
			Ref
			3.02 (1.33-6.83)
				0.01

Study
  AEWS0031
  INT-0154
	
	Ref
	1.69 (0.97-2.94)
		0.06	
			Ref
			1.68 (0.85-3.32)
				0.14

Abbreviations: HR=hazard ratio; Ref=reference group; CI=confidence interval.
[1]P-value, relative hazard and 95% confidence interval calculated using a proportional hazards regression model with the noted characteristic as the only variable in the model.

Characteristic	Extraskeletal	Skeletal	P-value[1]

Translocation			0.84
EWSR1-FLI1	22 (78%)	67 (81%)	
Other	3 (11%)	6 (7%)	
Negative	3 (11%)	10 (12%)	
EWSR1-FLI1			0.33
Translocation	15 (68%)	44 (66%)	
Type 1	6 (27%)	12 (18%)	
Type 2	1 (5%)	11 (16%)	
Other			
TP53 mutation			0.18
Mutation	3 (17%)	5 (7%)	
Normal	15 (83%)	70 (93%)	
CDKN2A mutation / loss			1.0
Yes	2 (9%)	10 (12%)	
No	21 (91%)	74 (88%)	

[1]Two-sided Fisher Exact test