VOLUME 28 · NUMBER 12 · APRIL 20 2010

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Current Treatment Protocols Have Eliminated the Prognostic Advantage of Type 1 Fusions in Ewing Sarcoma: A Report From the Children's Oncology Group

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See accompanying editorial on page 1973 and article on page 1982

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Submitted June 15, 2009; accepted December 8, 2009; published online ahead of print at www.jco.org on March 22, 2010.

Supported by National Institutes of Health (NIH) Strategic Partnering to Evaluate Cancer Signatures Grant No. 1U01CA11475-04, NIH Grant No. T32 CA09659, National Cancer Institute Grant No. CA98543-07, Children's Oncology Group Statistics and Data Center Grant No. SDC CA98413, and the My Brother Joey and T.J. Martell Foundations.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

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0732-183X/10/2812-1989/$20.00

DOI: 10.1200/JCO.2009.24.5845

ABSTRACT

Purpose
Ewing sarcoma family tumors (ESFTs) exhibit chromosomal translocations that lead to the creation of chimeric fusion oncogenes. Combinatorial diversity among chromosomal breakpoints produces varying fusions. The type 1 *EWS-FLI1* transcript is created as a result of fusion between exons 7 of *EWS* and 6 of *FLI1*, and retrospective studies have reported that type 1 tumors are associated with an improved outcome. We have re-examined this association in a prospective cohort of patients with ESFT treated according to current Children's Oncology Group (COG) treatment protocols.

Methods
Frozen tumor tissue was prospectively obtained from patients diagnosed with ESFT, and reverse transcriptase polymerase chain reaction (RT-PCR) was used to determine translocation status. Analysis was confined to patients with localized tumors who were diagnosed after 1994 and treated according to COG protocols. Translocation status was correlated with disease characteristics, event-free survival (EFS), and overall survival (OS).

Results
RT-PCR identified chimeric fusion oncogenes in 119 of 132 ESFTs. Eighty-nine percent of identified transcripts were *EWS-FLI1*, and of these, 58.8% were type 1. Five-year EFS and OS rates for patients with type 1 and non–type 1 fusions diagnosed between 2001 and 2005 were equivalent (type 1: EFS, 63% ± 7%; OS, 83% ± 6%; non–type 1: EFS, 71% ± 9%; OS, 79% ± 8%).

Conclusion
Current intensive treatment protocols for localized ESFT have erased the clinical disadvantage that was formerly observed in patients with non–type 1 *EWS-FLI1* fusions.

J Clin Oncol 28:1989-1994. © 2010 by American Society of Clinical Oncology

INTRODUCTION

The Ewing sarcoma family of tumors (ESFT) are highly malignant neoplasms of bone and soft tissue that affect approximately one per 2.9 million people younger than 20 years of age.[1] Despite multimodality therapy, overall survival (OS) is approximately 70% for patients with localized ESFT and only 20% to 30% for patients with metastatic disease.[2,3] Various clinicopathologic variables have been investigated as prognostic indicators, with hopes that clinicians may tailor therapy for tumors predicted to have favorable versus refractory response. Evidence of gross metastatic disease at the time of diagnosis is the worst prognostic factor, in particular for patients with extrapulmonary metastases.[2] Adverse outcome has also been associated with older age at presentation (age ≥ 14 years[2,4] or ≥ 18 years[5]), larger tumor volume,[4,6,7] poor response to induction therapy,[4] axial tumor location,[2,4] elevated lactate dehydrogenase,[8] secondary cytogenetic abnormalities,[9] deletion of p16,[10] and mutation of p53.[11,12] However, despite these associations, no pathologic or laboratory criteria exist in current clinical use that allow prediction of response to therapy.

Cytogenetically, ESFTs are identified by reciprocal chromosomal translocations. In nearly all cases, these translocations result in fusion of the amino-terminal domain of *EWSR1* (*EWS*) or, in rare cases, the related *TLS/FUS* gene to the DNA-binding domain of an *ETS* gene family member.[13] In 90% to 95% of cases, the *EWS* fusion partner is the

Table 1. Demographics and Clinical and Molecular Characteristics of Patients With ESFT

Characteristic	All Patients (N = 132)		Fusion (No. of patients)				
	No.	%	*EWS-FLI1* Type 1 (n = 70)	*EWS-FLI1* Type 2 (n = 19)	*EWS-FLI1* Other (n = 17)	*EWS-ERG* (n = 13)	No Fusion Detected (n = 13)
Positive patients, %			58.8	16.0	14.3	10.9	
Age at diagnosis, years							
Median	13		13	14	11	12	11
Range	1-25		2-25	1-17	3-19	1-19	1-19
Sex							
Male	72	54.5	36	12	9	7	8
Female	60	45.5	34	7	8	6	5
Region of tumor							
Pelvic	20	15.2	12	2	1	2	3
Nonpelvic	112	84.8	58	17	16	11	10
Systemic therapy							
AEWS0031	100	75.8	56	17	10	8	9
INT-0154	32	24.2	14	2	7	5	4
Treatment era							
1996-2000	31	23.1	14	0	7	5	5
2001-2005	103	76.9	56	19	10	8	10
Site of origin							
Bone	96	72.7	50	12	15	10	9
Soft tissue	36	27.3	20	7	2	3	4
Follow-up, years							
Median	4.2		4.3	3.8	4.0	5.0	4.1
Range	0.45-10.9		1.2-10.9	1.6-5.4	0.45-9.2	1.4-10.3	3.2-8.9

Abbreviation: ESFT, Ewing sarcoma family of tumors.

FLI1 gene, resulting in creation of an *EWS-FLI1* fusion oncoprotein.[13] Other *ETS* family members that pair with *EWSR1* include the *ERG* gene in up to 10% of cases and, in the remainder of cases, the *ETV1*, *E1AF*, and *FEV* genes.[14] Combinatorial diversity among the involved chromosomal breakpoints produces even more heterogeneity at the molecular level. Specifically, breakpoints in *EWS* can occur between introns 7 to 9, and breakpoints in *FLI1* can occur between introns 3 to 9.[15] Most often, exon 7 of *EWS* is fused to exon 6 (60%) or exon 5 (20%) of *FLI1*, creating type 1 and type 2 *EWS-FLI1* fusions, respectively. Other *EWS-FLI1* fusions occur less frequently, and to date, at least 18 variations of the fusion transcript have been described.[14]

In two independent retrospective studies published in 1996 and 1998, *EWS-FLI1* fusion type was found to be associated with significant differences in clinical outcome in patients with localized ESFT.[14,16] Specifically, these studies revealed that patients with nonmetastatic disease whose tumors harbored the most common fusion, type 1 *EWS-FLI1*, had a better event-free survival (EFS) and OS than patients with non–type 1 translocations. These studies together suggested that *EWS-FLI1* fusion type might be useful as a means to stratify patients into different risk groups at the time of diagnosis.

In the present study, we evaluated the prognostic significance of *EWS-FLI1* fusion type in a prospectively acquired group of localized ESFT samples obtained from patients treated on Children's Oncology Group (COG) clinical trials after 1994. Importantly, in contrast to earlier retrospective studies, fusion type was not found to be a significant prognostic variable in this recently treated cohort of patients. These data suggest that current treatment protocols, which routinely add ifosfamide and etoposide (IE) to the standard three-drug backbone (vincristine, doxorubicin, and cyclophosphamide [VDC]), have dramatically improved outcomes for tumors with non–type 1 fusions and have eliminated the previously reported prognostic advantage of type 1 fusion–positive tumors.

METHODS

Sample Accrual

ESFT biopsy specimens were obtained from the COG Biorepository in Columbus, OH (Cooperative Human Tissue Network) and the Childrens Hospital Los Angeles (CHLA) tumor bank. Prospectively acquired diagnostic samples from patients treated on clinical trials INT-0154 (Children's Cancer Group trial 7942 and Pediatric Oncology Group trial 9354) and AEWS0031 were assessed. These were the two most recent COG clinical protocols for patients diagnosed with localized disease. Study INT-0154 ran from 1995 to 1998, and AEWS0031 ran from 2001 to 2005. INT-0154 evaluated whether dose intensification of VDC/IE over 30 weeks would improve outcome compared with the standard 48-week regimen.[17] AEWS0031 was designed to determine whether delivery of the VDC/IE regimen in every-2-week rather than every-3-week cycles would improve outcome.[18] Inclusion criteria for the current molecular study included confirmation of localized disease, registration or treatment on either of the two COG trials, and availability of frozen tissue in either the COG or CHLA tumor bank.

Diagnosis of ESFT was reaffirmed by central pathologic review for COG specimens or by local pathologists for CHLA specimens. An estimate of percent viable tumor cells was made for all samples using hematoxylin and eosin–stained sections of the frozen tissue blocks. Tumors were assigned an anonymous identifier, and specimens and correlative data were obtained in compliance with the Health Insurance Portability and Accountability Act. Review and approval by the Committee for Clinical Investigations at CHLA was obtained in accordance with an assurance filed with and approved by the Department of Health and Human Services. Informed consent for use of

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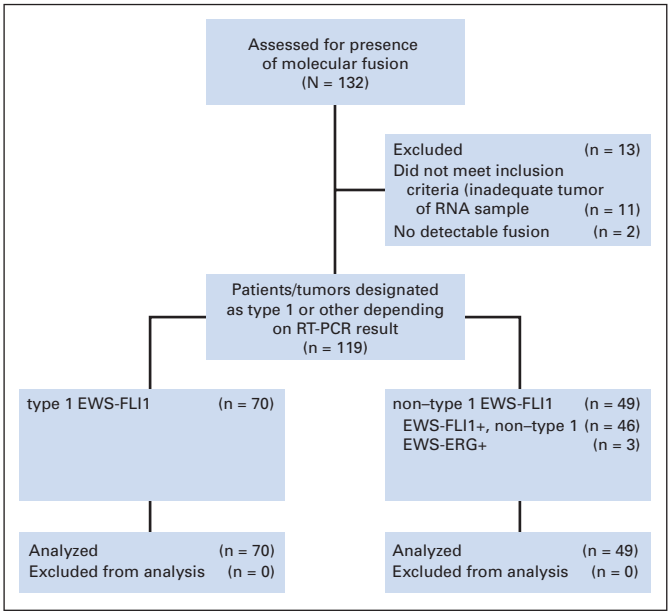

Fig 1. Translocation types of Ewing sarcoma family of tumor patients as determined by reverse transcriptase polymerase chain reaction (RT-PCR).

tumor samples for research purposes was obtained from each patient or the patient's guardian.

Translocation Type Determination

Total RNA was isolated from fresh-frozen tumor sections using RNAeasy or miRNAeasy kits (Qiagen, Valencia, CA) according to the manufacturer's instructions. Quantitative reverse transcriptase polymerase chain reaction (RT-PCR) was performed on first-strand cDNA using SYBR green incorporation and the following primer pairs: ESBP1(EWS) forward (CGACTAGTTATGATCAGAGCAGT) and ESBP2 (FLI1) reverse (CCGTTGCTCTGTATTCTTACTGA) or EWS3tqm forward (GTCAACCTCAATCTAGCACAGGG) and ERG3tqm reverse (CTGTCCGACAGGAGCTCCAG). These primer pairs will detect the most common fusions (ie, those that occur between *EWS* and exons 5 to 7 of *FLI1* or exons 4 to 8 of *ERG*, respectively).[15] RNA quality of each sample was determined by concurrent amplification of β-actin. Samples in which β-actin failed to amplify within 30 cycles were considered inadequate. In samples with amplifiable β-actin but no detectable *EWS-ETS* fusion using the aforementioned primers, RT-PCR was repeated using alternate primer pairs that will detect less common fusions between *EWS* and exons 8 and 9 of *FLI1*.[19,20] All amplified RT-PCR products were run on agarose gels, and bands were isolated and sequenced.

Statistical Methods

Correlations between translocation type and patient or disease characteristics were analyzed via logistic regression, Pearson's χ^2 test, or Fisher's exact test when the number of samples was limited. EFS was defined as the minimum interval from the date of diagnosis to the date of tumor recurrence, progression, death, or last follow-up. OS was defined as the interval from the date of diagnosis to the date of death or last follow-up. Two patients had a second malignancy before disease relapse/progression and were censored at the time of the occurrence of the second malignancy for both EFS and OS analyses. Estimates of EFS and OS rates were based on the product-limit (Kaplan-Meier) estimate with Greenwood SEs.[21] The association of EFS and OS with translocation type was tested using the log-rank test, either univariately or with stratification based on treatment era (1996 to 2000 *v* 2001 to 2005).

RESULTS

Population Characteristics

RNA from 132 patients with localized ESFT was analyzed by RT-PCR. Patient characteristics are listed in Table 1. The median age of all patients was 13 years (range, 1 to 25 years), and 54.5% were male. Tumors arising from bone and soft tissue tumors represented 72.7% and 27.3% of the study population, respectively. The majority of tumors included in this analysis arose outside the pelvis (n = 112). Importantly, comparison of the clinical characteristics of 100 patients from the AEWS0031 study who were included in this analysis with the 468 patients in AEWS0031 whose translocation status was not available for this study revealed no significant differences in age, sex, incidence of bone versus soft tissue origin, pelvic disease, or outcome (unpublished data). Thus, the patients whose tumors were analyzed for this study are representative of the larger population of patients with localized ESFT diagnosed and treated on COG clinical trials in the last 10 years.

Table 2. Association of *EWS-FLI1* Translocation Type With Other Predictors of Outcome

	Translocation Type (No. of patients)									
	EWS-FLI1				*EWS-FLI1* Type I			*EWS-ERG*		
Factor	Type 1	Type 2	Other	*EWS-ERG*	No. of Patients/Total	%	*P*	No. of Patients/Total	%	*P*
Age, years										
< 14	46	9	10	10	46/75	61	.41*	10/75	13	.62*
≥ 14	24	10	7	3	24/44	55		3/44	7	
< 18	66	19	15	11	66/111	59		11/111	10	
≥ 18	4	0	2	2	4/8	50		2/8	25	
Location										
Pelvic	12	2	1	2	12/17	71	.43†	2/17	12	.99†
Nonpelvic	58	17	16	11	58/102	57		11/102	11	
Site of origin										
Bone	50	12	15	10	50/87	57	.68†	10/87	11	.99†
Soft tissue	20	7	2	3	20/32	63		3/32	9	

*P value from logistic regression analysis with age as a continuous variable.
†P value from Fisher's exact test.

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Molecular Phenotypes

A fusion gene was identified by RT-PCR in 119 (90.2%) of 132 samples (Fig 1). Sequencing of the amplified fusion products revealed 106 *EWS-FLI1* fusions (89.1%) and 13 *EWS-ERG* fusions (10.9%; Table 1). Among *EWS-FLI1* fusions, 70 (66%) were type 1 (exons 7 and 6), 19 (18%) were type 2 (exons 7 and 5), and 17 (16%) had other variants including exons 10 and 5 (n = 6), exons 10 and 6 (n = 6), exons 7 and 7 (n = 3), and exons 10 and 7 (n = 2). In 13 samples, no fusion was detected. However, in three of these samples, the percentage of viable tumor on pathology review was less than 10%. Of the remaining 10 fusion-negative samples, seven required 30 or more RT-PCR cycles (range, 30 to 37 cycles) to amplify the control gene β-actin, indicating that the quality of the RNA sample was inadequate to reliably amplify a fusion gene even if one were present. In one additional fusion-negative sample, β-actin was detected at cycle 27, so although this sample met our criteria for RNA adequacy (< 30 cycles), low-level expression of a fusion transcript may have been missed in this RT-PCR assay. Therefore, it is highly probable that 11 of 13 samples with no detectable fusion were falsely negative as a result of inadequate tumor cell content (n = 3) and/or inadequate RNA quality (n = 8). In two samples, no fusion was detected with either *EWS-FLI1* or *EWS-ERG* primer pairs despite amplification of β-actin at 16 and 17 cycles. Although it is possible that these two samples represent true fusion-negative ESFT, it is more likely that they harbored rare variant translocations involving other fusion partners (eg, *TLS-ERG*, *EWS-ETV1*) that would not have been detected with the *EWS-FLI1*– and *EWS-ERG*–specific primer sets used in this study.

Non–Type 1 Fusion Type Is No Longer Associated With Adverse Outcome

Previous retrospective studies found that the type 1 *EWS-FLI1* fusion was associated with improved EFS and OS in patients with newly diagnosed localized disease.[14,16] To determine whether this observation was also true in our more recently diagnosed and treated prospective cohort of patients with ESFT, we first asked whether specific translocations occurred in conjunction with specific clinical or pathologic features. As shown in Table 2, no significant association was observed between age and translocation type, either in the proportion of tumors with *EWS-FLI1* type 1 fusion ($P = .41$) or in the proportion of tumors with *EWS-ERG* fusion ($P = .62$). Similarly, no significant association was found between translocation type and location of tumor or tissue of origin (bone *v* soft tissue; Table 2). These results are consistent with earlier data that showed no differences in clinical presentation between the different molecular phenotypes.[22]

Next, we evaluated whether fusion type was associated with outcome. Interestingly, survival analysis revealed no significant difference in outcome between patients with type 1 *EWS-FLI1* and non–type 1 fusions (Figs 2A and 2B). EFS and OS rates for 70 patients with a type 1 fusion were 65% ± 6% and 82% ± 5%, respectively, at 5 years. For the 49 patients with an alternate fusion, 5-year EFS and OS rates were 68% ± 7% and 74% ± 7%, respectively. Limiting the analysis to include only patients with an *EWS-FLI1* fusion also failed to identify a significant prognostic advantage of the type 1 fusion compared with other *EWS-FLI1* fusions (type 1: EFS, 65% ± 6%; OS, 82% ± 5% at 5 years; other *EWS-FLI1*: EFS, 63% ± 9%; OS, 71% ± 8% at 5 years; $P = .85$ for EFS; $P = .25$ for OS). Outcomes of patients with type 1 *EWS-FLI1* versus type 2 *EWS-FLI1*, the two most common fusion types, were also statistically equivalent (type 1: EFS, 65% ± 6%; OS, 82% ± 5% at 5 years; type 2: EFS, 67% ± 13%; OS, 78% ± 11% at 5 years; $P = .56$ for EFS; $P = .98$ for OS).

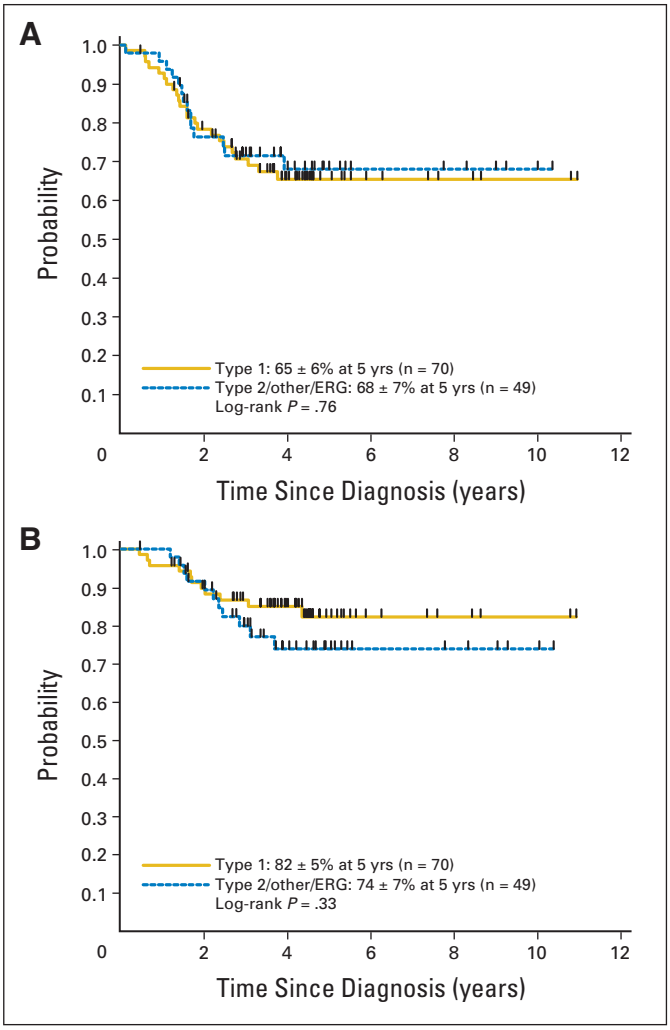

Fig 2. (A) Event-free survival and (B) overall survival for patients with Ewing sarcoma family tumors with type 1 *EWS-FLI1* fusions and non–type 1 fusions.

We hypothesized that the absence of observed difference in outcome in our study might be a result of treatment advances that had occurred since the time of the retrospective studies. To further address this possibility, we compared outcomes of patients diagnosed between 2001 and 2005 with outcomes previously reported in a multi-institutional study of patients diagnosed in the 1980s and 1990s.[14] For the 93 patients with identified translocations who were treated after the year 2000, 5-year EFS rates were 63% ± 7% and 71% ± 9% ($P = .32$) and OS rates were 83% ± 6% and 79% ± 8% ($P = .76$) for type 1 (n = 56) and non–type 1 (n = 37) fusions, respectively (Figs 3A and 3B). In contrast, the 5-year OS rate was approximately 70% for type 1 patients and only 20% for non–type 1 patients in the historical patient cohort.[14] Therefore, using historical cohorts as controls, a weighted improvement in outcome has been achieved in non–type 1 patients over the last decade.

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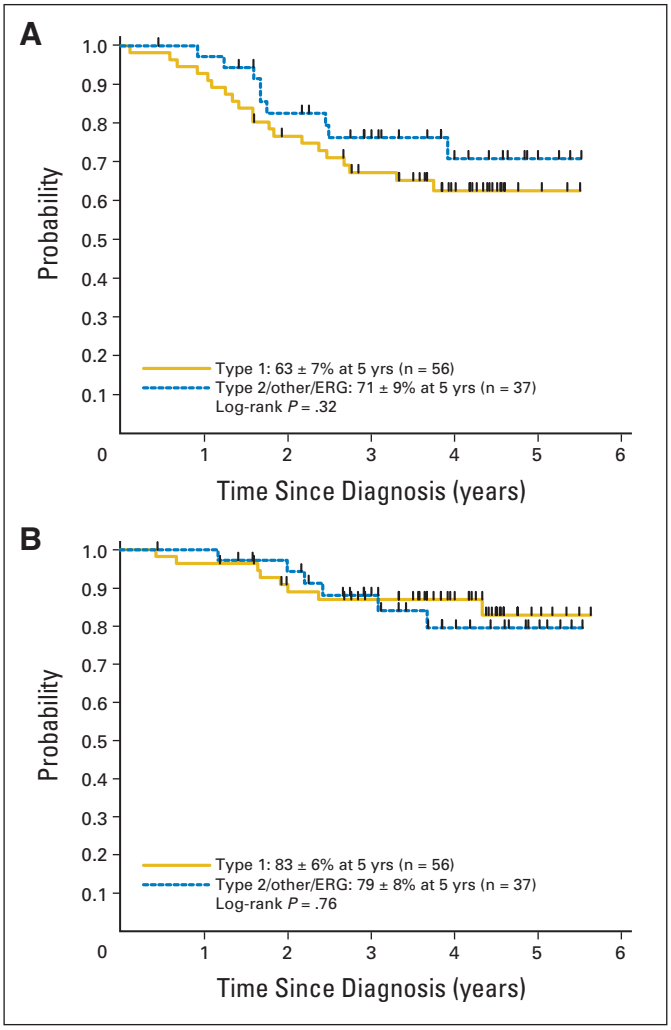

Fig 3. (A) Event-free survival and (B) overall survival for patients with Ewing sarcoma family tumors with type 1 *EWS-FLI1* fusions and non–type 1 fusions treated between 2001 and 2005.

DISCUSSION

This study shows that the outcome for patients diagnosed with localized ESFT after 1995 was not significantly impacted by the tumor's molecular phenotype. This result is in contrast to retrospective studies from the 1980s and early 1990s, in which the most common type 1 *EWS-FLI1* fusion was associated with significantly improved EFS and OS in patients with localized disease.[14,16] This raises the possibility that either the retrospective nature of the earlier studies in some way biased the patient and tumor characteristics or that changes in clinical protocols over the last decade have erased what was formerly a true prognostic advantage of the type 1 fusion. We propose that advances in therapy, in particular the routine inclusion of IE into ESFT protocols, have erased the prognostic disadvantage of non–type 1 fusions.

In a cohort of patients treated before April 1997, de Alava et al[14] found that patients with localized ESFT and non–type 1 *EWS-FLI1* fusions had a 5-year OS rate of only 20%, corresponding to a relative failure rate (RFR) of approximately 4.5 compared with type 1 patients. With the 70 type 1 patients and 49 non–type 1 patients available for survival analysis in the current study and accounting for censoring, there would be at least 95% power to detect an RFR of 4.5 between these two groups and at least 80% power to detect an RFR of 3.0, based on a two-sided log-rank test with 5% type I error and assuming 5-year survival rate in type 1 patients of 70% to 80%. Similar numbers apply to the comparison of EFS. Hence, there was sufficient power in this analysis to detect even a smaller difference than was observed in the previous study. Thus, there has been a statistically and clinically significant improvement in the outcome of patients with non–type 1 tumors over the last decade. By contrast, improvement in the outcome of patients with type 1 transcripts has been more modest. Localized patients with type 1 tumors in the study by de Alava et al[14] had an OS rate approaching 70%, whereas the same group treated between 2001 and 2005 in our study had an OS rate of 76%. This observation suggests that advances in treatment and supportive therapy in the intervening period have had their most profound effect on improving the survival of patients with non–type 1 tumors.

The reason for this marked and selective improvement in outcome for non–type 1 tumors is unknown, but our findings suggest that routine use of five-drug, intensified treatment protocols has likely overcome differences in the biology of these tumors that formerly portended a worse outcome. Interestingly, de Alava et al[23] reported that the proliferative index of ESFT with non–type 1 fusions is higher than type 1 fusions and that this is associated with differential activation of IGF1-R signaling. We have performed a preliminary comparison of the molecular profiles of ESFT samples expressing type 1 and non–type 1 *EWS-FLI1* and have also identified relative downregulation of cell cycle pathways in type 1 tumors (unpublished data). In addition, these molecular profiling studies suggest that host immune response pathways might be relatively upregulated in type 1 tumors. Further studies are now required to determine whether these differences could be exploited to develop novel, pathway-targeted therapies in the future that may further improve outcomes for patients with ESFT.

In summary, we have found that the most common type 1 *EWS-FLI1* translocation is no longer associated with a more favorable clinical outcome in patients with newly diagnosed localized ESFT. Instead, our data show that modern treatment protocols have resulted in a marked improvement in the survival of patients with non–type 1 *EWS-FLI1* fusion types and that this has eliminated the previously reported prognostic advantage of type 1 tumors. Eradication of translocation type as a prognostic variable in ESFT can be viewed as a tangible marker of success resulting from therapeutic advances of the last 20 years.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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REFERENCES

1. Bernstein M, Kovar H, Paulussen M, et al: Ewing's sarcoma family of tumors: Ewing sarcoma of bone and soft tissue and the peripheral primitive neuroectodermal tumors, in Pizzo PA, Poplack DG (eds): Principles and Practice of Pediatric Oncology (ed 5). Philadelphia, PA, Lippincott Williams & Wilkins, 2006, pp 1002-1032

2. Rodríguez-Galindo C, Liu T, Krasin MJ, et al: Analysis of prognostic factors in Ewing sarcoma family of tumors: Review of St. Jude Children's Research Hospital studies. Cancer 110:375-384, 2007

3. Rodriguez-Galindo C, Spunt SL, Pappo AS: Treatment of Ewing sarcoma family of tumors: Current status and outlook for the future. Med Pediatr Oncol 40:276-287, 2003

4. Jenkin RD, Al-Fawaz I, Al-Shabanah M, et al: Localised Ewing sarcoma/PNET of bone: Prognostic factors and international data comparison. Med Pediatr Oncol 39:586-593, 2002

5. Grier HE, Krailo MD, Tarbell NJ, et al: Addition of ifosfamide and etoposide to standard chemotherapy for Ewing's sarcoma and primitive neuroectodermal tumor of bone. N Engl J Med 348:694-701, 2003

6. Ahrens S, Hoffmann C, Jabar S, et al: Evaluation of prognostic factors in a tumor volume-adapted treatment strategy for localized Ewing sarcoma of bone: The CESS 86 experience—Cooperative Ewing Sarcoma Study. Med Pediatr Oncol 32:186-195, 1999

7. Paulussen M, Ahrens S, Dunst J, et al: Localized Ewing tumor of bone: Final results of the cooperative Ewing's Sarcoma Study CESS 86. J Clin Oncol 19:1818-1829, 2001

8. Bacci G, Ferrari S, Longhi A, et al: Prognostic significance of serum LDH in Ewing's sarcoma of bone. Oncol Rep 6:807-811, 1999

9. Hattinger CM, Potschger U, Tarkkanen M, et al: Prognostic impact of chromosomal aberrations in Ewing tumours. Br J Cancer 86:1763-1769, 2002

10. Wei G, Antonescu CR, de Alava E, et al: Prognostic impact of INK4A deletion in Ewing sarcoma. Cancer 89:793-799, 2000

11. Abudu A, Mangham DC, Reynolds GM, et al: Overexpression of p53 protein in primary Ewing's sarcoma of bone: Relationship to tumour stage, response and prognosis. Br J Cancer 79:1185-1189, 1999

12. de Alava E, Antonescu CR, Panizo A, et al: Prognostic impact of P53 status in Ewing sarcoma. Cancer 89:783-792, 2000

13. Lessnick SL, Dei Tos AP, Sorensen PH, et al: Small round cell sarcomas. Semin Oncol 36:338-346, 2009

14. de Alava E, Kawai A, Healey JH, et al: EWS-FLI1 fusion transcript structure is an independent determinant of prognosis in Ewing's sarcoma. J Clin Oncol 16:1248-1255, 1998

15. Zucman J, Melot T, Desmaze C, et al: Combinatorial generation of variable fusion proteins in the Ewing family of tumours. EMBO J 12:4481-4487, 1993

16. Zoubek A, Dockhorn-Dworniczak B, Delattre O, et al: Does expression of different EWS chimeric transcripts define clinically distinct risk groups of Ewing tumor patients? J Clin Oncol 14:1245-1251, 1996

17. Granowetter L, Womer R, Devidas M, et al: Dose-intensified compared with standard chemotherapy for nonmetastatic Ewing sarcoma family of tumors: A Children's Oncology Group study. J Clin Oncol 27:2536-2541, 2009

18. Womer R, Krailo M, Dickman P, et al: Randomized comparison of every-two-week vs. every-three-week chemotherapy in Ewing family tumors (ESFT). J Clin Oncol 26:554s, 2008 (suppl; abstr 10504)

19. Ishii N, Hiraga H, Sawamura Y, et al: Alternative EWS-FLI1 fusion gene and MIC2 expression in peripheral and central primitive neuroectodermal tumors. Neuropathology 21:40-44, 2001

20. Krams M, Peters J, Boeckel F, et al: In situ reverse-transcriptase polymerase chain reaction demonstration of the EWS/FLI-1 fusion transcript in Ewing's sarcomas and peripheral primitive neuroectodermal tumors. Virchows Arch 437:234-240, 2000

21. Cox DR, Oakes D: Analysis of Survival Data. Boca Raton, FL, CRC Press, 1984

22. Ginsberg JP, de Alava E, Ladanyi M, et al: EWS-FLI1 and EWS-ERG gene fusions are associated with similar clinical phenotypes in Ewing's sarcoma. J Clin Oncol 17:1809-1814, 1999

23. de Alava E, Panizo A, Antonescu CR, et al: Association of EWS-FLI1 type 1 fusion with lower proliferative rate in Ewing's sarcoma. Am J Pathol 156:849-855, 2000

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