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SYMPOSIUM: MOLECULAR GENETICS IN SARCOMA

Gene Translocations in Musculoskeletal Neoplasms

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Abstract Establishing the best diagnosis for musculoskeletal neoplasms requires a multidisciplinary approach using clinical, radiographic, and histologic analyses. Despite this rigorous approach, establishing accurate diagnoses and prognoses remains challenging. Improved diagnostic methods are expected as unique molecular signals for specific bone and soft tissue cancers are identified. We performed a systematic review of the best available evidence to explore three major applications of molecular genetics that will best benefit clinical management of musculoskeletal neoplasms: diagnostic, prognostic, and therapeutic applications. The specific questions addressed in this systematic review are: (1) What sets of histopathologic sarcoma subtypes will benefit from molecular evaluation and diagnosis? (2) What molecular methods are best applied to histopathologic sarcomas to distinguish between major subtypes? (3) How do the molecular patterns discovered on genetic diagnosis affect prognosis of certain sarcomas? (4) Which sarcoma translocations can benefit from an improved response and outcome using existing and forthcoming pharmacogenetic approaches targeting molecular events? This review summarizes recent advances in molecular genetics that are available and will soon be available to clinicians to better predict outcomes and subsequently help make future treatment decisions.

Each author certifies that he or she has no commercial associations (eg, consultancies, stock ownership, equity interest, patent/licensing arrangements, etc) that might pose a conflict of interest in connection with the submitted article.

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Introduction

Soft tissue and bone sarcomas are a rare and heterogeneous group of tumors that represent less than 1% of all adult and 15% of pediatric malignancies. The annual incidence in the United States, which remains relatively constant, is approximately 6000 to 7000 soft tissue and 2500 bone sarcomas [99]. The application of molecular genetics to musculoskeletal neoplasms has identified distinctive molecular features ranging from point mutations to chromosomal translocations. A comprehensive summary of molecular and cytogenetic lesions associated with musculoskeletal neoplasms is presented (Table 1). Knowledge obtained from these studies has translated into diagnostic, prognostic, and therapeutic applications for patient management.

The accurate diagnosis of musculoskeletal neoplasms is critical for clinical management. Accurate diagnosis requires integration of clinical findings, histologic evaluation, and new methods, including immunohistochemistry, cytogenetics, and molecular genetics. Molecular diagnostic techniques such as reverse transcription-polymerase chain reaction (RT-PCR) and fluorescence in situ hybridization (FISH) have become important tools for evaluating musculoskeletal neoplasms and increasing the diagnostic accuracy of histopathologic classification. Novel techniques with diagnostic potential continue to emerge such as cDNA microarray and expression profiling. These are still being evaluated to determine their clinical role in diagnosis. The identification of different molecular features in specific musculoskeletal neoplasm influences prognosis.

Table 1. Summary of genetic/molecular changes in musculoskeletal neoplasms

Type of tumor	Chromosomal abnormality	Gene involved or fusion gene	Prevalence	Molecular test	Prognosis
Benign musculoskeletal tumors					
Fibrous lesions [21]	12p13 aberrations (three cases) and trisomy 2 (three cases)	NA	NA	NA	Benign
Aneurysmal bone cyst [91]	16q22 and 17p–13	CDH11-USP6	NA	FISH or RT-PCR	Benign
Osteochondroma [16]	8q22–24.1	EXT1	NA	NA	Benign
Lipoma [14]	Majority have normal karyotype	NA	NA	NA	Benign
Lipoblastoma [31]	Rearrangement of 8q12, polysomy 8	PLAG1	70%	FISH	Benign
Desmoid tumor [80]	+8, +20, Deletion (5)(q21-22)	NA	NA	FISH	Benign
Benign and intermediate musculoskeletal tumors					
Giant cell tumor [91]	Telomere translocations–19q, 11p, 15p, 18p, 20q, and 21p	NA	NA	FISH	Benign, rarely malignant
Tenosynovial giant cell tumor [74]	t(1;2)(p11;q35-37)	NA	40%	FISH or RT-PCR	Benign, rarely malignant
Extraskeletal myxoid chondrosarcomas [94]	t(9;22)(q22;q12)	EWS-TEC(CHN)	75%	FISH	90%
	t(9;17)(q22;q11)	RBP56-TEC	NA	RT-PCR	5-year survival
	t(9;15)(q22;q21)	TAF2N-TEC	25%		
		TGF-TEC	Rare		
Extraskeletal mesenchymal chondrosarcoma [72]	t(9;17)(q22;q11)	RBP56-TEC	NA	FISH	90%
				RT-PCR	5-year survival
Chondromyxoid fibroma [88]	t(9;15)(q22;q21)	NA	NA	RT-PCR	Benign with 25% recurrence rate
Giant cell fibroblastoma (juvenile form of DFSP) [92]	t(17;22)(q22;q13)	COL1A1-PDGFB		FISH or RT-PCR	Benign
Malignant musculoskeletal tumors					
Osteosarcoma [83]	LOH in 3q, 13q, 17p and 18q and variable chromosomal changes	NA	NA	FISH	60%–85%
				RT-PCR	5-year survival
Parosteal osteosarcoma [83]	Supernumerary ring chromosomes	NA	NA	FISH	
Ewing's sarcoma/PNET [12]	t(11;22)(q24;q12)	EWS-FLI-1	85%–95%	FISH or RT-PCR	60%
	t(21;22)(q22;q12)	EWS-ERG			Others—20%
	t(7;22)(p22;q12)	EWS-ETV1			5-year survival
		EWS-E1AF			
		EWS-FEV			
		EWS-ZSG			
DSRCT [57]	t(11;22)(p13;q12)	EWS-WT1		RT-PCR	20%
					3-year survival
Chordoma [17]	Sporadic	NA	NA	NA	20–30%
					5yr survival – site dependent
Adamantinoma [40]	Numerical changes in 5 cases	NA	NA	NA	85%–87%
					5-year survival

Table 1. continued

Type of tumor	Chromosomal abnormality	Gene involved or fusion gene	Prevalence	Molecular test	Prognosis
Myxoid/round cell liposarcoma [14]	t(12;16)(q13;p11)	EWS-CHOP	Greater than 95%	FISH	17 months
	t(12;22)(q13;q12)	TLS-CHOP (Type I)	rare	RT-PCR or FISH	75 months
		TLS-CHOP (Type II)		RT-PCR	5 yr survival
Embryonal rhabdomysarcoma [33]	Gains of 2, 7, 8, 12, 13; losses of 1, 6, 9, 14, and 17	IGF2, GOK, PTCH, TP53	NA	NA	40% 5-year survival
Alveolar rhabdomysarcoma [29]	t(2;13)(q35;q14)	PAX3-FKHR	75%	FISH or RT-PCR	8%
	t(1;13)(p36;q14)	PAX7-FKHR	10%		75% 4-year survival
Clear cell sarcoma [87]	t(12;22)(q13;q12)	EWS-ATF1	90%	FISH or RT-PCR	33% 10-year survival
Synovial sarcoma [49, 96]	t(X;18)(p11;q11)	SYT-SSX1 (53%)	65%	FISH or RT-PCR	53%
		SYT-SSX2 (73%)	35%		73%
		SYT-SSX4	rare		5-year survival
Congenital/infantile fibrosarcoma [54]	t(12;15)(p13;q25)	ETV6-NTRK3	80%	FISH or RT-PCR	84% 5-year survival
Inflammatory myofibroblastic tumor [61]	t(1;2)(q25;p23)	TPM3-ALK	NA	FISH or RT-PCR	Inconclusive
	t(2;19)(p23;p13)	TPM4-ALK			
	t(2;17)(p23;q23)	CLTC-ALK			
	t(2;2)(p23;q13)	RANBP2-ALK			
Dermatofibrosarcoma protuberans [51, 92]	t(17;22)(q22;q13)	COL1A1-PDGFB	Greater than 99%	FISH or RT-PCR	86% 5-year disease-free survival
Alveolar soft part sarcoma [58]	t(X;17)(p11;q25)	NA	NA	RT-PCR	87% 5-year survival
Giant cell fibroblastoma (juvenile form of DFSP) [92]	t(17;22)(q22;q13)	COL1A1-PDGFB		FISH or RT-PCR	

DFSP = dermatofibrosarcoma protuberans; NA = not applicable/not known; FISH = fluorescence in situ hybridization; RT-PCR = reverse transcription-polymerase chain reaction.

Finally, with pharmacogenetics increasingly able to target specific molecular events, technology holds promise for additional novel treatment options in the future.

We provide a summary of recent advances in molecular genetics that clinicians can use to better predict outcomes and could subsequently be helpful in making future treatment decisions. The specific questions addressed in this systematic review are: (1) Which sets of histopathologic sarcoma subtypes will benefit from molecular evaluation and diagnosis? (2) Which molecular methods are best applied to histopathologic sarcomas to distinguish between major subtypes? (3) How do the molecular patterns discovered on genetic diagnosis affect prognosis of certain sarcomas? (4) Which sarcoma translocations can benefit from an improved response and outcome using existing and forthcoming pharmacogenetic approaches targeting molecular events and how?

Search Strategies and Criteria

We performed a systematic review of the best available evidence to explore three major applications of molecular genetics that will best benefit clinical management of musculoskeletal neoplasms: diagnostic, prognostic, and therapeutic applications. There already exist a number of reviews that summarize molecular diagnosis of sarcomas [5, 10, 26], existing methods for translocation detection [33, 67, 69], new trends in translocation detection [41, 47, 59], and new evidence for pharmacogenetic approaches to suppress products of gene translocations [60, 67, 84, 89, 108]. However, the literature lacks consolidation of this information addressed to the clinician to best apply laboratory developments in molecular genetics toward improving patient care.

We electronically searched the following databases: MEDLINE (1996 onward; accessed through OVID) and EMBASE (1996 onward; accessed through OVID). The search date was September 2007. The search strategy combined terms relating to translocations and to a limited set of musculoskeletal tumors. A list of the search strategies has been provided (Table 2).

In addition to the electronic search, we used a number of supplemental search strategies. We reviewed the reference lists of included papers, relevant papers, and related systematic reviews [5, 10, 26, 33, 41, 47, 59, 60, 67, 69, 84, 89, 108]. We used the "Related Articles" feature in PubMed to identify additional papers. Retrospective and nonrandomized studies were included in this systematic review, because they comprise the vast majority of the diagnostic and prognostic literature on the topic. Additionally, we searched the Cochrane Controlled Trials Register for any controlled studies involving therapeutic applications. We limited our search to English language articles. Preprint articles were also reviewed, particularly those in the previous 6 months, so that journal articles not yet contained in electronic databases were included.

The search strategy identified 666 citations. We excluded 411 case reports and 77 reviews using the search

Table 2. Search strategies for the electronic databases

Database	Search strategy
MEDLINE (1996 onward; accessed through OVID)	1. fibrous lesions.mp.
EMBASE (1996 onward; accessed through OVID)	2. aneurysmal bone cyst.mp. or exp Bone Cysts, Aneurysmal/
CENTRAL (The Cochrane Library 2007)	3. osteochondroma.mp. or exp Osteochondroma/
	4. giant cell tumor.mp. or exp Giant Cell Tumors/
	5. exp Chondrosarcoma, Mesenchymal/ or exp Chondrosarcoma/ or chondrosarcoma.mp.
	6. chondromyxoid.mp.
	7. exp Osteosarcoma, Juxtacortical/ or exp Osteosarcoma/ or osteosarcoma.mp.
	8. exp Sarcoma, Ewing's/ or ewing sarcoma.mp.
	9. Desmoplastic small-round cell tumour.mp.
	10. chordoma.mp. or exp Chordoma/
	11. adamantinoma.mp. or exp Adamantinoma/
	12. malignant fibrous histiocytoma.mp. or exp Histiocytoma, Malignant Fibrous/
	13. lipoma.mp. or exp Lipoma/
	14. exp Liposarcoma, Myxoid/ or exp Liposarcoma/ or liposarcoma.mp.
	15. exp Neoplasms, Adipose Tissue/ or lipoblastoma.mp.
	16. exp Lipoma/ or lipoblastoma.mp.
	17. rhabdomyoma.mp. or exp Rhabdomyoma/
	18. exp Rhabdomyosarcoma, Alveolar/ or rhabdomyosarcoma.mp. or exp Rhabdomyosarcoma/ or exp Rhabdomyosarcoma, Embryonal/
	19. clear cell sarcoma.mp. or exp Sarcoma, Clear Cell/
	20. synovial sarcoma.mp. or exp Sarcoma, Synovial/
	21. fibrosarcoma.mp. or exp Fibrosarcoma/
	22. inflammatory myofibroblastic.mp.
	23. exp Dermatofibrosarcoma/ or Dermatofibrosarcoma protuberans.mp.
	24. desmoid.mp. or exp Fibromatosis, Aggressive/
	25. Alveolar soft part sarcoma.mp. or exp Sarcoma, Alveolar Soft Part/
	26. Giant cell fibroblastoma.mp.
	27. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26
	28. exp Translocation, Genetic/
	29. 27 and 28
	30. limit 29 to case reports
	31. 29 not 30
	32. limit 31 to "review"
	33. 31 not 32

engine report. The title and abstract screening of the 334 remaining unique citations identified 83 as potentially eligible for this review. Two authors (BK, GK) independently screened the title and abstract of identified article citations for the potential eligibility. We retrieved the full text articles judged potentially eligible by at least one author. The two authors then independently screened the full text articles for eligibility with explicit inclusion and exclusion criteria. The two main criteria for inclusion were a 90% prevalence or greater of a molecular signature in the case set and the number of cases studied be greater than 40. For diagnostic studies we required at least 2 authors independently verify the histopathology and for prognostic studies we required a reproduction of the result by another independent study. Studies with a lack of description of data source, analysis of methods and case reports (not excluded electronically) were excluded.

Approaches to the Genetics of Tumors

Chromosomal translocation analysis has evolved substantially in the last two decades, slowly evolving from conventional chromosomal karyotyping and southern blot studies to more sophisticated molecular diagnostic techniques [8]. Much of this has been the result of the challenges in handling tissue samples, cost of testing, and long turnaround times. A number of cytogenetic abnormalities have been discovered, including (1) a recurrent characteristic translocation creating a fusion transcription factor; (2) point mutation; (3) translocation causing growth factor overexpression; (4) recurrent events within complex karyotypic changes; and (5) complex karyotypic changes without defined consistent events. These mechanisms have led to an improved understanding of the pathogenesis in individual neoplasms resulting from cytogenetic aberrations. Advances in molecular genetics have strengthened fusion gene detection, refined classification in several sarcoma groups, led to the identification of prognostic classes independent of conventional clinical risk factors, and yielded new insights into the treatment of these tumors.

Conventional karyotyping depends on the availability of fresh, sterile tumor tissue, the success of tumor cell growth in culture, and quality of metaphase cell preparations. It requires skilled personnel, mostly available in large centralized laboratories, and remains time-consuming, even with automated karyotyping systems. Conventional karyotyping is limited to detecting only large and predictable structural abnormalities. Cytogenetic techniques such as conventional karyotyping and southern blot have been supplanted in clinical laboratories by molecular diagnostic techniques such as RT-PCR and FISH to detect these fusion genes. Many other laboratory technologies are currently being researched for clinical application such as comparative genomic hybridization (CGH) [9], spectral karyotyping (SKY) [13], multiflorophore fluorescence in situ hybridization (M-FISH) [66], and cDNA microarray [1].

FISH offers several advantages over conventional karyotyping and RT-PCR. FISH technology detects a specific DNA target sequence in the nuclei of nondividing (interphase) cells and can be performed on fresh, frozen, or fixed samples. It can provide results when the tissue is insufficient for conventional cytogenetics or when only paraffin-embedded tissue is available and, as an overnight procedure, can be performed quickly with good sensitivity and specificity. In contrast to karyotyping, FISH is a targeted approach that requires knowledge of the suspected aberration; therefore, FISH is limited by what is known about the genetics of neoplasms and by the availability of commercial FISH probes. Only a handful of FISH probes have been made available for molecular diagnosis, including ALK, CHOP, FKHR, ETV6, EWS, and SYT [5]. Unsuccessful hybridization or detection can occur either when the number of tumor cells is inadequate or when there is improper fixation, as occurs when fixation is delayed or prolonged, or when a fixative is too stringent.

RT-PCR is a method for identifying genomic breakpoints by detecting fusion RNA transcripts. Although DNA is easier to handle and more readily obtained from paraffin-embedded tissue than RNA, most characteristic breakpoints are located within large introns. Progress has been recently made in developing a real-time PCR assay that is comparable in its results to RT-PCR and poses a lower risk of cross-contamination [79]. The advantage of RT-PCR is that a small amount of tissue is required and that it can be performed on fresh-frozen tissue or paraffin-embedded tissue. However, the diagnostic success rate is variable and dependent on multiple factors. The second impediment to RT-PCR methodology is the high risk of reagent contamination, mainly with PCR products, particularly in small laboratory spaces. Unexpected negative results may be the result of a variety of factors such as novel or undetected variant forms of the gene fusion, inappropriate primer design not covering the variability among fusion gene partners, questionable morphologic diagnosis, scant or necrotic tumor material, or poor RNA quality. In addition to detection, quantitative RT-PCR can also be used to quantify fusion transcripts, potentially an indicator of the neoplasm's aggressiveness.

Immunohistochemistry can be used to detect fusion gene proteins in translocation-associated sarcomas, exploiting the fact that only one portion of a given protein is overexpressed. Molecular features of musculoskeletal neoplasms have many similarities to those of hematopoietic neoplasms, including dysregulated kinases, overexpressed oncogenes, or fusion transcription factors. Hence, therapies developed for hematopoietic neoplasms have translated well into

therapies for sarcomas. Antibodies to the WT1 in desmoplastic round cell tumor and FLI1 in Ewing's sarcoma have been used with success in archival material [22]. One anti-ALK immunohistochemical study suggests upregulated ALK protein expression in approximately 60% of inflammatory myofibroblastic tumors (IMT) [100]. Immunohistochemistry may be helpful in the setting of small biopsies or suboptimal RNA preservation and in laboratories that are not set up to perform molecular genetic tests.

cDNA microarrays are showing great promise in classifying musculoskeletal neoplasms, particularly those with complex or multiple karyotypic changes [10]. cDNA microarrays have the capability of simultaneously examining the expression of more than 12,000 genes [95]. Using clustering analysis, researchers have been identifying signature sequences that uniquely categorize sarcomas. However, the technique produces more classifications in an already redundant classification system [95]. Multiple analyses have been conducted on various sarcomas to distinguish signature patterns that can further classify subtypes to establish a diagnosis [103].

Which Sets of Histopathologic Sarcoma Subtypes Will Benefit From Molecular Evaluation and Diagnosis?

One-third of all sarcomas are characterized by specific recurrent chromosomal translocations, resulting in highly specific gene fusions, usually encoding aberrant chimeric transcription factors [68]. The other two-thirds lack a recurrent genetic signature and are characterized by numerous aberrations, including chromosomal losses and gains [68]. The first group offers the best opportunity for molecular evaluation because these translocations are often the only cytogenetic abnormality and are most likely pathogenetically important.

The most common mechanism involves the EWS gene rearrangement, a specific translocation that juxtaposes the functional domain EWS gene with the DNA-binding domain FLI1, ERG, ATF1, DDIT3, WT1 genes [82]. Ninety-eight percent of small blue round cells will have the EWS gene rearrangement and are prone to misdiagnosis. These tumors have remarkable clinical diversity and often pose a diagnostic problem because they can be difficult to differentiate by light microscopy and sometimes as a result of nonspecific immunoresults. As an example, O13 (CD99) reactivity, initially believed to represent a reliable marker for Ewing's sarcoma/PNET diagnosis, has been described also in alveolar rhabdomyosarcoma, synovial sarcoma, desmoplastic round cell tumor (DRCT), and so on. Common immunohistochemical similarities among small blue round cell tumors are described (Table 3). The fusion transcripts created by these translocations serve as specific tumor markers that can now be detected by RT-PCR. Among round cell tumors, a distinction that should be made is between DRCT and Ewing's sarcoma/PNET. With DRCT, prognosis is very poor with 35% overall progression-free survival at 5 years and nonmetastatic Ewing's has a better prognosis. Desmoplastic small round cell tumor has a characteristic translocation, the EWS gene on chromosome 22 is fused with the WT1 gene (Wilms tumor suppressor gene) on chromosome 11 that clearly distinguishes it from Ewing's sarcoma [57]. Another distinction that should be made is between rhabdomyosarcoma and Ewing's sarcoma/PNET. Rhabdomyosarcoma and Ewing's sarcoma/PNET share two immunohistochemical markers, CD99 and MyoD, but can be distinguished through molecular translocations (Table 2) [18]. Even desmin positivity, once believed to represent a marker for rhabdomyosarcoma, is present in DRCT and in rare cases of Ewing's sarcoma [33]. Another important distinction is between poorly differentiated embryonal rhabdomyosarcoma (E-RMS) and solid-alveolar rhabdomyosarcoma (A-RMS) based on the PAX3/FKHR fusion [33]. A-RMS occurs predominantly in the extremities and the trunk, whereas E-ARMS occurs predominantly in the head and neck region, the genitourinary tract, and the retroperitoneum. Prognosis substantially differs for patients with A-RMS having a poorer survival than those with E-RMS. A-RMS is characterized by two pathognomonic translocations, t(2;13)(q35;q14) and t(1;13)(p36;q14), found in 80% and 15% of the cases, respectively, whereas E-RMS is not associated with recurrent structural chromosome rearrangement [81]. Further molecular identification by RT-PCR of the EWSR1-ATF1 translocation can also distinguish the two [110].

The second mechanism is a non-EWS gene-based functional domain translocation such as FUS and TLS resulting in chimeric fusion transcription factor overexpression. Based on the FUS-DDIT3 transcript, myxoid liposarcoma can be distinguished from other forms of liposarcoma (LS). Antonescu et al. [6] reported the TLS-CHOP fusion is highly sensitive and specific for myxoid/round cell LS. Other types of liposarcoma, even with a predominant myxoid component, lack the TLS-CHOP rearrangement, confirming they represent a genetically distinct group of LS. Approximately 5% of myxoid liposarcoma/round cell LS have cytogenetically, but not molecularly, indistinguishable 12;22 translocation that also has been identified as a characteristic aberration in clear cell sarcoma of the tendons and aponeuroses. However, histologic differentiation is sufficient despite molecular identification by RT-PCR of the EWSR1-ATF1 translocation also being able to distinguish the two. Low-grade fibromyxoid sarcoma (LGFMS) is an indolent, late-metastasizing malignant soft tissue tumor that is often mistaken for either more benign or more malignant tumor

Table 3. A practical algorithm for diagnostic evaluation of common musculoskeletal tumors

Cyto-/histomorphology		Immunohistochemistry	Confirmatory molecular aberrations
Round cell	Rhabdomyosarcoma	MyoD1 +	Alveolar Rhabdo:
		CD99 +	PAX3/FKHR: 75%
		Myogenin +	PAX7/FKHR: 10%
			Other Rhabdo:
			NA
	DSRCT	WT1+	EWS/WT1
		CK/EMA/Desmin +	
	Ewing/PNET	MyoD1 −	EWS/FLI-1—85%-95%
		CD99 +	EWS/ERG—5%–10%
		FLI1 +	EWS/ETVI
			EWS/EIA-F
			EWS/FEV
	Small cell osteosarcoma	Desmin +	Nondiagnostic
		CD99 −	
		S100 −	
Spindle cell	Synovial sarcoma	CK +/Vim +	SYT/SSX1—65%
			SYT/SSX2—35%
	Fibrosarcoma	CK −/Vim +/Desmin −/ S100 +	ETV6-NTRK3
		LCA +/CD 68 −	
		TrkC	
Epitheliod cell/spindle cell	Clear cell sarcoma	CK −/Vim +/Desmin −/ S100 +	EWS/ATF1
		HMB45 +	
	Liposarcoma	CK −/Vim +/Desmin −/S100 +	TLS/CHOP (Type I, II, and so on)
		HMB45 −	EWS/CHOP
		Leu 7 −	
Myxoid	Myxoid liposarcoma	CK −/Vim +/Desmin −/S100 +	TLS/CHOP (Type I, II, and so on)
		HMB45 −	EWS/CHOP
		Leu 7 −	
		MDM2/CDK4	
	Myxoid chondrosarcoma	S-100 +	EWS/CHN
	Lipoblastoma	Nondiagnostic	FUS/CHOP
DSRCT versus Ewing's sarcoma/PNET		Antibodies to the WT1 in desmoplastic round cell tumor and FLI1 in Ewing's sarcoma [22]	
Rhabdomyosarcoma versus Ewing's sarcoma/PNET		FISH probes or RT-PCR for the FKHR rearrangement [50]	
Embryonal rhabdomyosarcoma (E-RMS) versus solid-alveolar rhabdomyosarcoma (A-RMS)		RT-PCR of the PAX3/FKHR fusion [33]	
		RT-PCR of the EWSR1-ATF1 translocation [110]	
Myxoid liposarcoma versus liposarcoma		TLS-CHOP fusion or EWS-CHOP fusion	
		Southern blot analysis and reverse transcriptase-polymerase [6]	
Low-grade fibromyxoid sarcoma versus other sarcomas		*FUS*/CREB3L2 reverse transcriptase polymerase chain reaction (RT-PCR) and/or fluorescence in situ hybridization (FISH) [77]	
Benign versus well-differentiated lipomas		Gain of 12q15-q24 sequences with FISH [66]	
Angiomatoid fibrous histiocytoma versus malignant fibrous histiocytoma		Fusion of FUS and ATF-1 with FISH [104]	
Inflammatory myofibroblastic tumor versus leiomyosarcoma, rhabdomyosarcoma, and sarcomatoid carcinoma		Immunohistochemistry and ALK rearrangements detected by fluorescence in situ hybridization (FISH) [100]	
Giant cell tumors of bone versus aneurysmal bone cysts		Chromosome segments 17p11–13 and/or 16q22. G- band staining [91]	
Adamantinoma versus Ewing's sarcoma		t(11; 22) and t(21; 22) by RT-PCR [39]	
Clear cell sarcoma versus malignant melanoma		t(12;22)(q13;q12) [87]	

types. This can now be identified with a recurrent balanced translocation t(7;16)(q32-34;p11) (FUS/CREBL32 fusion gene) [77]. Some well-differentiated lipomas with minimal atypia reportedly show gain of 12q15-q24 sequences rather than rings and markers or balanced translocations of 12q13-15 (typical feature of benign, ordinary lipomas) [66]. Hence, it is important to make this distinction between benign and malignant lipomas. Distinction between angiomatoid fibrous histiocytoma and malignant fibrous histiocytoma can be made by detection of FUS-ATF1 fusion [104]. Prognostic criteria have changed substantially because the fibrohistiocytic tumor now is in a separate category, intermediate malignant (rarely metastasizing), occurring mainly in children and adolescents. This tumor was formerly considered a subtype of the broad category of malignant fibrous histiocytoma.

The third common mechanism involves the fusion of a catalytic domain of a tyrosine kinase receptor with a ubiquitously expressed protein providing a dimerization domain resulting in a constitutively activated, ligand-independent, chimeric tyrosine kinase. This latter mechanism is involved in the pathogenesis of inflammatory myofibroblastic tumor as a result of ALK rearrangements (TPM-ALK and so on) and congenital fibrosarcoma/cellular mesoblastic nephroma resulting from ETV6-NTRK fusion. Inflammatory myofibroblastic tumor of the urinary bladder is an unusual spindle cell neoplasm that displays cytologic atypia, infiltrative growth, and mitotic activity mimicking malignant tumors such as leiomyosarcoma, rhabdomyosarcoma, and sarcomatoid carcinoma. In inflammatory myofibroblastic tumor of the urinary bladder, positivity for ALK-1 by immunohistochemistry ranges from 33% to 89%, whereas ALK-1 protein expression in leiomyosarcoma and sarcomatoid urothelial carcinoma has not been reported, suggesting ALK-1 immunohistochemical studies may be useful in the differentiation of inflammatory myofibroblastic tumor from other spindle cell lesions in the urinary bladder [100]. In a similar mechanism, deregulation of the platelet-derived growth factor B-chain gene through fusion with collagen gene COL1A1 in dermatofibrosarcoma protuberans and giant cell fibroblastoma results in a chimeric autocrine growth factor that is diagnostic [92]. A histopathologic diagnosis is sufficient for dermatofibrosarcoma protuberans; however, the identification of this molecular event has important consequences in treatment, which is described later.

Lastly, a less common mechanism involves recurrent events within complex karyotypic changes. Very few neoplasms have been described in this category, including malignant peripheral nerve sheath tumor and dedifferentiated liposarcoma. Immunohistochemistry or FISH for detecting MDM2 or CDK4 alterations, two genes commonly amplified on 12q13-15 in atypical lipomatous tumor/well-differentiated liposarcoma [93], might be useful in the setting of a difficult differential diagnosis.

Most musculoskeletal tumors present with complex karyotypes lacking consistently identifiable specific genetic changes or expression profile signatures. These include most benign tumors and 60% of sarcomas [68]. Furthermore, these sarcomas tend to occur in older patients and exhibit high-grade pleomorphic cytology and p53 dysfunction. These include leiomyosarcoma, fibrosarcoma, myofibroblastic sarcoma, osteosarcoma, chondrosarcoma, pleomorphic rhabdomyosarcoma, and malignant fibrohistiocytic histiocytoma. Ongoing research could yet identify defining molecular events in these neoplasms.

Despite major advances in the cytogenetic characterization of benign and intermediate tumors of the bone, the incorporation of these alterations as molecular diagnostic tests has been less successful than in malignant tumors. This is because, in general, benign bone tumors are adequately treated by either an intralesional procedure (curettage and burr drilling, cryosurgery) or by marginal excision, depending on relevant anatomy. Furthermore, the recurrence rates of the chromosomal abnormality are not sufficient to achieve clinical molecular diagnostic specificity. Commercial FISH probes are still scarce and unavailable for routine molecular diagnosis.

Benign tumors fall broadly into one of the two of the previously mentioned mechanisms: (1) recurrent events within complex karyotypic changes; and (2) complex karyotypic changes without defined consistent events. Among benign tumors with recurrent events, FISH may be useful in distinguishing between the giant cell tumors of bone and aneurysmal bone cysts [91]. Telomeric associations are the most frequent chromosomal aberrations in giant cell tumor of bone, most commonly 19q, 11p, 16q, 17p, 18p, 20q, and 21p. All aneurysmal bone cysts exhibit involvement of chromosome segments 17p11-13 and/or 16q22 [91]. When confronted with a rearrangement, especially concerning 16q22 or 17p3, an associated aneurysmal bone cyst should be excluded. Chondroid lipoma is a rare tumor occurring in the subcutis or muscle of adults; it may be confused with liposarcoma and chondrosarcoma and shows microscopic features of both lipoma and hibernoma. It can be differentiated by identifying a recurrent translocation of t(11;16)(q13;p13) [11]. Upregulation of PTHrP and Bcl-2 expression characterizes the progression of osteochondroma toward peripheral chondrosarcoma and is a late event in central chondrosarcoma [15]. In this case, immunohistochemistry can be a useful tool in predicting prognosis if not in clarifying the diagnosis of a patient. Adamantinoma display Ewing-like histologic features described as “adamantinoma-like” Ewing’s sarcoma [39]. Recently, using RT-PCR on archival tissue, t(11;22) or t(21;22) was not found in any of 12 informative

adamantinomas [39]. Lipomas represent the most cytogenetically diverse benign tumors of fat tissue. Although 98% of lipomas have normal karyotypes, specific genetic abnormalities have been described in sporadic lipomas (12q13-15, t[3;12], 6p, 13q) [63], lipoblastoma (8q11-13) [31], hibernoma (11q13) [32], spindle cell/pleomorphic lipoma (13q12, 16q13) [20], and atypical lipomatous tumors (rings and giant markers secondary to 12q13-15 amplifications) [19]. Fibrous lesions, desmoplastic fibromas, desmoid tumors, and other miscellaneous tumors have cytogenetic abnormalities [80]; however, further distinguishing subtypes does not affect treatment criteria.

Molecular diagnosis should also be used in difficult distinctions between a benign and malignant diagnosis when the consequences of an incorrect interpretation are substantial. In this category, RT-PCR for detection of FUS-CREB3L2 fusion can be useful to distinguish a low-grade fibromyxoid sarcoma from other benign fibrous or neural proliferations when the immunohistochemical or ultrastructural findings are inconclusive [64]. A similar example includes the differential diagnosis between myxoid liposarcoma in children versus lipoblastoma, a diagnostic dilemma that can be settled by identifying the FUS-CHOP fusion (PLAG1 protein) by RT-PCR or the presence of an 8q abnormality by FISH [31]. A summary of common clinical scenarios described in this section in which molecular testing would be helpful is provided (Table 3).

Which Molecular Methods Are Best Applied to Histopathologic Sarcomas to Distinguish Between Major Subtypes?

Because detection of specific translocations or chimeric gene fusion products can be used reliably as disease-specific markers in diagnosing soft tissue tumors, an increasing number of practicing surgical pathologists or even treating physicians rely on molecular diagnostic validation [18, 70]. When evaluating the need to perform molecular evaluation for diagnosis, it is important to proceed along a practical algorithmic pathway before determining the need for molecular diagnostic tests. This is because the specificity of fusion, although reasonably high, is not absolute. Further drawbacks include high costs, low turnaround time, substantial numbers of test failures, and the limited number of FDA-approved probes or tests. Many existing pathologic techniques may be sufficient to establish a diagnosis without loss of accuracy and specificity. A practical diagnostic approach of integrating morphology, immunohistochemistry, and molecular genetics has been proposed by Chang and Shidham [18]. Initially, the specimens are evaluated for adequacy during fine-needle aspiration biopsy (FNAB) and/or core biopsy by immediate morphologic interpretation of the cytology smear or frozen section. The initial differential diagnosis based on morphologic interpretation is further refined using immunohistochemistry. At this point, the evaluation is often sufficient to yield a diagnosis. If the diagnosis remains inconclusive, then the use of molecular tools may be indicated for an accurate diagnosis. In most cases, molecular results should be used as validation of the morphologic differential diagnosis and corroborated with immunohistochemical findings and clinical information rather than as a challenge to the supremacy of histopathology.

Genetic testing is highly recommended to validate histologic diagnoses in unusual clinical presentations or unexpected immunohistochemical results. Even a specific diagnostic entity with a classic morphologic appearance can present a diagnostic challenge if it occurs in an unusual age group or location. A tumor with classic Ewing's sarcoma phenotype might need molecular confirmation if it occurs in an older individual or if it is present in a visceral location, for example. Similar examples might include a skeletal location of myxoid chondrosarcoma, gastrointestinal clear cell sarcoma, or renal synovial sarcoma. In the preceding diagnostic section, a summary of the appropriate molecular testing for different diagnostic dilemmas is provided. In the approach to molecular diagnostic, we describe classic molecular methods and more recent advances in molecular assays that are likely to be used in the future.

How do the Molecular Patterns Discovered on Genetic Diagnosis Affect Prognosis of Certain Sarcomas?

Recent studies have been conducted to study molecular aberrations as an independent marker of prognosis. The study of prognostic molecular markers, in particular, the type of fusion gene for determining prognosis in soft tissue sarcomas, has been addressed in four major types of sarcoma: (1) alveolar rhabdomyosarcoma [33]; (2) synovial sarcoma [34]; (3) Ewing's/peripheral neuroectodermal family of tumors [18]; and (4) myxoid liposarcoma [78].

Investigations regarding the prognostic value of the fusion genes PAX3-FKHR and PAX7-FKHR in alveolar rhabdomyosarcoma were initially carried out in a pilot study involving 34 patients [50]. The study reported better clinical outcomes for the PAX7-FKHR group by univariate analysis. These results were later confirmed in three other studies [3, 4, 22]. In patients presenting with metastatic disease, The Children's Oncology Group [98] reported there was a striking difference in outcome between PAX7-FKHR and PAX3-FKHR patient groups (estimated 4-year overall survival rate of 75% for PAX7-FKHR versus 8% for PAX3-FKHR). Furthermore, among metastatic ARMS, bone marrow involvement was higher in PAX3-FKHR-positive patients.

In nonmetastatic synovial sarcoma, patients with localized tumors and patients with the SYT-SSX2 fusion variant apparently have longer metastasis-free survival than those with the SYT-SSX1 variant [49]. A larger study confirmed the results reporting SYT-SSX fusion type appears to be the single most important prognostic factor by multivariate analysis in patients with localized disease at diagnosis [56]. Their results show the median and 5-year overall survivals for the SYT-SSX1 and SYT-SSX2 groups were 6.1 years and 53% and 13.7 years and 73%, respectively. However, these results are currently being debated after another study reported no association between the type of fusion gene and clinical outcome [36].

Two independent studies [23, 109] suggested the EWS-FLI1 type I fusion gene was associated with longer relapse-free (either metastasis or local recurrence) survival in patients with localized disease compared with other types of fusion gene in Ewing/PNET tumors. However, a third study has raised controversy by attributing no prognostic value to the fusion genes when evaluated for event-free and overall survival [36]. A single study has addressed the prognostic value of the type of fusion gene in myxoid liposarcoma, a common adult soft tissue sarcoma characterized by the presence of the TLS-CHOP fusion gene in 95% of cases. The authors were unable to find any association between the structure of the fusion gene and disease-specific survival but confirmed the value of careful histologic assessment for prognostication [24].

Despite the many issues involved in the study of molecular prognostic factors in sarcomas and despite the uncertainty that persists concerning the clinical relevance of fusion genes in these tumors, major biologic insights can be gained from this work. Recently, the identification of potentially therapeutically relevant molecular markers, including oncogenic protein tyrosine kinase, has been performed in studies addressing novel molecular prognostic factors. Expression of CYP3A4/5 was higher in primary biopsies of patients who developed distant metastatic disease compared with biopsies from patients with nonmetastatic disease [25].

Which Sarcoma Translocations Can Benefit From an Improved Response and Outcome Using Existing and Forthcoming Pharmacogenetic Approaches Targeting Molecular Events and How?

Optimal treatment and cure of patients with sarcomas remains an unsolved clinical problem. New pharmacogenetic approaches are being designed to target specific molecular events unique to individual sarcomas and to keep side effects to a minimum. Research in gene therapy [26, 27], stem cell biology [107], and nanotechnology [38, 44] will further enhance treatment options in the future. Targeting underlying molecular events in specific musculoskeletal neoplasias can provide dramatic benefits [62]. Fusion proteins generated by chromosomal translocations can function as tumor-specific antigens and are promising targets for immunotherapy. In a recent study, the induction of synovial sarcoma-specific cytotoxic T-lymphocytes from normal donor lymphocytes using in vitro stimulation with fusion peptide (derived from SYT-SSX fusion protein-pulsed dendritic cells) has been demonstrated [106]. These cytotoxic T-lymphocytes have the ability to lyse human synovial sarcoma tumor cells expressing the fusion protein. These findings suggest a peptide derived from the fusion protein may work as a neoantigen and induce a tumor-specific immune response. The identification of a new potential therapeutic target, ERBB2 (HER2.neu), has recently been reported for a subset of synovial sarcoma cases using cDNA microarray analysis [1].

Agents targeting receptor mechanisms, the cell cycle, and angiogenesis of soft tissue sarcoma and of those targeting osteoclasts in bone sarcomas are promising and in various phases of clinical trials. Imatinib's selective inhibition of a limited set of additional receptor tyrosine kinases has been effective in the management of locally extensive and malignant dermatofibrosarcoma protuberans (DFSP), a low-grade malignancy with a COL1A1-PDGFB translocation [92]. Flavopiridol is a cyclin-dependent kinase inhibitor (CDKI) drug that has strategically been used to interfere with determinants of crucial checkpoints in the cell cycle [90]. Well-differentiated and dedifferentiated liposarcoma and parosteal osteosarcomas harbor a characteristic 12q13-15 amplicon in which both cyclin-dependent kinase 4 (CDK4) and minute 2 gene (MDM2) reside [9]. Phase I studies with CDKIs together with chemotherapy are underway. Nutlins are a family of MDM2-specific agents, which by enhancing cytotoxicity of genotoxic agents, increase the efficiency of chemotherapy against p53 sarcoma cell lines such as osteosarcoma [2]. In colon, renal, lung, and breast cancer, antiangiogenic treatments are changing treatment options and improving patient outcomes. Recently, agents such as sorafenib and bevacizumab have been examined for usefulness as antiangiogenic agents in vascular sarcomas [30]. Bisphosphonates inhibit bone resorption through a number of pathways and have classically been used in the treatment of hypercalcemia, osteoporosis, and Paget's disease. Having shown antitumor activity in decreasing osteosarcoma metastasis in animals [43], a Phase II clinical trial is currently underway examining zoledronic acid and chemotherapy for patients with osteosarcoma. Phase III trials are also underway for inhibitors of RANKL as alternatives to bisphosphonates in treating patients with osteosarcoma [48].

Inherited genetic variations can serve as biomarkers for individual differences in response and toxic effects of

chemotherapeutic drugs and can even affect disease outcome. The 677TT genotype is associated with decreased enzymatic activity and can serve as a marker for methotrexate toxic effects in patients with osteosarcoma [110]. In patients with myxoid lipomatous sarcoma (MLS) with susceptible DNA-repair mechanism, adding the natural product alkylating agent trabectedin produces better responses to chemotherapy [35]. Recently, the ETV6-NTRK3 gene fusion has been identified in both infantile fibrosarcoma and cellular mesoblastic nephroma [7]. For both these tumors, standard curative treatment has been primarily surgical with wide local excision. This has frequently involved radical and even mutilating surgery. Three patients with identified ETV6-NTRK3 gene fusions were treated with preoperative chemotherapy, which produced excellent responses negating the need for amputation in two patients.

Agents inhibiting signaling pathways have been studied such as inhibitors of hedgehog signaling in chondrosarcoma, inhibitors of wnt/β-catenin in osteosarcoma and aggressive fibromatosis, and inhibitors of histone deacetylases in synovial sarcoma and Ewing's sarcoma. Several studies demonstrate chondrosarcomas and enchondromas exhibit activation of the hedgehog signaling pathway and blocking the pathway reduces cell proliferation and tumor size [42, 102]. Triparanol and cyclopamine, both hedgehog inhibitors, decrease tumor volume in animals by 60%, cellularity by 30%, and proliferation rate by 20%; however, the side effects currently limit clinical applications (birth defects such as limb malformations and holoprosencephaly) [102]. There is hope because when newer agents with lesser side effect profiles are developed, these agents can be used in patients with chondrosarcomas and other tumors with active hedgehog signaling [53]. Inhibitors of Wnt receptors such as frizzled homologue 10 receptor (FZD10) [72] or low-density lipoprotein receptor-related protein (LRP5) [37] reduce both local tumor growth and metastases in osteosarcomas in animal models. β-catenin is mutated in two-thirds of fibromatosis cases and all desmoid tumors exhibit β-catenin-mediated transcriptional activation [55]. Cyclooxygenase and matrix metalloproteinase inhibitors have shown promise in animal models [55]. Cyclooxygenase inhibitors are currently in clinical trials. Histone deacetylase inhibitors have been effective against synovial sarcoma [46], Ewing's sarcoma [85, 97], and chondrosarcoma [86] in preclinical studies. Clinical studies are expected in the near future.

Discussion

Survival from sarcomas is poised to greatly improve in the coming decade with the continued growth of literature and applications of molecular genetics. Molecular translocations are redefining and clarifying the classification of musculoskeletal sarcomas with greater specificity and accuracy. The potential benefits of this type of classification include improved diagnoses, improved prognostication, and improved treatment. We have described in this article the recent state of knowledge in molecular diagnosis of sarcomas, the application of that knowledge in prognostication, the appropriate technology in determining molecular patterns, and finally the development of novel therapeutics that has led to improved response rates and clinical outcomes with fewer side effects than standard cytotoxic chemotherapy.

Major issues exist with each of the questions presented in this article. Current definitions of molecular signatures are based on histopathologic classification. This raises the question of the need to use cytogenetics in diagnosis versus using histopathologic diagnosis if the two are equivalent. In this article, we stress that the use of molecular diagnosis should be reserved for diagnostic dilemmas, particularly when there are considerable differences in prognosis and the choice of treatment. Another important question is the sensitivity and specificity of the molecular signatures in identifying neoplasms. Few papers describe an analysis of sensitivity and specificity of a molecular diagnosis because the histopathologic diagnosis remains the gold standard for classification. A histopathologic diagnosis is required a priori to identify a molecular diagnosis. It is difficult to design a study to search for a molecular anomaly and to then assign a histopathologic diagnosis. Most papers have chosen to address this issue by determining the prevalence of a molecular signature. Another issue is the existence of a mixed tumor with combined features, which sometimes yields controversial molecular diagnostic results. This is well highlighted with the TLS-CHOP fusion transcript, which is described earlier as having a strong and specific association with myxoid liposarcoma (with similar translocations it is present in pure round cell LS and combined myxoid and round cell liposarcoma) allowing it to be distinguished from well-differentiated LS (WDLS) and pleomorphic liposarcoma, which contain no specific recurrent translocation [28]. Nonetheless, a single recent report has suggested TLS-CHOP fusion transcripts may also be present in pleomorphic LS and WDLS [105]. The existence of a mixed tumor with combined features of myxoid liposarcoma and WDLS has been proposed based on cases of liposarcoma showing histologic features of both [101]. This again highlights the phenomenon of developing diagnostic tools based on imperfect gold standards. Given interobserver bias and the lack of complete sampling, many such tumors would be considered myxoid liposarcomas. Identifying a round cell component can be very challenging in these situations and is not necessary for the diagnosis.

Yet, identifying the phenomenon of a "true myxoid liposarcoma" is critical because they are highly metastatic [52].

The data on the best diagnostic tools to use when identifying a particular translocation have been limited to the methods chosen by individual studies that first identified the molecular translocations. Very few studies justify the efficacy of using their tool of choice versus using other existing methods. There exists a large opportunity in quantifying the sensitivity and specificity of using a particular tool in determining specific molecular translocations.

Molecular markers promise a predictive framework for prognosis; however, some of these data are also generating controversial and, not uncommonly, contradictory findings. The recent debate around the prognostic value of synovial sarcoma fusion genes is an example of many of the issues encountered in studies of molecular prognostic markers in cancer. In a groundbreaking retrospective pilot study published in 1998, Kawai et al. [49] found patients with localized tumors harboring SYT-SSX1 fusion transcripts had decreased metastasis-free survival. Similar results were later obtained in four other retrospective studies [45, 65, 73, 76] and were further supported by a large multicenter study by Ladanyi et al. [56]. However, the same question readdressed in another multiinstitutional study by Guillou et al. [36] reported no association between the type of fusion gene and clinical outcome. So what factors can account for these differing results, and what valuable lessons can be taken from these studies? A summary by Oliveira et al. [75] highlights the deficiencies in the use of retrospective design in prognostic studies and their unavoidable shortcomings, including missing data and the possibility of several selection biases. As described by the authors, validation of putative prognostic factors should be more rigorous and be performed in three major phases: exploratory studies, retrospective confirmatory investigations, and prospective studies.

Many additional hurdles beyond the understanding of the biology of different tumor subtypes still need to be overcome. Even with newer, more specific agents for systemic treatment, the key molecules downstream of specific targets, which signify a good response to treatment, still need to be identified. Additionally, the identification of individual patients who are most likely to respond to a specific treatment, ie, a novel targeted drug, a standard chemotherapeutic, or a combination, will rely on the development of robust biomarkers that can account for genetic variability in the tumor, the tumor's surrounding microenvironment, and each patient's germline. Even if a sarcoma subtype is associated with an activated molecular pathway, the development of methods to accurately identify pathway activation will be imperative before patient enrollment in a targeted drug trial.

This article reviews molecular translocations for 30 musculoskeletal tumors that have been described in the literature and molecular technologies that can be used to identify these translocations. We identify 14 unique scenarios that have been described in the literature in which a molecular diagnosis will benefit a clinician in making decisions. We review 4 musculoskeletal tumors for which prognostic studies have been conducted and the potential use of in clinical decision-making. We finally analyze the state of literature in the treatments that are being developed based on molecular translocations. We review 13 potential therapeutic agents that are in various phases of development.

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