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Review

Biological Prognostic Factors in Adult Soft Tissue Sarcomas

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Abstract. *Adult soft tissue sarcomas (STSs) are a rare group of highly heterogeneous neoplasms arising in different tissues. They are locally aggressive and can produce recurrence and distant metastasis. The most common metastatic sites are lung, lymph nodes, liver, bone and soft tissues. Staging for STSs has been based on some prognostic information: grade (low vs. intermediate/high grade), size (small vs. large tumors), depth of infiltration (superficial vs. deep neoplasms) and presence or not of distant metastasis. In the last 10 years, a plethora of new markers (proliferation markers and DNA alteration, P-gp, p53, TLS-CHOP, cyclins, survivin, TERT, PAX3-PAX7/FKHR, Syt-SSX1/2, VEGF, E-cadherin and beta-catenin, nm23, SKP-2, p27, CD40) has been studied with regard to their role in promoting progression (in a laboratory setting) and then determining prognosis and therapy (in a clinical setting). In the present survey, we focused on the role of new biological prognostic factors in STSs and also reported the quality of such studies with an ad hoc designed questionnaire.*

Adult soft tissue sarcomas (STSs) are a rare group of highly heterogeneous neoplasms arising in different tissues and characterized by cells that can range from spindled to fusiform in shape. They are locally aggressive and can produce recurrence and distant metastasis. The most common metastatic sites are lung, lymph nodes, liver, bone and soft tissues (1,2).

STS depends on many different clinical and pathological characteristics. Traditionally, staging for STSs has been based on some prognostic information: grade (low *vs.* intermediate/high grade), size (small *vs.* large tumors), depth of infiltration (superficial *vs.* deep neoplasms) and presence or not of distant metastasis (2-8). In the last 10 years, a plethora of new molecules has been studied with regard to their role in promoting progression (in a laboratory setting) and then determining prognosis (in a clinical setting). In the near future, these new prognostic factors will allow for the identification of different degrees of risk and, in many cases, the administration of new target-based therapies.

In the present survey, we focused on the role of new biological prognostic factors in STSs. We also reported the quality of such studies with an *ad hoc* designed questionnaire.

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Materials and Methods

Literature selection criteria and quality evaluation questionnaire. This survey focuses on all prognostic studies of biologic/molecular markers in STSs (with the exception of gastrointestinal stromal tumors, [GIST]) published between June 1992 and June 2004. By prognostic factors, we mean a marker that correlates with survival and disease-free survival. Studies lacking a formal time-to-event analysis were excluded. Articles following searches of PubMed with the terms "soft tissue sarcomas" and "prognostic factors" were identified, and references from relevant articles were also included. Journals with an impact factor <1 during the selected time-period were excluded. Studies reporting data on <45 patients were also excluded. Each article was checked with regard to the biological rationale underlining the prognostic potential of the selected molecule. An *ad hoc* quality evaluation questionnaire (Table I) was designed, which was based on a literature review (1-7). Each trial was given a score with a maximum of 100 points by two independent reviewers who were not included in the authorship (MDM and LDM) and blinded to the journal and authors' names.

Results

New prognostic factors. In the last 10 years, the prognostic value of new molecular factors has been tested in STSs. In addition to providing new prognostic and diagnostic markers, these molecules, in some cases, are important to

Table I. *Criteria list for methodological quality assessment of selected prognostic studies of biological / molecular factors in soft tissue sarcomas.*

Study population
- A. Trial size: 0 points if total number of patients is <80; 15 points if >80.

Study characteristics
- B. 5 points if retrospective; 15 points if prospective.
- C. Basic background: 0 points if preclinical evidence is not cited; 10 points if yes.
- D. Time-to-event descriptions: 10 points if overall survival and disease-free survival are reported; 0 if no.
- E. Follow-up: 10 points if information about follow-up is reported (median and range); 0 if no.
- F. 10 points if methodology for marker evaluation (technique, reagents, negative controls) is clearly presented; 0 if no.
- G. 10 points if the expression of the prognostic marker is confirmed with two different techniques (or more); 0 points if no.

Statistical analysis
- H. 0 points if the number of analyzed patients is ≤75% of total patients included into the study; 10 points if >75%.
- I. 10 points if multivariate analysis is performed with adequate no. of patients (>80), follow-up and events (if median time-to-event is reached); 0 if no.

the biology of the tumors, so that they might be used, in the very near future, as targets of anticancer therapies.

In the following section, we propose a list of new prognostic factors developed in recent years. Each one is briefly described with regard to its role in tumor biology and then in determining the prognosis of STSs. In Table II, studies dealing with these factors are reviewed with regard to methodological features and conclusions.

Proliferation markers and DNA alteration. Carcinogenesis is linked to the development of proliferative abnormalities, and these have been found to have prognostic significance in a variety of human tumors. In general, growth rate is a principal determinant of the aggressiveness of a tumor and an important prognostic factor. Several methods are available for assessing proliferation in human tumors (in routine sections as well as in fresh neoplastic tissues or cells). The most common methods used in recent years have been immunohistochemical detection of the proliferation-associated antigen Ki-67, proliferating cell nuclear antigen (PCNA) and flow cytometric measurement of the S-phase fraction (SPF).

Ki-67 is a DNA-binding nuclear protein expressed throughout the cell cycle in proliferating cells, but not in quiescent (G0) cells (9). MIB-1, an antibody against Ki-67 antigen, is able to detect proliferating cells in formalin-fixed, paraffin-embedded tissue sections after microwave antigen retrieval. Ki-67 expression by a such technique has been correlated with prognosis in a series of STSs and synovial sarcomas (10-14).

Proliferating cell nuclear antigen (PCNA) is a 36-kDa nuclear protein acting as a cofactor for DNA polymerase delta (15). Synthesis of PCNA is reported to correlate directly with DNA replication and cell proliferation. The expression of PCNA increases in late G1, reaches its maximum in S1 and then declines (16). The expression of PCNA has been found to be related to prognosis in synovial sarcoma and high-grade malignant fibrous histiocytoma (17,18).

Chromosomal and DNA ploidy changes frequently occur in malignancies. It is well recognized that several solid tumors have an abnormal cellular DNA content and that there is a relationship between the cellular DNA content and prognosis. Conflicting results have been reported on the role of the DNA content in determining the prognosis of STSs. Three studies found no prognostic power for the determination of DNA content (19-21), while 7 studies reported that the DNA content was associated with prognosis (10, 12, 17, 22-25).

P-gp. Resistance to chemotherapy is a common clinical problem that is encountered in many different neoplasms. The multi-drug resistance phenotype (*i.e.* resistance to a variety of drugs) is induced by the *MDR-1* gene, which is a single-copy gene located on chromosome 7. It encodes for a 1280 amino acid trans-membrane glycosylated protein of 170-kDa, p-glycoprotein (P-gp). P-gp is an energy-dependent pump that removes lipophilic agents from the cell (26). Many different chemotherapy drugs are P-gp substrates: vinca alkaloids, epipodophyllotoxins, mitomycin, paclitaxel, anthracycline, *etc*. These drugs are active in a plethora of tumors. Induction of P-gp in chemotherapy-sensitive tumor cells makes them resistant to chemotherapy. Furthermore, the presence of P-gp has been related to poor prognosis in several malignancies. The expression of P-gp has been evaluated by Levine *et al.* in adult STSs (10), in a study of a total of 65 patients for which P-gp staining by immunohistochemistry was performed in 50 patients. P-gp was expressed in 48% of cases. Interestingly, the glycoprotein was present in 3 out of 3 rhabdomyosarcomas and in 1 out of 12 desmoid and fibrosarcomas (these differences were statistically significant). With a mean follow-up duration of 83 months, the P-gp expression was an independent prognostic indicator that correlated with poor outcomes. The median survival time for P-gp-positive patients was 30.5 months *versus* 56.5 months for P-gp-negative patients. Disease-free survival was also significantly better for P-gp-negative tumors: 9.7 *versus* 28.5 months. Only 12 patients were assessable for response to chemotherapy so that the important question of the

Table II. *Characteristics of selected studies.*

Author, Year	No. of analyzed patients/ total patients	Molecular markers	Stages	Period of follow-up Range, Median	Multivariate analysis	Histology	Methodology	Conclusions
Gustafson, 1992	48/48	DNA content	All	3-24 y, 8 y	Yes	L	FC	Negative
Oda, 1993	51/56	PCNA, SPF, DNA content	All	3-245 m, ?	Yes	SS	IHC	Positive (PCNA)
Wynaendts, 1993	95/98	DNA content, SPF	All	1-19 y, 6 y	Yes	RMS	FC	Positive
Kawai, 1994	96/96	p53	All	ns, 5.1 y	No	All STS	IHC	Positive
Dreinhofer, 1994	48/48	PCNA	All	2-7 y, 4 y	No	MFH	IHC	Positive
Kuratsu, 1995	44/151	DNA content	All	2-297 m, 47 m	Yes	All STS	FC	Negative
Huuhtanen, 1996	155/193	DNA content, SPF	All	0,3-35,8 y, 3,5 y	Yes	All STS	FC	Negative
De Zen, 1997	59/59	DNA content	All	7-107 m, 35 m	Yes	RMS	FC	Positive
Levine, 1997	38/65	Ki-67, P-gp, DNA content	All	ns, 38 m	Yes	All STS	IHC, RT-PCR	Positive
Gustafson, 1997	160/160	DNA content, SPF	All	6-31 y, 16 y	Yes	All STS	FC	Positive
Royds, 1997	88/88	nm23	All	minimum of 3 y	Yes	All STS	IHC	Negative
Collin, 1997	83/132	DNA content, SPF	All	1-123 m, 44 m	Yes	All STS	FC	Positive
Kim, 1998	84/84	Cyclins	All	ns, 2.4 y	Yes	All STS	IHC and Southern blot	Positive (cyclin D1)
Heslin, 1998	121/121	Ki-67, Rb, p53, mdm2	All	ns, 64 m	Yes	All STS	IHC	Positive (Ki-67)
Kawai, 1998	45/45	Syt-SSX1/2	All	2-180 m, 26 m	Yes	SS	RT-PCR	Positive
Huuhtanen, 1999	123/123	DNA content, Ki-67	All	22-447 m, 92 m	No	All STS	IHC, FC	Positive
Skytting, 1999	86/86	Ki-67, p53	All	2-11 y, 6 y	Yes	SS	IHC	Positive
Saito, 2000	62/72	E-cadherin, A-catenin	All	1-232 m, 55.5 m	Yes	SS	IHC	Positive
Oliveira, 2000	47/47	P27	All	0.7-3 y, 6.3 y	Yes	MR Lip	IHC	Positive
Hoos, 2001	47/47	Ki-67	All	7-129 m, 39 m	No	All STS	IHC	Positive
Yudoh, 2001	115/115	VEGF	All	63-176 m, ?	Yes	All STS	ELISA	Positive
Kim, 2001	79/79	Cyclin D1	All	0.3-35.8 y, 3.5 y	Yes	All STS	IHC	Positive
Antonescu, 2001	82/82	TLS-CHOP, p53	All	3-288 m, 44 m	Yes	Mix Lip	RT-PCR	Positive (p53)
Wurl, 2002	66/89	Survivin, TERT	All	ns	Yes	All STS	RT-PCR	Positive
Sorensen, 2002	78/171	PAX3-PAX7/FKHR	All	ns, 3.9 y	Yes	RMS	RT-PCR	Positive
Ladanyi, 2002	160/243	Syt-SSX1/2	All	0.05-25.5 y, 2.7 y	Yes	SS	RT-PCR	Positive
Kohler, 2002	62/82	BCL-2 gene family	All	32 m (4-120)	Yes	All STS	RT-PCR	Positive
Oliveira, 2003	47/47	Skp-2	All	0.7-23 y, 6.3 y	Yes	All STS	IHC	Positive

(y=years, m=months, L=leiomyosarcoma, SS=synovial sarcoma, RMS=rhabdomyosarcoma, All STS=all soft tissue sarcomas, MR Lip=myxoid and round-cell liposarcomas, Mix Lip=myxoid liposarcoma, MFH=malignant fibrous hystiocytoma).

predictive power of P-gp with regard to chemosensitivity was not evaluated in this study.

p53. p53 is one of the most widely studied oncogenes. Overexpression of *p53* appeared to cause oncogenic transformation of cells, and *p53* mutations occur with unusually high frequency in tumor tissues. Wild-type *p53* genes, when introduced into cells, were found to be growth suppressive. Furthermore, mice that are homozygous null for *p53*, although developmentally competent, are highly predisposed to tumors. p53 contains a strong transcriptional activation domain within its amino terminus and it is a tetrameric, sequence-specific DNA-biding protein. Although the p53 protein acts as a transcriptional activator of genes containing p53-binding sites, it is also capable of strongly inhibiting transcription from many genes lacking p53-binding sites (27). Several oncogenic DNA viruses express viral gene products that associate with and inhibit the trans-activation function of *p53*, notably SV40 large T antigen, the adenovirus E1B 55-kDa protein, and the E6 protein of oncogenic forms of human papillomavirus (HPV E6) (28). In cells, p53 can associate with a 90-kDa protein, identified as the product of the *mdm-2* oncogene, which is amplified in some types of tumors. When bound to *mdm-2*, p53 can no longer function as an activator of transcription. *p53* plays multiple roles in cells. Expression of high levels of wild-type *p53* has two outcomes: cell cycle arrest or apoptosis. The observation that DNA-damaging agents induce levels of *p53* in cells led to the definition of *p53* as a checkpoint factor. While dispensable for viability, in response to genotoxic stress, *p53* acts as an "emergency brake" inducing either arrest or apoptosis, protecting the genome from accumulating excess mutations. Consistent with this

notion, cells lacking *p53* were shown to be genetically unstable and thus more prone to malignancy. Three studies have reported that p53 is a prognostic factor in STSs (13, 29, 30), and only one study failed to show any prognostic power (11).

TLS-CHOP. The *CHOP* gene is a member of the CHOP family of leucine zipper transcription factors, implicated in adipocyte differentiation and growth arrest (31). The *CHOP* gene is normally expressed at very low levels in most cells, including adipocytes; however, it is markedly activated by perturbations that induce cellular stress. In >95% of cases of myxoid liposarcomas (MLS) and round-cell (RC) liposarcomas the t(12;16)(q13;p11) translocation occurs, resulting in the hybrid *TLS-CHOP* gene. The *TLS* gene is also known as *FUS*. The hybrid protein consists of the 5' portion of TLS fused to the entire coding region of CHOP. The TLS-CHOP protein functions primarily as an aberrant transcriptional regulator that interferes with adipocyte differentiation, favoring proliferation over terminal differentiation (32). On the basis of different portions of TLS included into the hybrid gene product (TLS exons 5, 7, and 8, respectively, to exon 2 of CHOP) three major recurrent fusion transcript types have been reported in cases of MLS: type 7-2 (also known as type I); type 5-2 (also known as type II); and type 8-2 (also known as type III).

The prognostic impact of different TLS-CHOP fusion transcripts has been investigated in MLS, but the molecular variability of this fusion transcript structure seems not to be associated with clinical outcome (30, 39). The majority of p53-positive MLS contains the type 5-2 TLS-CHOP fusion, and none of the type 7-2 or type 8-2.

Cyclins. Cyclins are proteins that govern transitions through distinct phases of the cell cycle by regulating the activity of the cyclin-dependent kinases. *Cyclin D1*, one of the key cell cycle regulators, is a putative proto-oncogene over-expressed in a wide variety of human neoplasms, and was originally cloned as an oncogene responsible for parathyroid adenomas (33). In mid- to late G1, cyclin D1 shows a maximum expression following growth factor stimulation. Binding of G1 cyclins to cyclin-dependent kinases leads to phosphorylation of the retinoblastoma protein and progression through the G1- and S-phases of the cell cycle. Cyclin D1 has also been successfully employed and is a promising tool for further studies in both cell cycle biology and cancer-associated abnormalities (34, 43). Two studies reported the prognostic role of cyclin D1 expression in STSs. In the first, the authors reported, with a median follow-up of 3.5 years, that the cyclin D1 expression was associated with a high rate of local recurrence and with a poor prognosis in retroperitoneal sarcomas (35). In the second, overexpression of cyclin D1 was associated with poor prognosis in 84 patients affected with extremity STSs (36).

Survivin and TERT. Recently, Wurl *et al.* published their work on the prognostic role of the co-expression of survivin and TERT in STSs (37). Survivin is an inhibitor of apoptosis (IAP), containing one baculovirus IAP repeat (BIR) domain and has been reported to be capable of regulating both cellular proliferation and apoptotic cell death. Survivin expression has been also described during embryonic development and in adult cancerous tissues, with greatly reduced expression in normal adult differentiated tissues, particularly if their proliferation index is low. This makes survivin a potentially attractive target for cancer therapeutics. Survivin has been defined as a universal tumor antigen and is the fourth most significant transcriptosome expressed in human tumors.

Telomeres are primarily controlled by a highly specialized DNA polymerase, termed telomerase. In early studies, high levels of telomerase activity were detected in cancer cells, but no activity was found in most normal somatic cells, leading to the speculation that telomerase might be required for tumor growth. Recent studies have demonstrated that introduction of the human telomerase reverse transcriptase (hTERT) into telomerase- negative cells activates telomerase and extends cell lifespan. These findings suggest that telomerase plays an important role in long-term cell viability and cell proliferation (38). The expression of survivin and TERT was investigated in 89 adult soft tissue sarcomas. The cumulative 2-year survival rate was 27.9% for patients with increased expression of survivin and TERT compared with 100% of patients with negative expression.

PAX3-PAX7/FKHR. Chromosomal analyses have demonstrated the frequent presence of 2 translocations associated with alveolar rabomyosarcomas (ARMS): t(2;13)(q35;q14) and t(1;13)(p36;q14). These translocations fuse the *FKHR* locus on chromosome 13 to either *PAX3* on chromosome 2 or the chromosome 1 *PAX7* gene. *PAX3-FKHR* and *PAX7-FKHR* gene fusions produce chimeric proteins that combine transcriptional domains from the corresponding wild-type proteins and thereby acquire oncogenic activity (39). *PAX3-FKHR* and *PAX7-FKHR* mRNAs can be assessed by reverse transcriptase polymerase chain reaction (RT-PCR) in primary tumor tissues of ARMS. *PAX3-FKHR* is 4.5-fold more frequent than *PAX7-FKHR*. These gene fusions are also found in a small proportion of embryonal rhabdomyosarcomas (ERMS) cases but not in other "small round-cell tumors", thus allowing for the use of these proteins in the diagnostic work-up of ARMS. In patients presenting with metastatic ARMS, the estimated 4-year survival for patients with *PAX7-FKHR* was 75%, while for patients with *PAX3-FKHR* the same

survival was 8%. Again, *FAX3-FKHR* was associated with a significantly higher bone marrow involvement (40).

Syt-SSX1/2. Synovial sarcomas (SS) represent 10% of all STSs. In essentially all cases, SSs contain a t(X;18;p11;q11) representing the fusion of *SYT* (at 18q11) with either *SSX1* or *SSX2* (both at Xp11), or rarely, with *SSX4* (also at Xp11). Neither SYT nor the SSX proteins contain DNA-binding domains. Instead, they appear to be transcriptional regulators the actions of which are mediated primarily through protein-protein interactions. The *SSX1* and *SSX2* genes encode 188 amino acid proteins that are highly similar. Between SSX1 and SSX2, the COOH-terminal 78 amino acids of SSX proteins included in SYT-SSX differ at 13 residues. The significance of the resulting amino acid differences between SYT-SSX1 and SYT-SSX2 to their putative roles as aberrant chimeric transcriptional proteins are presently unknown. *SYT-SSX1* and *SYT-SSX2* appear to be mutually exclusive gene fusions in SS. Interestingly, the fusion type is concordant in primary tumors and metastases and constant over the course of the disease (41).

A large study by Ladanyi *et al.* found a significant relationship between fusion type and histological subtype (biphasic SS with the SYT-SSX1 fusion transcript and monophasic SS with the SYT-SSX2 transcript). In addition, the type of transcript was significantly correlated to prognosis in localized tumors: patients with *SYT-SSX2* had better survival than those with *SYT-SSX1*. Thus, the identification of the SYT-SSX chimeric transcript can provide a sensitive diagnostic test for SS, as well as prognostic information (42).

VEGF. Several data indicate that the aggressiveness of solid tumors depends on angiogenesis. Neovascularization supports tumor growth in both primary and secondary sites. Vascular endothelial growth factor (VEGF) is a strong angiogenic factor and correlates in several neoplasms, as well as in experimental models, with tumor progression. The VEGF family of growth factors are highly conserved secreted glycoproteins that regulate vasculogenesis, hematopoiesis, angiogenesis, lymphangiogenesis and vascular permeability and are implicated in many physiological and pathological processes. To date, the VEGF family is comprised of VEGF-A, -B, -C and -D and Orf virus VEGFs (also called VEGF-E). Of the three VEGF tyrosine kinase receptors identified to date (VEGFR-1, -2 and -3), VEGFR-1 binds VEGF-A and -B, VEGFR-2 binds VEGF-A, -C, -D and -E, and VEGFR-3 binds VEGF-C and -D. VEGFRs differ with respect to their mechanisms of regulation and patterns of expression. For example, VEGFR-1 and -2 are expressed almost exclusively by vascular endothelial cells and hematopoietic precursors, whereas VEGFR-3 is widely expressed in the early embryonic vasculature, but becomes restricted to lymphatic endothelium at later stages of development and in post-natal life (43).

Yudoh *et al.* studied the prognostic relevance of neovascularity assessed by microvessel density and the concentration of VEGF in the tumor tissue of patients with STSs (by ELISA). Only the tissue concentration of VEGF was an independent prognostic factor for the disease outcome. The 5-year survival rate for patients with high VEGF level (≥2.5 μMol/mg protein) was 40.2%, while the rate for patients with low VEGF level was 66.6% (44).

E-cadherin and beta-catenin. E-cadherin is one of the most important molecules in cell-cell adhesion. It is localized on the surfaces of cells in regions of cell-cell contact known as adherens junctions. The human epithelial *(E)-cadherin* gene maps to chromosome 16q22.1. As a member of a large family of genes coding for calcium-dependent cell adhesion molecules (CAMs), the cadherin glycoproteins are expressed by a variety of tissues. It is essential for the formation and maintenance of epithelia (45). Besides its role in normal cells, this highly conserved gene can play a major role in malignant cell transformation, and especially in tumor development and progression. The suppression of *E-cadherin* expression is regarded as one of the main molecular events responsible for dysfunction in cell-cell adhesion. Most tumors have abnormal cellular architecture and loss of tissue integrity can lead to local invasion. In other words, loss of function of *E-cadherin* correlates with increased invasiveness and metastasis of tumors, resulting in it being referred to as a "suppressor of invasion" gene (46, 47). On the cytoplasmic side of the membrane, a bundle of actin filaments is linked to the E-cadherin molecules *via* a protein complex. Alpha-catenin and either beta- or gamma-catenins are included in this complex. Beta- and gamma-catenins share significant homology and bind to a specific domain at the E-cadherin C-terminus. Alpha-catenin links the bound beta- or gamma-catenin to the actin cytoskeleton. The mechanism that renders E-cadherin functional is unknown, but it does include phosphorylation of the protein. Cadherin-mediated adhesion is a dynamic process that is regulated by several signal transduction pathways. There is also evidence that cadherins are not only targets for signaling pathways that regulate adhesion, but may themselves send signals that regulate basic cellular processes, such as migration, proliferation, apoptosis and cell differentiation (48-51).

Reduced expression of E-cadherin and alpha-catenin and widespread aberrant expression of beta-catenin within the cytoplasm and/or the nuclei were significantly correlated with a poor survival rate in synovial sarcomas (52).

nm23. The *nm23* gene was first identified by differential screening of melanoma cell lines of high and low metastatic potential (nm means non metastatic). Two human homologs

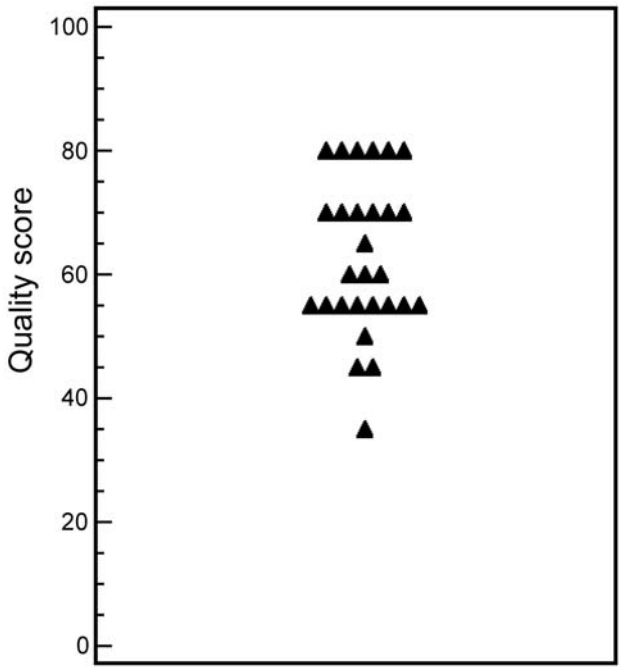

Figure 1. *Dot-plot distribution of quality scores of selected prognostic studies.*

of the *nm23* gene (located on cromosome 17) have been isolated and designated *nm23-H1* and *nm23-H2* (98% of homology). They may have arisen by a tandem duplication. The products of these genes have been identified as nucleoside diphosphate kinase A (NDPK-A) and NDPK-B, respectively. *nm23-H1* and *nm23-H2* are metastasis-suppressor genes implicated in the control of the metastatic process of malignant cells. nm23 proteins may act in the regulation of signal transduction by complexing with G proteins, causing activation/inactivation of developmental pathways. Expression of nm23 has been investigated in a number of tumors and is associated with a less aggressive phenotype (53).

A study by Royds *et al.* in 88 STSs suggests that the expression of nm23 in sarcomas is variable and has no value as a prognostic indicator (54).

SKP-2 and p27. Human SKP1 and SKP2 were identified as components of a stable quarternary KP1/SKP2/DK2/ cyclin A/Cks (cyclin-dependent kinase subunit) complex that was present in elevated levels in human transformed cell lines compared to their non-transformed counterparts (55).

Skp2 (S-phase kinase-associated protein 2) is a member of the F-box family and is implicated in the ubiquitin-mediated degradation of several key regulators of G(1) point progression. It positively regulates the G(1)-S transition by controlling the stability of several G(1) regulators, such as the cell cycle inhibitor p27. SKP2 functions as a critical component in the PTEN/PI 3-kinase pathway for the regulation of p27 and cell proliferation (56).

The protein is oncogenic and overexpressed in several human cancers, its expression correlating directly with the grade of malignancy. p27 is a member of the Waf1/Cip1 family of cyclin-dependent kinase inhibitors, which also includes p21 and p57. p27 binds and inhibits the cyclin E-CDK2 enzymatic complex, which causes a cell block in the G1-phase of the cell cycle. High Skp2 expression has been demonstrated to be an independent predictor for decreased local recurrence-free, disease-free and overall survival in stage II and III STSs (57). By contrast, low expression of p27 has been shown to predict decreased metastasis-free and overall survival in myxoid and round-cell liposarcomas (58).

CD40. CD40 is a member of the nerve growth factor/tumor necrosis factor (TNF) receptor superfamily. CD40 is expressed by B cells, dendritic cells, monocytes, epithelial cells including thymocytes, several exocrine glands (salivary glands, sweat glands, mammary glands and pancreas) and endothelial cells. A variety of non-lymphoid cell types express both receptor and CD40 ligand (CD40L), including hematopoetic and non-hematopoetic cells, such as monocytes, basophils, eosinophils, dendritic cells, fibroblasts, smooth muscle and endothelial cells. The CD40 signal is critical for B-cell proliferation, growth and differentiation, but several studies have recently shown that it mediates a diverse array of biological processes. It plays a critical role in humoral and cellular immune responses and has been implicated in biological pathways involving epithelial cells, fibroblasts and platelets. Recently, it has been demonstrated that CD40 activation may lead to the promotion of many phenomena involved in cancer invasiveness and metastatization (neoangiogenesis, matrix-metalloproteinase induction, proliferation, motility, *etc.*) (59).

The expression of CD40 was studied in 82 patients with STSs. With 61 patients (74.4%) progressed and 31 (37.8%) died, CD40 expression was a significant prognostic factor for disease-free and overall survival on univariate and multivariate analysis. Patients with tumors expressing CD40 in more than 50% of cells had a dramatically unfavorable prognosis with median disease-free and overall survival of 7 and 17 months, respectively; and hazard ratios of relapse and death as compared to patients with CD40-negative tumors of 2.89 (95% CI: 1.26-6.60) and 6.92 (95% CI: 2.18-22.0), respectively (60).

Quality of studies. The studies reviewed presented a very heterogeneous quality score ranging from 35 to 80 points (median score of all studies: 60) (Figure 1). No significant increase of quality scores was registered during the analyzed period (data not shown).

Conclusion

In the present survey, two important issues were raised. The first finding we would like to emphasize is that the quality of prognostic studies is very heterogeneous. In some cases there is heterogeneity of results between studies that could depend on the extreme diversity of tumor biology, but also on the methodological pitfalls of such studies. We noticed, for example, that prognostic studies in STSs are frequently retrospective analyses of archival tissues; in other words, the tissues are not collected specifically to address the prognostic power of a marker. Studies lack distinction between the potential prognostic *versus* predictive role. Patients may have received one of many types of therapy, but frequently such therapies are not clearly described. Again, not all patients included in the initial trial were later included in the prognostic analysis study. Finally, the heterogeneity of technical issues (methods of detection, different reagents between assays, different positive/negative cut-off levels, different specimen preparations, *etc.*) could be a source of variability. The second issue of concern is that the quality scores did not improve over the selected period. Both of the issues above could be, in part, due to the lack of guidelines available to the oncologist conducting prognostic studies.

Recent research has focused on the investigation of the prognostic role of many different molecules in STS, because of the increasing perception that cancer invasion and metastasis are multifactorial processes and that the molecular characteristics of neoplasms determine their clinical behavior. Such approaches will become increasingly feasible with the improvement of methodological issues and biomolecular techniques (61). Ideally, the analysis of samples obtained by surgery with regard to the expression of selected proteins, mRNA or DNA by gene profiling techniques will play an important role in determining prognosis and treatments.

References

1 Weiss SW and Goldblum JR: Enzinger and Weiss's Soft Tissue Tumors. 4th ed. St. Louis, Mo: Mosby, 2001.

2 Komdeur R, Hoekstra HJ, van den Berg E *et al*: Metastasis in soft tissue sarcomas: prognostic criteria and treatment perspectives. Cancer Metastasis Rev *21*: 167-183, 2002.

3 Maltoni M and Amadori D: Prognosis in advanced cancer. Hematol Oncol Clin North Am *16*: 715-729, 2002.

4 Montserrat E: Prognostic factors in aggressive lymphoma: the contribution of novel biological markers. Ann Hematol *80*: B42-44, 2001.

5 Gospodarowicz M, Mackillop W, O'Sullivan B *et al*: Prognostic factors in clinical decision making: the future. Cancer *91*: 1688-1695, 2001.

6 Yamauchi H, Stearns V and Hayes DF: When is a tumor marker ready for prime time? A case study of c-erbB-2 as a predictive factor in breast cancer. J Clin Oncol *19*: 2334-2356, 2001.

7 Gospodarowicz M and O'Sullivan B: Prognostic factors in cancer. Semin Surg Oncol *21*: 13-18, 2003.

8 Greene FL, Page DL, Fleming ID, Fritz AG, Balch CM, Haller DG and Morrow M (eds.): Soft tissue sarcoma. *In*: American Joint Committee on Cancer: AJCC Cancer Staging Manual. 6th ed. New York, NY: Springer, pp. 193-197, 2002.

9 Gerdes J: Ki-67 and other proliferation markers useful for immunohistological diagnostic and prognostic evaluations in human malignancies. Semin Cancer Biol *1*: 199-206, 1990.

10 Levine EA, Holzmayer T, Bacus S *et al*: Evaluation of newer prognostic markers for adult soft tissue sarcomas. J Clin Oncol *15*: 3249-3257, 1997.

11 Heslin MJ, Cordon-Cardo C, Lewis JJ, Woodruff JM and Brennan MF: Ki-67 detected by MIB-1 predicts distant metastasis and tumor mortality in primary, high grade extremity soft tissue sarcoma. Cancer *83*: 490-497, 1998.

12 Huuhtanen RL, Blomqvist CP, Wiklund TA *et al*: Comparison of the Ki-67 score and S-phase fraction as prognostic variables in soft-tissue sarcoma. Br J Cancer *79*: 945-951, 1999.

13 Skytting BT, Bauer HC, Perfekt R, Nilsson G and Larsson O: Ki-67 is strongly prognostic in synovial sarcoma: analysis based on 86 patients from the Scandinavian Sarcoma Group Register. Br J Cancer *80*: 1809-1814, 1999.

14 Hoos A, Stojadinovic A, Mastorides S *et al*: High Ki-67 proliferative index predicts disease specific survival in patients with high-risk soft tissue sarcomas. Cancer *92*: 869-874, 2001.

15 Maga G and Hubscher U: Proliferating cell nuclear antigen (PCNA): a dancer with many partners. J Cell Sci *116*: 3051-3060, 2003.

16 Hall PA, Levison DA, Woods AL *et al*: Proliferating cell nuclear antigen (PCNA) immunolocalization in paraffin sections: an index of cell proliferation with evidence of deregulated expression in some neoplasms. J Pathol *162*: 285-294, 1990.

17 Oda Y, Hashimoto H, Takeshita S and Tsuneyoshi M: The prognostic value of immunohistochemical staining for proliferating cell nuclear antigen in synovial sarcoma. Cancer *72*: 478-485, 1993.

18 Dreinhofer KE, Akerman M, Willen H, Anderson C, Gustafson P and Rydholm A: Proliferating cell nuclear antigen (PCNA) in high-grade malignant fibrous histiocytoma: prognostic value in 48 patients. Int J Cancer *59*: 379-382, 1994.

19 Gustafson P, Willen H, Baldetorp B, Ferno M, Akerman M and Rydholm A: Soft tissue leiomyosarcoma. A population-based epidemiologic and prognostic study of 48 patients, including cellular DNA content. Cancer *70*: 114-119, 1992.

20 Kuratsu S, Tomita Y, Myoui A, Uchida A, Ono K and Aozasa K: DNA ploidy pattern and cell cycle stage of tumor cells in soft-tissue sarcomas: clinical implications. Oncology *52*: 363-370, 1995.

21 Huuhtanen RL, Blomqvist CP, Wiklund TA, Virolainen MJ, Elomaa AI, Pan Y and Tribukait B: S-phase fraction of 155 soft tissue sarcomas: correlation with clinical outcome. Cancer *77*: 1815-1822, 1996.

22 Wijnaendts LC, van der Linden JC, van Diest P *et al*: Prognostic importance of DNA flow cytometric variables in rhabdomyosarcomas. J Clin Pathol *46*: 948-952, 1993.

23 De Zen L, Sommaggio A, d'Amore ES *et al*: Clinical relevance of DNA ploidy and proliferative activity in childhood rhabdomyosarcoma: a retrospective analysis of patients enrolled onto the Italian Cooperative Rhabdomyosarcoma Study RMS88. J Clin Oncol *15*: 1198-1205, 1997.

24 Gustafson P, Ferno M, Akerman M *et al*: Flow cytometric S-phase fraction in soft-tissue sarcoma: prognostic importance analysed in 160 patients. Br J Cancer *75*: 94-100, 1997.
25 Collin F, Chassevent A, Bonichon F, Bertrand G, Terrier P and Coindra JM: Flow cytometric DNA content analysis of 185 soft tissue neoplasms indicates that S-phase fraction is a prognostic factor for sarcomas. French Federation of Cancer Centers (FNCLCC) Sarcoma Group. Cancer *79*: 2371-2379, 1997.
26 Gottesman MM, Fojo T and Bates SE: Multidrug resistance in cancer: role of ATP-dependent transporters. Nat Rev Cancer *2*: 48-58, 2002.
27 Sigal A and Rotter V: Oncogenic mutations of the p53 tumor suppressor: the demons of the guardian of the genome. Cancer Res *60*: 6788-6793, 2000.
28 Kaelin WG Jr: The emerging p53 gene family. J Natl Cancer Inst *91*: 594-598, 1999.
29 Kawai A, Noguchi M, Beppu Y *et al*: Nuclear immunoreaction of p53 protein in soft tissue sarcomas. A possible prognostic factor. Cancer *3*: 2499-2505, 1994.
30 Antonescu CR, Tschernyavsky SJ, Decuseara R *et al*: Prognostic impact of P53 status, TLS-CHOP fusion transcript structure, and histological grade in myxoid liposarcoma: a molecular and clinicopathologic study of 82 cases. Clin Cancer Res *7*: 3977-3987, 2001.
31 Tang QQ and Lane MD: Role of C/EBP homologous protein (CHOP-10) in the programmed activation of CCAAT/enhancer-binding protein-beta during adipogenesis. Proc Natl Acad Sci USA *97*: 12446-12450, 2000.
32 Perez-Losada J, Sanchez-Martin M, Rodriguez-Garcia MA *et al*: Liposarcoma initiated by FUS/TLS-CHOP: the FUS/TLS domain plays a critical role in the pathogenesis of liposarcoma. Oncogene *19*: 6015-6022, 2000.
33 Stacey DW: Cyclin D1 serves as a cell cycle regulatory switch in actively proliferating cells. Curr Opin Cell Biol *15*: 158-163, 2003.
34 Lamb J and Ewen ME: Cyclin D1 and molecular chaperones: implications for tumorigenesis. Cell Cycle *2*: 525-527, 2003.
35 Kim SH, Cho NH, Tallini G, Dudas M, Lewis JJ and Cordon-Cardo C: Prognostic role of cyclin D1 in retroperitoneal sarcomas. Cancer *91*: 428-434, 2001.
36 Kim SH, Lewis JJ, Brennan MF, Woodruff JM, Dudas M and Cordon-Cardo C: Overexpression of cyclin D1 is associated with poor prognosis in extremity soft-tissue sarcomas. Clin Cancer Res *4*: 2377-2382, 1998.
37 Wurl P, Kappler M, Meye A *et al*: Co-expression of survivin and TERT and risk of tumour-related death in patients with soft-tissue sarcoma. Lancet *359*: 943-945, 2002.
38 Nicol Keith W, Jeffry Evans TR and Glasspool RM: Telomerase and cancer: time to move from a promising target to a clinical reality. J Pathol *195*: 404-414, 2001.
39 Barr FG: Gene fusions involving PAX and FOX family members in alveolar rhabdomyosarcoma. Oncogene *20*: 5736-5746, 2001.
40 Sorensen PH, Lynch JC, Qualman SJ *et al*: PAX3-FKHR and PAX7-FKHR gene fusions are prognostic indicators in alveolar rhabdomyosarcoma: a report from the children's oncology group. J Clin Oncol *20*: 2672-2679, 2002.
41 Ladanyi M: Fusions of the SYT and SSX genes in synovial sarcoma. Oncogene *20*: 5755-5762, 2001.
42 Ladanyi M, Antonescu CR, Leung DH *et al*: Impact of SYT-SSX fusion type on the clinical behavior of synovial sarcoma: a multi-institutional retrospective study of 243 patients. Cancer Res *62*: 135-140, 2002.
43 Ferrara N, Gerber HP and LeCouter J: The biology of VEGF and its receptors. Nat Med *9*: 669-676, 2003.
44 Yudoh K, Kanamori M, Ohmori K, Yasuda T, Aoki M and Kimura T: Concentration of vascular endothelial growth factor in the tumour tissue as a prognostic factor of soft tissue sarcomas. Br J Cancer *84*: 1610-1615, 2001.
45 Brackenbury R: Molecular mechanisms of cell adhesion in normal and transformed cells. Cancer Metastasis Rev *4*: 41-58, 1985.
46 Berx G and Van Roy F: The E-cadherin/catenin complex: an important gatekeeper in breast cancer tumorigenesis and malignant progression. Breast Cancer Res *3*: 289-293, 2001.
47 Bremnes RM, Veve R, Hirsch FR and Franklin WA: The E-cadherin cell-cell adhesion complex and lung cancer invasion, metastasis, and prognosis. Lung Cancer *36*: 115-124, 2002.
48 Nelson WJ and Nusse R: Convergence of Wnt, beta-catenin, and cadherin pathways. Science *303*: 1483-1487, 2004.
49 He TC, Sparks AB, Rago C, Hermeking H, Zawel L, da Costa LT, Morin PJ, Vogelstein B and Kinzler KW: Identification of c-MYC as a target of the APC pathway. Science *281*: 1509-1512, 1998.
50 Michaelson JS and Leder P: Beta-catenin is a downstream effector of Wnt-mediated tumorigenesis in the mammary gland. Oncogene *20*: 5093-5099, 2001.
51 van de Wetering M, Sancho E, Verweij C *et al*: The beta-catenin/TCF-4 complex imposes a crypt progenitor phenotype on colorectal cancer cells. Cell *111*: 241-250, 2002.
52 Saito T, Oda Y, Sakamoto A *et al*: Prognostic value of the preserved expression of the E-cadherin and catenin families of adhesion molecules and of beta-catenin mutations in synovial sarcoma. J Pathol *192*: 342-350, 2000.
53 Salerno M, Ouatas T, Palmieri D and Steeg PS: Inhibition of signal transduction by the nm23 metastasis suppressor: possible mechanisms. Clin Exp Metastasis *20*: 3-10, 2003.
54 Royds JA, Robinson MH, Stephenson TJ, Rees RC and Fisher C: The association between nm23 gene expression and survival in patients with sarcomas. Br J Cancer *75*: 1195-1200, 1997.
55 Pagano M: Control of DNA synthesis and mitosis by the Skp2-p27-Cdk1/2 axis. Mol Cell *14*: 414-416, 2004.
56 Lin DI and Alan Diehl J: Mechanism of cell-cycle control: ligating the ligase. Trends Biochem Sci *29*: 453-455, 2004.
57 Oliveira AM, Okuno SH, Nascimento AG and Lloyd RV: Skp2 protein expression in soft tissue sarcomas. J Clin Oncol *21*: 722-727, 2003.
58 Oliveira AM, Nascimento AG, Okuno SH and Lloyd RV: p27(kip1) protein expression correlates with survival in myxoid and round-cell liposarcoma. J Clin Oncol *18*: 2888-2893, 2000.
59 Younes A and Kadin ME: Emerging applications of the tumor necrosis factor family of ligands and receptors in cancer therapy. J Clin Oncol *21*: 3526-3534, 2003.
60 Ottaiano A, De Chiara A, Perrone F *et al*: Prognostic value of CD40 in adult soft tissue sarcomas. Clin Cancer Res *10*: 2824-2831, 2004.
61 Bertucci F, Viens P, Tagett R, Nguyen C, Houlgatte R and Birnbaum D: DNA arrays in clinical oncology: promises and challenges. Lab Invest *83*: 305-316, 2003.

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