A general aspect on soft-tissue sarcoma and c-kit expression in primitive neuroectodermal tumor and Ewing's sarcoma

Is there any role in disease process?

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ABSTRACT

Objective: Within soft-tissue sarcoma, primitive neuroectodermal tumors have been shown to cover a wide spectrum of small round cell sarcomas, including Ewing's sarcomas (ES) and primitive neuroectodermal tumors (PNET). The role of the stem cell factor/kit pathway has been investigated in different human tumors especially in chronic myelocytic leukemia and gastrointestinal stromal tumor and an autocrine loop has been assumed in small cell lung carcinoma, and recently in ES and PNET. Our aim is to investigate the c-kit expression in ES and PNET and also to assessed if c-kit has any role in disease process.

Methods: We thoroughly searched the archives of the Department of Pathology, Faculty of Medicine, Cukurova University Turkey, between 2000 and 2004; and found 14 ES and 14 PNET paraffin embedded tissues. We carried out the detection of the c-kit expression by immunohistochemical staining.

Results: The patient's median age was 23.7 ±14.6 (12 male and 16 female). Five were diagnosed as metastatic disease whereas 23 were diagnosed as non-metastatic disease at admission. The mean follow up period was 38.9±22.3 months. The main localization of the disease was lower extremity (32.1%), and others were as follows: head and neck 25%, thorax and abdomen 14.3%, pelvic and upper extremity 7.1% (11 were localized skeletal and 17 were extraskeletal). According to treatment modalities, 10 were treated with surgery alone, 11 with surgery and chemotherapy, and 7 with surgery, radiation therapy and also with chemotherapy. The primary tumor was lower than 5 cm in its dimension in 21 patients. While in 5 patients, tumor was more than 5 cm but did not exceed 10 cm, it was >10 cm in 2 patients. The c-kit expression was positive in 7 patients both cytoplasmic and membranously, whereas 8 patients were positive cytoplasmically. In 5 PNET patients, c-kit expression were stained immunohistochemically in over 50% and in 3 of ES patients. There was no significant correlation between c-kit expression and gender, localization, metastatic status, treatment modalities and tumor. Although in survival analysis, patients treated with surgery alone lived longer, while >50% of patients treated with c-kit expression lived for a shorter period.

Conclusion: We suggest that therapy with tyrosine kinase inhibitor for PNET and ES patients may be an alternative in addition to standard therapy modalities, especially in patients non-responsive to standard therapy.

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Sarcomas of non-osseous tissues, known traditionally as soft-tissue sarcomas, comprise a group of relatively rare malignancies that exhibit tremendous diversity of anatomic site and

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histopathologic characteristics. These tumors share a common embryologic origin, arising primarily from mesodermal tissues. The notable exceptions are sarcomas of the neural tissues and possibly the Ewing's sarcoma (ES)/primitive neuroectodermal tumor (PNET) family of tumors, which are believed to arise from ectodermal tissues. Approximately 7,800 new cases of soft-tissue sarcoma are diagnosed in the United States each year, with 4,400 deaths annually.[1] Despite their rarity, a thorough understanding of these tumors is important as patient outcomes might be compromised if initial management is anything less than ideal. Furthermore, biologic insights on sarcomas are providing new strategies for the detection, treatment, and prevention of other more common malignancies.

Within soft-tissue sarcoma, PNET have been shown to cover a wide spectrum of small round cell sarcomas, including ES, PNET and neuroblastoma. These tumors are phenotypically related, but with a different grade of neuronal differentiation;[2,3] thus, supporting the theory of a common origin of this neoplasm from a primitive cell that is specifically a pluripotential neural crest cell.[4] A thorough understanding of the clinicopathologic factors known to affect outcome is essential in formulating a treatment plan for the patient with soft-tissue sarcoma. Several multivariate analyses of prognostic factors for patients with localized sarcoma have been reported.[5,6-20] However, with few exceptions, most studies have analyzed <300 patients (range 82-297 patients).[6,12,21,22] Recently, larger reports have established the clinical profile of what is now accepted as the high-risk patient with extremity soft-tissue sarcoma: the patient with a large (≥5 cm), high-grade and stage (**Table 1**), deep lesion.[6,21,22] In addition, the previously unappreciated prognostic significance of certain histologic subtypes and the increased risk for adverse outcome associated with a microscopically positive surgical margin or presentation with locally recurrent disease were noted. Specific molecular parameters evaluated for prognostic significance in soft-tissue sarcoma have included p53, mdm2 mutations, Ki-67 status, altered expression of the Rb gene product in high-grade sarcomas, and the presence of specific molecular subtypes of SYT-SSX fusion transcripts in synovial sarcoma or EWS-FL11 fusion transcripts in ES.[23-28]

Stem cell factor (SCF) and its receptor c-kit have been shown to play major roles in the survival, expansion and differentiation of hematopoietic progenitor cells of various lineages.[29] It has also been demonstrated to play critical roles in cells derived from the neural crest.[30,31] Stem cell factor in fact decreases spontaneous apoptosis in neuroblastoma and in soft tissue sarcomas cultured in serum-free medium;[29-32] in addition, treatment with c-kit blocking antibody induces a significant apoptosis in neuroblastoma, ES and PNET.[29–32] The treatment of soft tissue sarcoma as for the ES and PNET are mainly consisted of surgery with or without irradiation and chemotherapy. Especially in advanced disease the treatment was difficult and required different agent or agents and at this point the physicians should present an alternative to those patients. In this study, we retrospectively investigate the c-kit expression with immunohistochemical staining method in 14 ES and 14 PNET patients. The question was explain if there is any relation of c-kit expression in ES and PNET; and if there would be any role of thyroxine kinase inhibitory treatment in these diseases, in the future alone or in combination of standard therapy.

Methods. The archives of the Department of Pathology, Faculty of Medicine, Cukurova University, Turkey between 2000 and 2004 were searched and 28 patients specimen were found. Fourteen were ES (9 female and 5 male) and 14 were PNET (7 male and 7 female). The patients' median age were 23.7 ±14.6, 12 were male and 16 were female. Five of the patients were diagnosed as metastatic disease whereas 23 of them diagnosed as non-metastatic disease at admission. The mean follow up period was 38.9±22.3 months. The main localization of the disease was in the lower extremity (32.1%) and others were as follows head and neck 25%, thorax and abdomen 14.3%, pelvic and upper extremity 7.1% (11 were localized skeletal and 17 were extraskeletal). According to treatment modalities, 10 were treated with surgery alone, 11 with surgery and chemotherapy and 7 with surgery, radiation therapy (RT) and also with chemotherapy. The primary tumor was lower than 5 cm in its great dimension in 21 patients. While in 5 patients the tumor was >5 cm but not exceed 10 cm, and it was >10 cm in 2 patients (**Tables 2 & 3**).

Five micrometer sections from undecalcified, formalin-fixed, paraffin-embedded tissue samples were placed on poly-L-lysine–coated slides. The avidin-biotin-peroxidase procedure was used for immunostaining. The primary antibody anti–c-kit C-19, the c-Kit ligand, rabbit polyclonal antibody c-Kit (C-19) sc-168 (Dako, Autogen-Bioclear UK Ltd) diluted in 1:50 was applied overnight in a moist chamber at 4°C. The following day, the tissue sections were incubated with a secondary-biotinylated antigoat antibody and with an avidin-biotin-peroxidase complex. The final reaction product was revealed by exposure to 0.03% diaminobenzidine, and the nuclei were counter-stained with Mayer's hematoxylin. Specimens in which the incubation with the primary antibody had been omitted were used as a negative control. The cases were scored as negative when there was a complete absence of staining for c-kit or when

Table 1 - American Joint Committee on Cancer System for staging soft-tissue sarcoma.

Stage	Grade	TNM		
Stage IA	G1, 2	T1a, b	N0	M0
Stage IB	G1, 2	T2a	N0	M0
Stage IIA	G1, 2	T2b	N0	M0
Stage IIB	G3, 4	T1a, b	N0	M0
Stage IIC	G3, 4	T2a	N0	M0
Stage III	G3, 4	T2b	N0	M0
Stage IV	Any G	Any T	N1	M0
Any G	Any T	Any N	M1	

T1 = less than 5cm, T1a - superficial to muscular fascia, T1b - deep to muscular fascia, T2 = ≥5cm
T2a - superficial to muscular fascia, T2b - deep to muscular fascia, N0 - no regional nodal involvement, N1 - regional nodal involvement, M0 - no distant metastasis, M1 - distant metastasis
G1 - well differentiated, G2 - moderately differentiated, G3 - poorly differentiated, G4 - undifferentiated

Table 2 - Demographic features of Ewing's sarcoma patients.

Age	Gender	c-kit %	Anatomic site	Status
8	Female	>50	Intra orbital	Metastatic
15	Male	>50	Paravertebral	Non-metastatic
21	Female	Negative	Pelvic	NA
2	Female	Negative	Head-neck	NA
4	Female	<50*	Head-neck	Local relapsed
12	Male	Negative	Lower extremity	Non-metastatic
5	Female	<50*	Head-neck	Local relapsed
12	Female	Negative	Head-neck	NA
22	Female	>50	Lower extremity	NA
33	Male	Negative	Thoracic wall	NA
17	Male	Negative	Pelvic	Metastatic
26	Female	<50*	Scapular	NA
20	Female	<50	Lower extremity	NA
23	Female	<50	Upper extremity	Non-metastatic

*stained both membranously and cytoplasmic, NA - not assessed

Table 3 - Demographic features of primitive neuroectodermal tumor patients.

Age	Gender	c-kit %	Anatomic site	Status
50	Female	>50*	Upper extremity	Non-metastatic
23	Female	Negative	Lower extremity	Metastatic
30	Male	Negative	Right kidney	Non-metastatic
49	Female	<50	Nasal cavity	Metastatic
25	Male	>50*	Intra abdominal	Non-metastatic
20	Male	>50	Gluteal	Non-metastatic
15	Male	Negative	Lower extremity	NA
47	Female	Negative	Lower extremity	Non-metastatic
53	Male	<50	Retroperitoneal	Non-metastatic
12	Female	>50*	Pelvic	Metastatic
11	Female	Negative	Intracerebral	NA
25	Female	Negative	Lower extremity	Metastatic
43	Male	>50	Gluteal	NA
20	Male	Negative	Left lung	NA

*stained both membranously and cytoplasmic, NA - not assessed

scattered positive cells were observed, and positive when the positivity was diffuse, which is independent of the level of immunostaining. All the specimens were evaluated by 3 pathologist that were specialized on sarcoma diseases.

Results. The specimens were evaluated by 3 pathologist and revealed that the c-kit expression was positive in 7 patients both cytoplasmic and membranously, whereas 8 patients were positive cytoplasmically. In 5 PNET patients, c-kit expression were stained immunohistochemically over 50% and in 3 of ES patients. There was no significant correlation between c-kit expression and gender (p=0.691), localization (p=0.746), metastatic status (p=0.552), treatment modalities (p=0.455), disease subgroup (p=0.403) and tumor (p=0.178). Although in survival analysis, patients treated with surgery alone lived longer, while >50% of patients treated with c-kit expression lived for a shorter period. (**Figures 1 & 2**). In each group, c-kit staining were positive both membranously and cytoplasmic in 3 patients (**Figures 3 & 4**).

Discussion. We have found that in 28 patients (14 ES and 14 PNET) the c-kit expression were 50% positive. As we discuss, the c-kit positivity was reported in different publications. In this study, we focused on the general treatment of soft-tissue sarcoma and c-kit; and its ligand SCF in different neuroectodermal cell line and also the targeted therapy that will be a cornerstone of cancer therapy in the future.

In general, the surgical resection remains the cornerstone of therapy for localized primary soft-tissue sarcoma. With the widespread application of multimodality treatment strategies, the vast majority of patients with localized soft-tissue sarcoma of the extremities undergo limb-sparing treatment, wherein <10% of patients undergo amputation.[33,34] In selected patients, limb-sparing can be approached with surgery alone. The use of RT in combination with surgery for soft-tissue sarcomas is supported by 2 Phase III clinical trials and is based on 2 premises: 1. microscopic nests of tumor cells can be destroyed by RT, and 2. less radical surgery can be performed when surgery and RT are combined.[35,36] Although the traditional belief was that soft-tissue sarcomas were resistant to RT, radiosensitivity assays performed on sarcoma cell lines grown in vitro have confirmed that the radiosensitivity of sarcomas is similar to that of other malignancies.

Although local or locoregional recurrence is a problem for a small subset of patients following primary therapy, the major risk to life in sarcoma patients is uncontrolled systemic disease. The field of adjuvant systemic therapy for sarcomas is

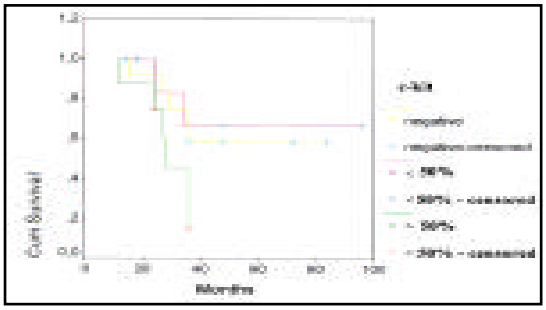

Figure 1 - Survival analysis of patients' with c-kit positive and c-kit negative.

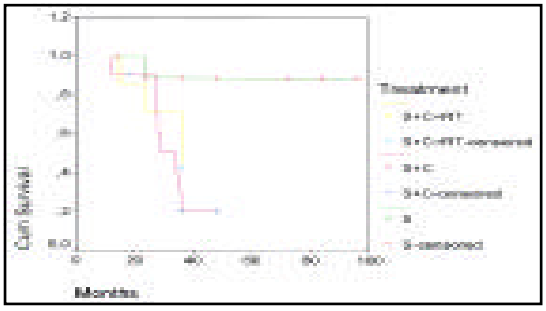

Figure 2 - Survival analysis of patients' treated with surgery alone and treated with multi-modalities. S - surgery, C - chemotherapy, RT - radiation therapy

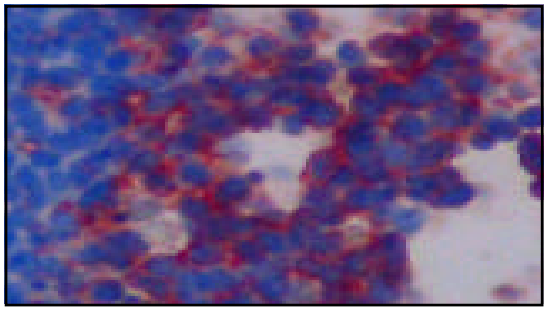

Figure 3 - C-kit expression in Ewing's sarcoma, immuno-histochemistry (x100).

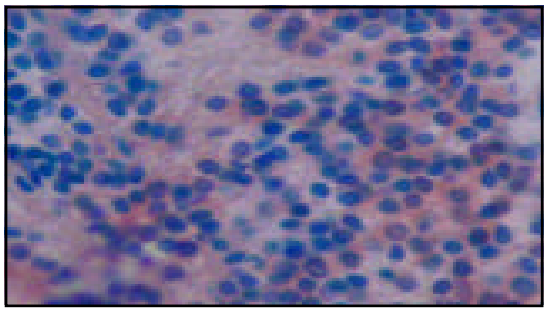

Figure 4 - C-kit expression in primitive neuroectodermal tumor, immunohistochemistry (x100).

plagued by underpowered studies that reveal tantalizing clinical benefits, even with older chemotherapy regimens. It is clear that non-randomized studies are of little benefit in determining the value of adjuvant chemotherapy, because its variables in patient selection, tumor definition and grade, and surgical and radiation techniques can drive outcomes significantly. In most trials, the use of adjuvant chemotherapy was associated with decreased local recurrence rates and, therefore, a prolongation of disease-free survival. However, this did not translate into overall survival benefits in all studies. In particular, one of the larger adjuvant therapy studies by the European Organization for the Research and Treatment of Cancer (EORTC) found no overall survival benefit when using doxorubicin-based multi-agent chemotherapy in the adjuvant setting.[37] This finding along with the toxicities of combination chemotherapy, has led to question the value of routine adjuvant chemotherapy for primary sarcomas.

The diagnosis of recurrent or metastatic disease in patients with soft-tissue sarcomas is devastating. Patients and physicians are aware that, in general, such a diagnosis significantly worsens the expected outcomes. Both surgery and systemic chemotherapy can play an important role in improving these outcomes in selected patients. Overall, it is important to recognize that unresectable metastatic sarcomas of soft tissues are, with rare exceptions, eventually fatal and that chemotherapy is given with the palliative aim of prolonging life and improving quality of life. The optimal treatment of patients with metastatic soft-tissue sarcoma requires an understanding of the natural history of the disease and individualized selection of treatment options based on patient factors, disease factors, and limitations imposed by previous treatment. The EORTC has made a major contribution in defining the expected course of unresectable metastatic sarcoma by publishing their large series of more than 2,000 patients with advanced sarcomas of soft tissues to describe prognostic features and the response to anthracycline-based chemotherapy.[38] Thus, one interpretation that is reasonable is that the most important predictors of survival with metastatic sarcoma are variables dependent on the tumor biology itself, as well as certain patient factors such as age and comorbid disease. These data are critical to understand; thus, information regarding the impact of new drugs and treatments can be interpreted appropriately, based on a comparison with the correct expectations for the natural or treated history of the disease in past clinical trials. Management of sarcomas is increasingly being driven by the specific nature of the disease entity, most importantly the patho-physiologic subtype. The work of Pasteur and

Koch[39] was fundamental for the recognition and definition of pathogenic microbes; similarly, many laboratories today are identifying molecular and cellular lesions that will redefine the field of sarcoma research. An example of this work is in the recognition of the ES/PNET family of tumors as extraosseous soft-tissue sarcomas rather than sarcomas of bone. These tumors should be treated with curative intent using an aggressive multimodality approach that begins with multi-agent chemotherapy. If primary surgery has removed measurable disease, adjuvant chemotherapy is definitely indicated, with consideration of adjuvant RT if surgical margins were suboptimal. By adopting similar strategies for PNETs and Askin's tumors as for conventional ES, outcomes have improved.[39,40] The molecular similarities between tumors of this family have led to the current convention of considering them morphologic and clinicopathologic variants of the same underlying molecular disease process.[28,41]

The role of the SCF/kit pathway has been investigated in different human tumors and an autocrine loop has been assumed in small cell lung carcinoma in gynecological tumors and in neuroblastoma.[32,42] Autocrine/paracrine stimulation of c-kit has been observed also in some other human cancers,[42-44] including ES.[29,45] In particular, the analysis of several ES cell lines indicated that ES cells express c-kit and SCF; that SCF is capable of protecting the tumor cells against apoptosis, giving them a growth advantage; and that SCF/c-kit system may be associated with, or even partly responsible for, the peculiar pattern of ES metastasis in patients,[46] which includes the appearance of bone metastases in one third of patients in the absence of lung metastases.[47] Interaction of c-kit with its ligand, SCF has been implicated in the growth, survival, and metastatic potential of Ewing's sarcoma.[29,46] Cell lines and fresh isolates from the Ewing's sarcoma family of tumors express cell surface c-kit[29] and SCF in both soluble and membrane-bound forms.[46] The homogeneous expression of c-kit in all Ewing tumor cell lines suggested that Ewing tumors may be good targets for treatment with imatinib mesylate, particularly as c-kit positive gastro-intestinal stromal tumors[30] and small-cell lung cancer cell lines[48] have been shown to be responsive to imatinib mesylate. A central role of c-kit and its ligand SCF in survival and metastasis of Ewing tumors has been indicated.[49] Stem cell factor was shown to induce down regulation of c-kit on the cell surface in all 6 cell lines tested in that study indicating functionality of c-kit. Stem cell factor was also shown to be a chemoattractant in 3 of the 6 cell lines. However, only one cell line was SCF found to stimulate proliferation and reduce apoptosis.[49] Thus, the exact role of SCF and its receptor in the biology of Ewing's tumors needs further investigation.

STI-571 was previously identified as a potent inhibitor of the c-abl protein kinase and shown to have similar activity against v-abl and both the p210 and p190 forms of bcr-abl. Additionally, STI-571 was found to inhibit the kinase activity of the and chains of PDGF-R and c-kit.[50] As a consequence, this compound is being evaluated in clinical trials for the treatment of chronic myeloid leukemia[51,52] expressing the fusion product bcr-abl, and among solid tumors, gastrointestinal stromal tumor (GIST)[53,54] expressing high levels of c-kit. These promising clinical results recently obtained in GIST have raised enthusiasm and new hopes for ES patients. However, the 2 neoplasms featured quite differently with respect to c-kit expression, and this should be carefully considered to optimize clinical results.

It is clear that soft-tissue sarcoma management has come a long way in a short time. In less than 3 decades, the standard of care has shifted toward coordinated multimodality care in specialty centers, with increased rates of function sparing surgery and better outcomes for patients. Judicious use of aggressive multimodality approaches show promise to decrease relapse rates and improve survival rates. New scientific approaches are furthering the fundamental understanding of these unusual diseases and providing novel approaches for diagnostic techniques, which will banish the vagaries and lack of consistency that have plagued this field of clinical investigation. New therapeutic initiatives are attacking the basic mechanisms of sarcomatous transformation of cells in some subtypes of soft-tissue sarcoma, and it is hoped that these initiatives will improve outcomes for patients with less morbidity than current treatments entail. Large collaborative studies will further this work tremendously.

In this study, we demonstrate c-kit expression 50% in ES and PNET samples and found no any correlation with localization of the primary tumor, metastatic status. But in patients that expressed c-kit over 50%, we observed short survival time, and it is seems to be an independent factors that play on disease process. The baseline of this study is to start the clinical-prospective trial especially in patients non-responsive to standard therapy. We suggest that therapy with tyrosine kinase inhibitor may be an alternative in addition to standard therapy modalities.

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