KIT protein expression in uterine sarcomas: an immunohistochemical study and review of the literature

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Summary

Purpose: The aim of the present study was to investigate the possibility of treating uterine sarcomas with imatinib mesylate. Imatinib mesylate, a selective tyrosine kinase inhibitor, is very efficient against mesenchymal tumors of the gastrointestinal tract, known as GISTs. Imatinib mesylate acts against a tyrosine kinase encoded by the *KIT* gene in GISTs, and is more effective in tumors expressing this protein. *Methods:* Expression of *KIT* was analyzed immunohistochemically (n = 12) in formalin-fixed paraffin-embedded primary uterine sarcomas. *Results:* Using a semi-quantitative immunohistochemical score we found that KIT expression was very weak in the majority of tumors, while none of the uterine sarcomas tested showed strong expression. Overall, published studies addressing this issue in small series of uterine sarcomas yielded similar results. *Conclusion:* Current data suggest that it is unlikely that imatinib mesylate could be used effectively as a single agent in patients with uterine sarcomas.

Key words: Uterine sarcoma; KIT; Imatinib mesylate.

Introduction

Uterine sarcomas are rare malignant mesenchymal tumors of the female reproductive tract, comprising less than 1% of gynecologic malignancies [1]. Like most malignant mesenchymal tumors in other organ systems, uterine sarcomas have a very poor prognosis. According to published series, although they represent only 2-5% of the total, they account for more than 25% of deaths due to malignancies of the uterine corpus [1, 2]. This is largely due to the fact that uterine sarcomas are commonly diagnosed in advanced stages, making complete surgical resection virtually impossible in most of these cases [2, 3]. Moreover, the majority of uterine sarcomas respond poorly to chemotherapy and radiation, if at all [3-5]. Thus, development of new therapeutic strategies for the treatment of uterine sarcomas is needed.

A recent advance in cancer treatment has been the administration of novel targeted therapeutic agents in patients whose tumors have specific molecular characteristics, as determined by molecular analyses prior to initiation of therapy. One of the most promising among these novel agents is imatinib mesylate, which has shown very encouraging results when given to patients with mesenchymal tumors of the gastrointestinal tract - gastrointestinal stromal tumors (GISTs) [6-8]. Imatinib mesylate is a selective inhibitor of tyrosine kinases, acting in GISTs against a protein, which is expressed on the surface of tumor cells and has tyrosine kinase activity; this protein, also known as CD117, is encoded by the *KIT* gene [9]. To evaluate the possibility of applying imatinib mesylate in uterine sarcomas, we have analyzed immunohistochemically the expression of KIT in a series of these tumors.

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Materials and Methods

Archived tumor specimens from 12 patients with uterine sarcomas were obtained from the Pathology Department of "Hippokration" General Hospital in Thessaloniki Greece. All tumor specimens were fixed in formalin and embedded in paraffin. All patients were surgically treated at the 1st Department of Obstetrics and Gynecology. Patient age ranged between 40 and 69 years. Hematoxylin and eosin stained slides were reviewed to confirm histological diagnoses. Tumors were classified according to the World Health Organization (WHO) classification (2003) for uterine sarcomas. The tumors included four leiomyosarcomas, four mixed mullerian mesenchymal tumors (MMMT), two low-grade endometrial stromal sarcomas (LGESS), and two high-grade endometrial stromal sarcomas (HGESS).

Representative tissue blocks were selected for immunohistochemistry. Immunoperoxidase staining for KIT (CD117) was performed in 4.0-μm-thick tissue sections from all tumors. The BioGenex Automatic Staining System (BioGenex, San Ramon, CA) was used. In brief, tissue sections were deparaffinized, rehydrated, and soaked in 0.6% hydrogen peroxide for 30 min in order to block endogenous peroxidase activity. Microwave antigen retrieval in citrate buffer with pH 6.0 (BioGenex, San Ramon, CA) for 25 min followed. Tissue sections were incubated with the polyclonal rabbit anti-KIT antibody A4502 (Dako, Glostrup, Denmark) at a dilution of 1:250 for 30 min. Incubation with a peroxidase-streptavidin conjugate (BioGenex, San Ramon, CA) for 20 min followed. Diaminobenzidine tetrahydrochloride was then used as a chromogen and sections were counterstained with hematoxylin, dehydrated and mounted. Tissue sections from a gastrointestinal stromal tumor (GIST) with strong membranous and cytoplasmic staining for KIT were used as a positive control.

For evaluation of immunohistochemical data a semi-quantitative scoring system was used, as described previously [10]. In brief, staining intensity was characterized using the following scale: 0 = negative, 1+ = low, 2+ = middle and 3+ = strong. The percentage of stained cells varied between: 0 = negative, 1 = < 10%, 2 = 10-50%, 3 = 51-80% and 4 = > 80% positive cells. According to the scores, tissues were classified as having low (0 to 2 points), middle (3 to 6 points) or strong (8 to 12 points) KIT expression.

Results

Immunostaining results for *KIT* expression are summarized in Table 1 according to sarcoma histological type. *KIT* expression was detected in all four leiomyosarcomas (two 2+/10-50%, one 1+/10-50%, and one 1+/<10%), and both LGESS (both 1+/10-50%) tested. Three of the four MMMTs were KIT-negative and one was positive (1+/10-50%). One of the two HGESSs tested was KIT-negative and one was positive (1+/<10%). Altogether, the majority of uterine sarcomas showed weak to moderate intensity (1+ or 2+ in 8 out of 12 tumors) and focal (< 10%) to moderate (10-50%) staining distribution (8 out of 12 tumors) of KIT. Four of the 12 tumors were entirely KIT-negative. Neither strong intensity (3+) nor extensive intensity distribution was observed in any of the tumors tested. Cytoplasmic staining was seen in all positive sarcomas, while staining of the cell membrane was also seen in most, but not all, positive tumors. Figure 1 shows a representative section of sarcoma cells positive for KIT.

Results of the semi-quantitative immunohistochemical scores are presented in Table 2. Ten out of 12 tumors had a low immunohistological staining score (0-2), and two had a moderate score, while none of the tumors showed strong *KIT* expression.

Table 1. — *KIT expression in a panel of uterine sarcomas*.

Histological classification	n	Staining intensity 0	1+	2+	3+	Tissue staining distribution 0	< 10%	10-50%	> 50%
Leiomyosarcoma	4	–	2	2	–	–	1	3	–
MMMT †	4	3	1	–	–	3	–	1	–
LGESS ‡	2	–	2	–	–	–	–	2	–
HGESS §	2	1	1	–	–	1	1	–	–

† MMMT: mixed mullerian mesenchymal tumor, ‡ LGESS: low-grade endometrial stromal sarcomas, § HGESS: high-grade endometrial stromal sarcomas.

Table 2. — *Immunohistochemical scores of KIT expression in uterine sarcomas*.

Histological classification	n	IHC-score†† Low (0-2 points)	Moderate (3-6 points)	Strong (8-12 points)
Leiomyosarcoma	4	2	2	–
MMMT †	4	4	–	–
LGESS ‡	2	2	–	–
HGESS §	2	2	–	–

† MMMT: mixed mullerian mesenchymal tumor, ‡ LGESS: low-grade endometrial stromal sarcomas, § HGESS: high-grade endometrial stromal sarcomas. †† IHC-score = immunohistochemical score.

Discussion

Imatinib mesylate (Glivec, Novartis International AG, Basel, Switzerland) is a selective inhibitor of the enzymatic activity of several tyrosine kinases. Its main advantages include oral administration and favorable safety profile, with minimal side-effects under standard doses [7-9, 11]. Imatinib mesylate was first used in chronic myeloid leukemia, in which a new gene is created by chromosomal translocation, leading to fusion of two genes (*bcr* and *abl*); the new gene encodes a protein kinase, whose spontaneous activity is responsible for leukemia [9]. In the case of GISTs, a mutation of the *KIT* gene leads to production of an activated protein kinase and subsequent uncontrolled cell growth and proliferation [9,12]. Imatinib mesylate is active against KIT-positive tumors, and has not shown any activity in KIT-negative GISTs [9]. Since 2001, more than 2,000 patients with GISTs have been included in therapeutic trials with imatinib mesylate, with a clinical benefit of 80-90% in patients whose chance of survival had been less than 30% at one year [8, 9].

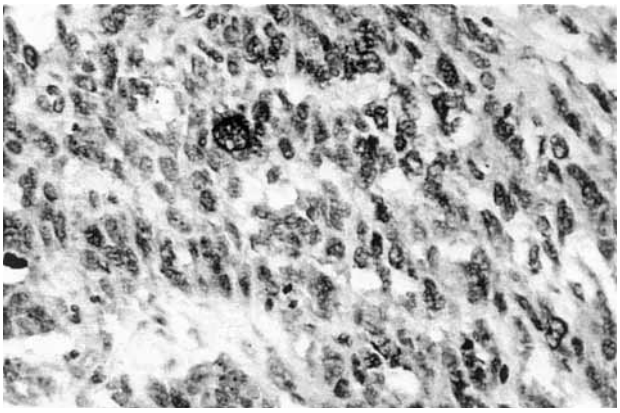

Figure 1. — Positive sarcoma cells for KIT protein (CD 117) immunostaining (x 400).

Based on the fact that both GISTs and uterine sarcomas are mesenchyme-derived tumors, the application of imatinib mesylate in uterine sarcomas seems to be a reasonable treatment option. The expression of *KIT* in uterine sarcomas has been previously analyzed [13-22], but due to the rarity of this pathological entity the total number of tumors studied so far is limited, not allowing definitive conclusions to be drawn. Thus, we analyzed immunohistochemically the expression of *KIT* in a panel of archival formalin-fixed paraffin-embedded tissue specimens of this rare entity. For this purpose we used an anti-KIT antibody, which was found to be the most sensitive in a recent study comparing seven different antibodies with the use of tissue microarrays [23]. The majority of the tumors we tested had a low immunohistological staining score (10 out of 12, with 4 entirely negative), only two had a moderate staining score, while none showed strong *KIT* expression.

Our results are in line with those of previous studies, most of which found only rare expression of KIT in uterine sarcomas by immunohistochemistry [13-22]. Winter *et al*. [13] found *KIT* immunopositivity in nine of 21 MMMTs, and one of 17 leiomyosarcomas. Likewise, Klein and Kurman [14] found *KIT* expression in one out of 24 and Nakayama *et al*. in four out of 26 uterine sarcomas [15]. In three studies *KIT* expression was analyzed only in MMMTs. Sawada *et al*. [16] found KIT overexpression in the mesenchymal component in six out of 16 cases, Menczer *et al*. [17] did not find any KIT-stained sarcoma cells (n = 20), and Raspollini *et al*. [18] found KIT expression only in four of 24 uterine MMMTs. In two other studies, KIT expression was analyzed only in leiomyosarcomas. Raspollini *et al*. [19] found immunopositivity in 17 out of 32 cases, while Serrano *et*

al. [19] did not find any positive tumors (18 cases). In contrast to the above studies [13-20], Rushing *et al*. [21] and Leath *et al*. [22] found *KIT* to be positive in all uterine sarcomas tested (25 cases and 11 cases, respectively).

The differences among various studies in *KIT* expression, as determined by immunohistochemistry, are most likely due to different antibodies used, differences in staining methods, and different patient populations [20]. A rough overall estimate from the above studies, with the total number of uterine sarcomas tested hardly exceeding 200 cases, is that *KIT* is expressed was no more than 36%. However, this could well be an overestimate, since as previously shown [20] mast cells infiltrating uterine sarcomas stain strongly for KIT and possibly lead to false-positive results. Such non-specific staining was ruled out in the present study by careful histological evaluation. Thus, it seems unlikely that patients with uterine sarcomas could benefit from imatinib mesylate treatment. Furthermore, molecular analyses suggest that even *KIT*-positive uterine sarcomas would probably not respond to imatinib mesylate: tumors that respond frequently to imatinib mesylate have mutation(s) in exon 11, and *KIT* needs to be phosphorylated in order to start its signaling cascade, but neither mutations [19-21], nor *KIT* phosporylation [21] were found in uterine sarcomas.

Conclusion

Our data together with those from previous studies, as presented above, suggest that it is unlikely that patients with uterine sarcomas might respond to imatinib mesylate. However, treatment of uterine sarcomas with imatinib mesylate might be feasible in a small subgroup of patients with KIT-positive tumors, possibly in combination with other therapeutic modalities.

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