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ErbB-4 expression in limb soft-tissue sarcoma: correlation with the results of neoadjuvant chemotherapy

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Abstract

ErbB-4 is a recently described growth factor receptor. Relatively little is known about its expression in human tumours. In this study, we assessed the possible role of erbB-4 as a tissue marker for soft-tissue sarcomas (STS) and its correlation with the response to chemotherapy. The histological specimen of 29 patients with STS of a limb who had received preoperative doxorubicin (ADR)-based chemotherapy were studied for the degree of necrosis and the expression of erbB-4 (by an avidin–biotin–peroxidase technique). ErbB-4 expression in the preoperative tissue samples was compared with the expression in the postchemotherapy resected tumour. The true objective response rate to preoperative chemotherapy was 34%. Wide resection of the tumour was done in 12 patients, marginal in 14, amputation in 2 and no surgery in 1. The tumour necrosis was above 90% in 9 patients, 60–90% in 12, and less than 60% in 7 patients. An increase in erbB-4 expression was more common in cases with no response to chemotherapy, while no change or a decrease in erbB-4 was more common in responsive tumours ($P=0.004$). No correlation could be found between the degree of necrosis or the chemotherapeutic regimen and the change in expression of erbB-4. The median disease-free survival (DFS) was longer for patients with a decrease or no change in expression of erbB-4 than for patients with increased expression. It is believed that postchemotherapy new expression or no downregulation of the erbB-4 molecule represents tumour aggressiveness and increased capability of growth and spread. © 2002 Published by Elsevier Science Ltd.

Keywords: erbB4; Soft-tissue sarcoma; Neoadjuvant chemotherapy

1. Introduction

The most important factors affecting control of disease and survival of patients with soft tissue sarcomas (STS) are the tumour size and tumour grade. Additional accepted prognostic factors are age, sex, tumour site, depth, status of surgical margins, and presence or absence of distant metastases [1–4]. Immunohistochemical search for cellular markers such as DNA ploidy, bcl-2 oncoprotein, mutant p-53, proliferating cell nuclear antigen (PCNA), Ki-67, and P-glycoprotein (Pgp), has been widely applied for more precise prognostication of STS. The bcl-2 oncoprotein plays a role in the inhibition of 'programmed cell death'. Overexpression of the mutant *TP53* tumour suppresser gene represents a loss of tumour suppression and an increase in cell proliferation. Expression of p53 and bcl2 did not significantly correlate with the proliferative activity or histological features of the tumours. Neither mutant p53 protein nor bcl-2 oncogene alone were sufficient to induce increased proliferation [5]. Pgp (multidrug resistance gene product) may be used for the prediction of resistance to chemotherapy and is strongly correlated with the degree of multidrug resistance (MDR) and poor outcome irrespective of response to chemotherapy in STS [6,7]. However, the role of Pgp as a prognostic factor remained controversial [8,9]. In a previous study, we investigated the correlation between histopathological markers and the clinical course of STS, including the response to neoadjuvant chemotherapy. No correlation was found between the expression of PCNA, MDR, mutant p53, bcl2, Ki67 and the degree of

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chemotherapy-related tumour necrosis, nor between these parameters and the patient status or the disease-free period [10].

A breakthrough in the field of molecular markers occurred during the last decade, with the characterisation of erbB gene family, of which erbB-2 is the best-known representative. The ErbB/HER family of transmembrane receptor tyrosine kinases includes four members that bind more than two dozens ligands sharing an epidermal growth factor-like motif. This family plays a crucial role in cell lineage determination in a variety of tissues, including mesenchyme-epithelial inductive processes. Certain ligands and receptors of the family, especially the ErbB-2 receptor tyrosine kinase, contribute to a relatively aggressive character of some human cancers. This large variety of biological signals is generated through a combinatorial network of signal transduction [11].

The erbB-2 encodes the human epidermal growth factor receptor 2 (HER2). Its actual ligand has not yet been identified [12]. HER2 overexpression may have a direct role in the pathogenesis and poor clinical course of certain human tumours [13–22]. The prognostic value of erbB-2 has been shown primarily in breast cancer [23,24]. The clinical significance of erbB-2 in sarcoma of bone or soft tissue is poorly investigated. The erbB-2 expression product is probably not detectable in STS [25]. Similarly, in a recent study carried out by our group (J. Issakov, Tel-Aviv Sourasky Medical Centre, Israel), specimens from 230 patients with various types of primary STS, and 48 patients with recurrent or metastatic disease were negative for the expression of erbB-2. In a study of 26 patients with osteosarcoma, erbB-2 was expressed in 42%, and was associated with the presence of pulmonary metastases, decreased survival, and poor histological response to preoperative chemotherapy [26,27]. ErbB-2 may be detected in cartilaginous tissue [28,29].

ErbB-4 is a recently described member of the epidermal growth factor receptor (EGFR) family. Unlike the EGFR and erbB-2, relatively little is known about the expression of erbB-4 in human tumours [30]. The pattern of erbB-4 expression in normal tissues and cancers suggests that it tends to be associated with the differentiated compartment [31]. In this study, we assessed the possible role of erbB-4 expression product as a tissue marker for STS, and its correlation with the response to chemotherapy.

2. Patients and methods

2.1. Patients

Patient eligibility criteria for preoperative chemotherapy included the following: biopsy-proven intermediate- or high-grade STS of the extremity, an option of limb-sparing surgical (LSS) approach, disease stage [American Joint Committee on Cancer (AJCC)] [32] either IIB, IIIA or IIIB, any histological subtypes of STS excluding extraskeletal osteosarcoma or chondrosarcoma, age younger than 60 years, Karnofsky's performance status (KPS) above 60%, adequate pretreatment renal and liver functions, adequate bone marrow reserve, and normal left ventricular ejection fraction (LVEF). Patients had to sign an informed consent form. Ineligibility criteria included low-grade STS, evidence of metastatic disease, poor KPS, no option of LSS, impaired cardiac function, inadequate reserve of bone marrow (white blood cell (WBC) count $<4\times10^9$/l, platelet count $<100\times10^9$/l, haemoglobin <100 g/l), impaired liver function tests (bilirubin $>$ 27.36 μmol/l), decreased renal function (creatinine clearance test (CCT) <0.83 ml/s), and LVEF $<50\%$. Patients with recurrent STS after previous LSS with or without radiation therapy were eligible provided that the recurrence was out of the previously irradiated volume, and that the patients were chemo-naïve.

After having a core needle or open biopsy proving an intermediate- or high-grade STS, all the patients underwent baseline evaluation that included computerised tomography (CT) studies of the chest, abdomen, pelvis, and magnetic resonance imaging (MRI) and CT of the involved limb, bone scan, MultiGated blood-pool imaging (MUGA) scan for evaluation of the LVEF, blood count, blood chemistry and CCT. Response and toxicity were scaled according to the World Health Organization (WHO) criteria. Evaluation of tumour response was made on the basis of physical examination, radiological studies and pathological examination of the surgical specimen for adequacy of surgical margins, evidence of vascular invasion, percentage of tumour necrosis (by a mapping technique) and the expression of the *erbB-4* gene.

2.2. Chemotherapy

Three doxorubicin adriamycin (ADR)-based induction regimens were given to patients with extremity STS at various time periods:

1. One regimen included three preoperative 3-weekly cycles of chemotherapy. The first cycle included continuous intravenous (c.i.v.) infusion of ADR 25 mg/m^2/day for 3 days, and ifosfamide (IFX) 2.25 g/m^2/day days 1–4 by intravenous (i.v.) infusion over 2 h. Mesna (M) was given at a dose of 0.5 g/m^2 15 min before, and 4 and 8 h after IFX started. Subcutaneous (s.c.) injection of granulocyte-colony stimulating factor (G-CSF) (Neupogen) 5 mcg/kg was given daily starting 24 h after the end of

chemotherapy. The second and third cycles were based on administration of c.i.v infusion of ADR 25 mg/m^2/day for 3 days, followed by intra-arterial infusion of cisplatin 120 mg/m^2 over 6 h on day 4. S.c. injection of G-CSF (Neupogen) 5 mcg/kg was given daily, starting 24 h after chemotherapy was finished. After a post-operative recovery period of 3–4 weeks, three 3-weekly cycles of adjuvant chemotherapy were administered. The postoperative courses were similar in composition and schedule to the first preoperative course.

2. The second regimen that was used was based on three 3-weekly identical cycles of ADR and IFX. ADR was infused at a dose of 50 mg/m^2/day on day 1 over 15 min, and IFX 2.5 g/m^2/day on days 1 and 2 by c.i.v. infusion. M was given at a dose of 2.5 g/m^2/day on days 1 and 2 and half the dose on day 3. S.c. injection of G-CSF (Neupogen) 5 mcg/kg was given daily, starting 24 h after the chemotherapy was finished only if the patient had already experienced neutropenic fever. Adjuvant chemotherapy consisted of 3-weekly cycles of ADR and IFX using the same schedule as in the preoperative treatment.
3. The third was based on i.v. ADR (37.5 mg/m^2/day on days 1 and 2 over 15 min) and ifosfamide (3 g/m^2/day on days 1 and 2 by c.i.v. infusion), followed by s.c. injection of G-CSF (Neupogen; 5 mcg/kg/day on days 4–13). Adjuvant chemotherapy consisted of 3-weekly cycles of ADR and IFX using the same schedule as in the preoperative treatment.

2.3. Limb-sparing surgery (LSS)

Limb-sparing surgery (LSS) was attempted in all the cases by the same surgical team, and performed 1–2 weeks after the end (i.e. day 21) of the last preoperative course of chemotherapy. Preoperative evaluation included MRI of the involved extremity, chest CT scan, MUGA scan, and blood count and chemistry.

2.4. Radiation therapy (RT)

Radiation therapy (RT) was delivered through an 8 MV linear accelerator photon beam to the target volume by two opposed fields. When feasible, we preferred to use two tangential fields (to the scar area) in order to have a maximal effect in the scar. Otherwise, 1-cm thick and 4-cm wide wax bolus covered the surgical scar in opposed perpendicular fields. The preferred schedule was 1.8 Gy/fraction, five fractions per week to a midplane dose of 63 Gy, or 70 Gy in the case of marginal excision. A midplane dose of 45 Gy was given to the tumour bed marked by surgical clips, to the surgical scar, and to either the whole compartment or to proximal and distal margins of 10 cm in non-compartmental lesions. A coned-down field of 5-cm margin proximally and distally received a dose up to 54 Gy, and a further coned-down field of the tumour bed plus 2-cm margins received a dose up to 63 or 70 Gy.

2.5. Follow-up

The follow-up schedule included history, physical examination, blood count and lactate dehydrogenase (LDH) every 3 months, chest plain film every 3 months, and chest CT and limb CT every 6 months, for the first 2 years. During the next 3 years' history, physical examination, blood count and serum LDH, chest CT and limb MRI or CT were performed every 6 months.

2.6. Pathology and immunohistochemistry

The extent of tumour necrosis was evaluated histologically, and presented as the percentage of necrosis out of the original tumour mass. Two slices from the largest diameter of the tumour mass (maximal sagittal and tangential cross-sections) were entirely embedded in paraffin and histologically examined by a mapping technique [33]. The grading of tumour necrosis was classified as follows: $>90\%$ necrosis = complete or near complete response; 60–90% necrosis = partial or incomplete response, and $<60\%$ necrosis = no response.

Paraffin blocks of preoperative incisional biopsy were available for immune staining from 29 patients, and blocks of the surgical specimen after preoperative chemotherapy were available from 27 patients. Immunohistochemical stains were performed on paraffin sections using the avidin–biotin–peroxidase method. Anti Erb-4(C-18) antibodies (Santa Crus Biotechnology, Inc., USA) that are reactive with Erb-4 of mouse, rat and human origin by western blotting, immunoprecipitation and immunohistochemistry, were applied. Three-μm sections mounted on Super Frost/Plus glass (Menzel, Glaser, Braunschweig, Germany) were processed by a labelled-(strept) avidin–biotin (LAB-SA) method using the HISTOSTAINTM plus kit (Zymed, San Francisco, USA). Heat-induced antigen retrieval was performed by temperature-controlled microwave treatment using an H2800 model processor (Energy Bean Sciences, Inc., Agawan, MA, USA) in 10 mM citrate buffer, pH 6.0, for 12 min at 97 °C. The sections were treated with 3% H_2O_2 for 5 min, followed by a 10-min incubation with the universal blocker, CAS-BLOCKTM (Zymed, San Francisco, USA). The sections were incubated for 1 h with 1:150 diluted anti Erb-4(C-18) antibody. A biotinylated second antibody was applied for 10 min, followed by incubation with horseradish peroxidase-conjugated streptavidin (HRP-SA) for 10 min. The slides were washed thoroughly with

Optimax wash buffer (Biogenix, San Ramon, CA, USA) following each incubation. The immunoreaction was visualised by a horseradish peroxidase (HRP)-based chromogen/substrate system, including 3,3′-Diaminobenzidine Tetrahydro-chloride-plus kit (DAB) (brown) chromogen (Liquid DAB substrate kit, Zymed, San Francisco, USA). The sections were then counterstained with Mayer's haematoxylin (which stains the nuclei a blue colour), dehydrated in ascending ethanol concentration, cleared in xylene and mounted for microscopic examination. For negative controls, pre-immune serum was substituted for the primary antibody.

Paraffin sections of normal skeletal muscle were used as positive controls. Negative controls consisted of consecutive tissue sections of each case. Two independent observers read each slide. Immunopositivity was subjectively graded according to the staining intensity and recorded as negative (−), weak (+), moderate (++), or strong (+++). For the statistical analysis, all patients were divided in two groups: (a) 'negative': negative or weak staining, versus (b) 'positive': moderate or strong positive staining. For studying the correlation between the stain and the response to chemotherapy, all clinical complete, partial and minimal responses were grouped together, while stabilisation and disease progression constituted a second group. The grouping was based on the possible discrepancy between clinical and pathological response in STS.

3. Results

The histological specimen of 29 patients with STS of a limb who had received preoperative ADR-based chemotherapy were available for erbB-4 staining. Taken altogether, there were 12 females and 17 males, whose age ranged from 18 to 68 years (median 45 years). The histological types of the STS were: epithelioid (1 case), liposarcoma (14 cases), malignant fibrous histiocytoma (MFH, 5 cases), malignant peripheral nerve sheath tumour (MPNST, 4 cases), synovial (3 cases) and unclassified spindle cell sarcoma (2 cases). Combined i.v. and intra-arterial approach was carried in eight patients, while 21 were treated by i.v.-only regimens. The clinical response to preoperative chemotherapy was complete (CR) in 2 patients, partial (PR) in 8, minimal (MR) in 7, stabilisation of disease (SD) in 7 patients, and disease progression (PD) in 5. The true objective response rate was 34%, and 59% if MR is added to CR + PR. Wide resection of the tumour was feasible in 12 patients, marginal resection was performed in 14 cases, amputation was carried out in 2 patients with disease progression, and no surgery was done in 1 case because of rapid progression and early death. The tumour necrosis was above 90% in 9 patients, 60–90% in 12, and less than 60% in 7 patients. ErbB-4 expression in tumour specimen before and after the administration of chemotherapy is presented in Table 1, and depicted in Fig. 1(a) and (b). Positive staining for erbB-4 was observed in 18/29 patients before induction was given and in 19/27 after induction chemotherapy. In 2 patients, only the pretreatment specimens were available for immunostaining. The change in expression of erbB-4 was assessed in view of the clinical response (Table 2) and the percentage of tumour necrosis. It was found that an increase in erbB-4 expression was more common in cases with no response to chemotherapy, while no change or a decrease in erbB-4 was more common in the responsive tumours ($P = 0.004$). No correlation could be found between the degree of necrosis and the change in expression of erbB-4, neither between the type of the chemotherapeutic regimen and the change in level of expression of erbB-4. There was no correlation between the type of STS and the expression of erbB-4 or with the change of expression of the antigen. Survival analysis (Fig. 2) showed that the median DFS was longer for patients with a decrease or no change in expression of erbB-4 (30 months) than for patients with an increased expression (14 months). The curves, however, were based on relatively small number of cases, and did not differ significantly.

4. Discussion

The introduction of the erbB gene family into daily oncological practice in the form of prognostic factors or direct targeting for a therapeutic use raised our enthusiasm to evaluate the prognostic role of the family in STS. This study was designed to assess the expression of erbB-4 in specimens of extremity STS in relation to preoperative chemotherapy in order to try to characterise a new prognostic factor. A possible association between the *change* in the level of expression of erbB-4 due to ADR-based neoadjuvant chemotherapy and the clinical response of the tumour was noted. A second interesting observation was a trend towards an improved disease-free survival in patients with a chemotherapy-related decrease in expression of erbB-4. The long-term disease-free survivors belonged to the group with no increase in erbB-4 expression.

While different reports have been published on the overexpression of erbB-2 and its clinical significance in malignant tumours, the data on erbB-4 remains very scarce. The erbB-4 receptor has been recently identified in non-small cell lung cancer. Its overexpression was associated with an inferior response to gemcitabine–cisplatin chemotherapy [34]. The expression of erbB-4 has also been reported in thyroid [35], endometrial [36] and breast cancers [37]. No significant associations were found between the presence of the erbB-4 receptor and the disease-free interval or survival in breast cancer

Table 1
Patient characteristics and results of neoadjuvant chemotherapy (ChT)

Tx arm	Pt no.	Sex	Age (years)	Site	Histology	St.	Response[b]	Resection	% nec	DFS (months)	Pre-ChT erbB4	Post-ChT erbB4	Net result of staining	Sites of relapse
1	1	f	35	Proximal thigh	Synovial sarcoma, monophasic	IIIA	CR	Marginal	99[a]	24	3	3	NC	Lung
1	2	f	56	Proximal thigh	Liposarcoma myxoid	IIIB	MR	Marginal	80	22	0	0	NC	Lost to follow-up
1	3	f	46	Proximal thigh	Liposarcoma, round cell	IIIB	MR	Marginal	75	55	3	3	NC	
1	4	f	26	Proximal thigh	MFH	IIIB	MR	Wide	70	11	3	2	Decreased	Local, lung
1	5	m	57	Shoulder, scapula	Epithelioid sarcoma	IIIB	PD	FQA	60	0	0	1	Increased	Local, lung
1	6	f	46	Proximal arm	MPNST	IIIB	PD	Marginal	5	30	2	2	NC	Pelvis, sacrum
1	7	m	34	Thigh	Liposarcoma, round cell	IIIB	SD	Marginal	30	12	0	1	Increased	Pelvic mass
1	8	m	59	Gluteus	Unclassified high-grade spindle cell	IIIB	SD	Marginal	80	2	2	1	Decreased	Local and pelvic
2	9	m	52	Thigh	Liposarcoma, round cell	IIIB	MR	Wide	90	23	1	1	NC	
2	10	m	63	Gluteus	Liposarcoma, pleomorphic	IIIB	PD	NA	NA	0	0	NA		DWD
2	11	m	48	Poplitea	Liposarcoma, myxoid	IIIB	PR	Wide	95	32	0	3	Increased	
2	12	f	29	Thigh	Liposarcoma, myxoid	IIIB	PR	Wide	95	31	3	0	Decreased	
2	13	m	49	Thigh	Liposarcoma, round cell	IIIB	PR	Wide	40	12	1	1	NC	Gluteus, liver
2	14	f	42	Thigh	MFH	IIIB	PR	Wide	60	33	1	3	Increased	Lung
2	15	f	58	Gluteus	Liposarcoma, pleomorphic	IIIB	SD	Marginal	80	12	0	3	Increased	Sacrum
2	16	f	42	Gluteus	Liposarcoma, round cell	IIIB	SD	Marginal	75	6	1	1	NC	Groin, abdomen
2	17	m	33	Thigh	MFH	IIIB	SD	Wide	40	14	0	1	Increased	Thigh
3	18	m	47	Knee	Synovial sarcoma, monophasic	IIB	CR	Wide	100	9	0	0	NC	
3	19	m	39	Leg anterolateral	Liposarcoma myxoid	IIB	MR	Marginal	95	5+	2	0	Decreased	
3	20	m	68	Ankle	Liposarcoma myxoid	IIIB	MR	Marginal	60	2+	2	1	Decreased	
3	21	m	32	Thigh	MFH	IIIB	MR	Wide	90	20	1	0	Decreased	NED
3	22	m	34	Proximal thigh + pelvis	MPNST	IIIB	PD	Marginal	80	3	1	2	Increased	
3	23	m	19	Calf	Unclassified high-grade spindle cell	IIIB	PD	AK amp	40	3	2	3	Increased	Local and lung
3	24	f	40	Proximal thigh + groin	Liposarcoma, pleomorphic	IIIB	PR	Wide	80	10	0	0	NC	
3	25	f	62	Chest wall	MFH	IIIA	PR	Wide	100	6	1	0	Decreased	
3	26	m	45	Proximal thigh + pelvis	MPNST	IIIB	PR	Marginal	85	7	3	1	Decreased	
3	27	m	18	Shoulder	Synovial sarcoma, monophasic	IIIB	PR	Marginal	30	12+	3	NA		
3	28	f	59	Thigh	Liposarcoma, myxoid	IIIB	SD	Wide	95	19	0	1	Increased	
3	29	m	45	Forearm	MPNST	IIIB	SD	Marginal	10	13	0	0	NC	NED

Note that numbers expresses the intensity of staining for erbB-4 in specimen before and after the induction chemotherapy: 0 for negative, 1 for weak, 2 for moderate and 3 for strong positive.

DFS, disease-free survival; ChT, chemotherapy; CR, complete response; PR, partial response; MR, minimal response; SD, stable disease; PD, progressive disease; NA, not assessed; NED, no evidence of disease; DWD, dead with disease; FQA, forequarter amputation; Tx, therapy; Pt, patient; MFH, malignant fibrous histiocytoma; MPNST, malignant peripheral nerve sheath tumour; f, female; m, male; % nec, % necrosis; NC, no change, St., Stage; Akamp, above knee amputation.

[a] CR was documented by clinical and radiographical examinations, but the tissue that was resected from the tumour bed during definitive surgery contained a few viable cells, that were stained with the same intensity as before induction. Therefore, it was concluded as 99% necrosis.

[b] Note that 'no change' could be associated with stable molecular response, e.g. staining intensity of 0 before and after induction, or with an increased staining intensity, e.g. from 0 to 1 (reflecting molecular progression).

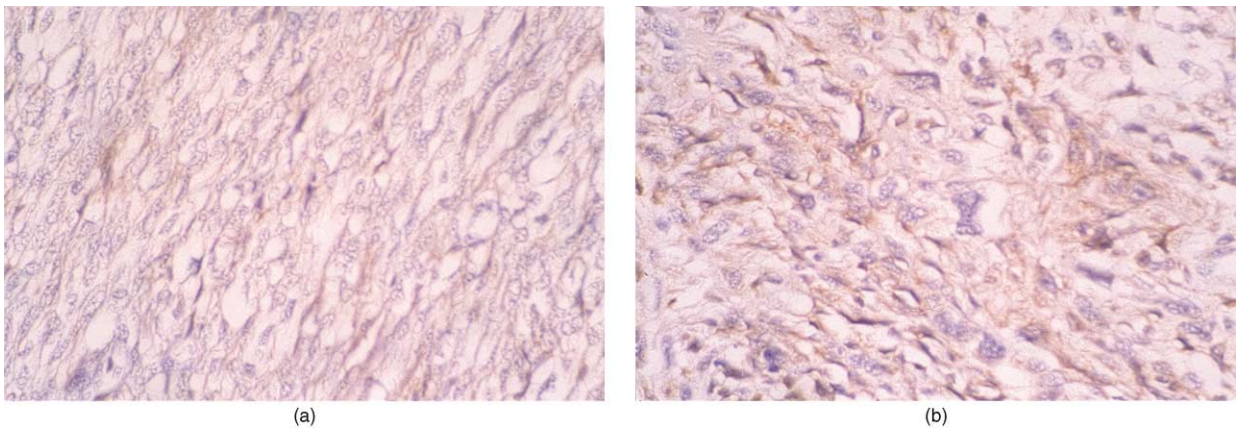

Fig. 1. (a) Membranous staining for erb-4 in synovial sarcoma (×400). (b) Membranous staining for erb-4 in malignant fibrous histiocytoma (×400).

patients [38]. The role of erbB-4 in the evolution of STS is as yet unknown.

In this study, the expression of erbB-4 at a certain point of time, ie pre- or postchemotherapy had no prognostic significance, only the change in intensity of staining was related to outcome. If the intensity of staining increased following chemotherapy, the treatment outcome was worse.

Three chemotherapy combinations have been used in the present study, reflecting the different protocols used during the recent years. The common drugs for all the protocols were ADR and IFX, given i.v., in different doses and schedules. ADR was given in a dose of 50–75 mg/m^2, and IFX 5–9 g/m^2. Since these drugs are considered as the most potent agents used nowadays in STS, and they were given to all of the patients in the study, we grouped the patients altogether. Since the number of patients in each regimen was small, we could not define the best regimen according to the molecular response of erbB-4. Other differences between the protocols have been discussed elsewhere [10].

In this series, the true clinical and radiographical response rate of STS to induction chemotherapy was 34%. For statistical analysis, we combined minimal response to the true objective partial and complete responses. Minimal response is not regarded as response according to standard criteria. It is, however, better than no response at all. It is known that there is a discrepancy between the clinical and radiological response of STS and the pathological response to chemotherapy. Clinically stable disease may be associated with a significant percentage of necrosis, while clinical partial response may be associated with a high percentage of residual and viable cells. More than that, it has been claimed that the radiographical response to preoperative chemotherapy in STS should not be used to make clinical decisions regarding the postoperative treatment or predict local and distant tumour control [39]. In addition, it has been demonstrated recently that treatment-induced pathological necrosis was the strongest predictor for local control and long-term survival in patients with STS [40]. Data from both series suggest that the traditional criteria for response (such as the WHO criteria) are not necessarily applicable in evaluating the response to induction chemotherapy in STS, and alternative endpoints are required.

Table 2
Response of STS to preoperative chemotherapy according to the change in expression of erbB-4

	Increased expression	Decreased expression	No change
Response			
CR + PR + MR	2	8	7
SD + PD	7	1	3
			$P = 0.004$

For the purpose of the analysis, decreased expression and no change (= no increase) were grouped together. CR, complete response; PR, partial response; MR, minimal response; SD, stable disease; PD, progressive disease.

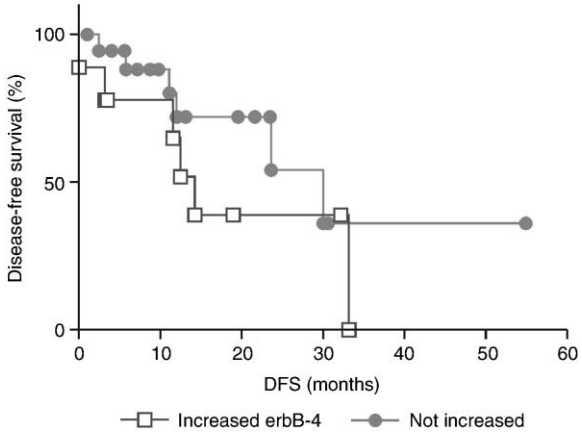

Fig. 2. Disease-free survival (DFS) curve according to erbB-4 expression. The median DFS is longer for patients with a decrease or no change in expression than for patients with an increased expression.

In this series, a molecular response rate could be defined as a reduction or no change in the expression of erbB4 following induction chemotherapy, while upregulation of erbB-4 might reflect tumour progression. Proceeding with this definition, the molecular response rate was 33% (9/27), molecular no-change 37% (10/27), and molecular progression 30%. The rates of molecular and clinical responses were eventually similar, but did not result from the same cases. It was observed, however, that clinical PD was associated with molecular failure or no change, while molecular response was more common in clinical PR or MR. It remained unclear why in some cases clinical PR was associated with 95% necrosis (as expected), but with molecular progression.

It is assumed that postchemotherapy new expression of the erbB-4 molecule represents tumour aggressiveness and increased capability of growth and spread. The same may be true when chemotherapy failed to downregulate the expression of erbB-4. In this case, it is assumed that the tumour characteristic was not affected by the treatment, and it keeps its potential to grow and metastasise. If the increase in the expression of erbB-4 was due to the selection of biologically aggressive tumour cells following chemotherapy, which would be a reasonable explanation, and if this would therefore result in shorter survival, which again would be a reasonable assumption, an *a priori* high degree of expression, especially if it is not changed by chemotherapy, might predict for shorter survival as well. In such instances, there was in fact no molecular response.

These assumptions may be supported by the observation that the clinical response and disease-free survival was better (although it did not reach statistical significance) in tumours that showed a downregulation of the erbB-4 expression in response to chemotherapy. It is conceivable that erbB-4 is not the only important molecule that influences the course of STS. Many other factors also play an important role in the life cycle of the malignant cell. The correlation of DFS with the change in erbB-4 expression is obviously explained by the correlation of DFS with the clinical response. Whether the change in erbB-4 expression is an independent factor remains open, and may be answered in a large-scale prospective trial of induction chemotherapy.

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