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Topoisomerase II-alpha protein expression and histological response following doxorubicin-based induction chemotherapy predict survival of locally advanced soft tissues sarcomas

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ABSTRACT

Purpose: Neoadjuvant chemotherapy for locally advanced soft tissue sarcomas (LASTS), although not standard, represents a promising option for resectable tumours. Current lack of biological predictors of chemotherapy response led us to establish a relationship between Topoisomerase II-alpha (Topo2A), HER2, excision repair cross-complementing group 1 (ERCC1) protein expression, histological response and clinical outcomes of LASTS patients.

Patients and methods: A retrospective study based on clinical data and archival paraffin-embedded tumour tissue at diagnosis from 78 consecutive LASTS patients treated with neo-adjuvant chemotherapy in our institution enabled analysis of ERCC1, HER2 and Topo2A protein expression by immuno-histochemistry.

Results: Disease free survival (DFS) and overall survival (OS) were 48% and 64%, respectively. The annual risk of relapse increased with a higher percentage of residual identifiable cells (RIC). A higher Topo2A protein was associated with an improved rate of good HR ($r = 0.416$) and with a decreased risk of relapse. Median DFS decreased with low Topo2A ($p \leqslant 0.042$). The ERCC1 status had no impact on histological response while ERCC1 positive tumours correlated with a favourable OS ($p \leqslant 0.058$). Patients with LASTS co-expressing Topo2A and ERCC1 had a significant better outcome ($p = 0.018$). Topo2A was the only independent variable linked to a good HR ($p \leqslant 0.017$); histological grade 3 was the only independent adverse prognostic variable linked to both DFS ($p \leqslant 0.04$) and OS ($p \leqslant 0.004$).

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Conclusions: While histological response predicts better DFS, Topo2A protein expression correlates with histological response and better DFS. The combination of an early predictive factor for chemosensitivity (Topo2A) and for survival (ERCC1) highlights the possibility to develop individualised therapeutic approaches (CONTICANET program).

1. Introduction

Soft tissue sarcomas (STS) are uncommon mesenchymal neoplasms with variations in natural history and response to treatment suggesting future specific and individual trials. The 5-year survival rate according to American Joint Committee on Cancer stage III tumours is approximately 50%.[1] Following surgery and radiotherapy, about 50% of patients with an initially localised STS will relapse with metastasis and die of the disease, especially the high-grade tumours.[2]

The impact of adjuvant chemotherapy remains controversial. About one third of STS treated in reference centres present as locally-advanced tumours (LASTS) that do not allow optimally radical non-mutilating tumour resections. Induction chemotherapy for LASTS in reducing tumour size enables subsequent negative resection margins (R0).[3]

The histological response (HR) after induction chemotherapy is a powerful predictive factor for survival in primary bone sarcomas.[4] However regarding STS, similar histoprognostic classification is hampered by the difficulty of evaluating histological response in soft tissue, since good histological response varies from 50% to more than 95% of pathological necrosis (PN).[3,5]

The induction API-AI doxorubicin-based regimen has been used in our institution for all patients referred as high-grade LASTS. The interim analysis of this cohort reported a 5-year disease-free survival (DFS) significantly higher for good HR patients (>95% PN, GHR) than those without.[5] Necrosis may result from both treatment and natural course of tumour evolution rendering the term pathological necrosis ambiguous. Therefore, percentage of residual identifiable cells (RIC) would be preferable for evaluating chemosensitivity in LASTS but this criterion was not validated yet. Moreover, no predictive biological surrogate markers of HR have been identified.

Chemoresistance to doxorubicin and cisplatinum might be sustained by specific biochemical anomalies involved both in drug–DNA interactions and in DNA repair. Thus, we hypothesise that expression of Topoisomerase II-alpha (Topo2A) and excision repair cross-complementing group 1 (ERCC1) could provide markers of response to chemotherapy. Indeed, Topo2A, an important target for anthracyclines, when overexpressed in advanced breast cancer, has been shown to be associated with a higher probability of response to doxorubicin.[6,7] The vicinity of *TOP2A* and *HER2* genes in a 17q12 and 17q21 chromosome locus as well as the correlation between HER2-overexpression/response to anthracycline-containing therapies enabled to propose *HER2* amplification as a surrogate marker for *TOP2A* amplification.[8] Regarding ERCC1, involved in the repair of cisplatin-induced DNA damage, its expression was associated with response to cisplatin in lung cancer.[9]

The aim of this study was to retrospectively analyse the data of all LASTS patients treated with the API-AI regimen, in order to determine a potential threshold level for HR and to investigate the predictive value of ERCC1, HER2 and Topo2A expression on HR, relapse and survival.

2. Material and methods

2.1. *Patient population*

Patients with LASTS treated during the period 1995–2005 in the Gustave Roussy Institute underwent induction chemotherapy until the last follow-up or known status on 31st December 2007.

All patients received two cycles (four courses) of induction chemotherapy including doxorubicin 60 mg/m^2 (d1 and d15), cisplatin 100 mg/m^2 (d1) and ifosfamide 5 g/m^2 (d2 and d15). The second cycle was at day 28. To maintain the dose intensity, all patients received granulocyte colony-stimulating factor after each course of chemotherapy (day 6–11 and day 20–25). Disease was evaluated by MRI at diagnosis and during the last course of chemotherapy before surgery and response was evaluated according to RECIST.

Surgery was planned 3–4 weeks after the fourth course of induction chemotherapy. The surgical margins and the HR were evaluated.[4]

Patients receiving adjuvant radiotherapy were followed up every 4 months during the first 2 years, every 6 months during 3 years and once a year afterwards. Follow-up consisted of a clinical examination, thoracic CT scan and MRI of the tumour resection site.

2.2. *Immunohistochemical (IHC) analysis of protein expression*

Paraffin-embedded tissue enabled standard immunostaining used automated DAKO equipment.

2.3.1. *Topoisomerase II-alpha*

Monoclonal antibody raised against the human 170 kd alpha isoform protein of Topo2A (mouse, clone KI-SI, working dilution 1:5000, Chemicon International) was used with epidermoid oropharyngeal cancer as external positive control. The percentage of cells with positive nuclear staining for Topo2A was assessed with median percentage as cutoff that discriminates high-expressing from low-expressing tumours.

2.3.2. *HER2*

Polyclonal antibody raised against the HER2 protein (rabbit, A0485, working dilution 1:500, DAKO) was used with

intra-ductal breast cancer tissue as the external positive control. Staining was interpreted as described for breast cancer.[10]

2.3.3. ERCC1

Monoclonal antibody raised against the full-length human ERCC1 protein (mouse, clone 8F1, working dilution 1:400, Neomarkers) was used with normal tonsil tissue as positive control and endothelial cells as internal positive controls. Nuclear staining intensity (scale of 0–3) multiplied by proportion scores[9] yielded a final semi-quantitative *H* score from which a cutoff point discriminates positive tumours from negative tumours.

Two independent observers analysed immunohistochemical staining.

2.4. Statistical analysis

The chief study's end-point was the HR to chemotherapy evaluated in the tumour specimen after surgery. Secondary end-points were the efficacy of the API-AI regimen for clinical/radiological response (RECIST criteria), disease free survival (DFS) and overall survival (OS), as defined from the first cycle of preoperative chemotherapy administered until next event. Descriptive statistics assessed patient characteristics and IHC data, using median values and 95% confidence intervals (CI).

3. Results

3.1. Response to induction chemotherapy

Seventy-eight patients had received the API-AI regimen. The median age of the 43 males and 35 females was 42 years (range 18–68 years), the median tumour size was 110 mm (range 10–250 mm), given 65% of patients elicited at diagnosis a grade 3 tumour[2] (Table 1)

Most patients (79%) had received a median of four chemotherapy cycles (range 1–6 cycles). Radiological response according to RECIST was evaluated as complete in 4 patients, partial in 25 patients (objective response rate: 37%), stable disease in 44 patients (56%) and progressive disease in 5 patients (6%) (Table 2). There was no correlation between the clinical response and the tumour site, grade, size or the histological subtype.

3.2. Histological response

All patients underwent surgery of their primary/local relapse STS. A conservative resection was achieved in 96% of patients (two amputations) with a R0 resection in 82% of patients. In addition, 92 % of patients had received conventional postoperative radiotherapy (median dose 60 Gy).

The median RIC after chemotherapy, 30% (Table 3), was chosen as the cutoff that discriminates a good (<30%) from a poor HR (>30%).

About 75% of poorly differentiated sarcomas obtained a good HR, contrasting with synovialosarcoma showing 78% poor HR. The remaining STS subtypes equally balanced between the good and poor HR. A good HR was more frequently achieved in patients older than 45 years ($p \leqslant 0.041$, OR 1.63 [1–2.69]), in grade 3 tumours ($p \leqslant 0.011$, OR 1.59 [1–2.1]) and was also associated with R0 resections ($p \leqslant 0.003$, OR 1.37 [1–1.7]). Clinical responses did not correlate with any type of histological pattern: 3 out of 4 obtained complete (75%), 15/25 partial (60%), 20/44 stable disease (45%) and 1/5 progressive disease (20%). Good HR correlated with a lower incidence of relapses ($p \leqslant 0.023$, OR 2.85 [1.14–7.16]) and metastases ($p \leqslant 0.04$, OR 1.52 [1–6.4]), and annual risk of relapse increased with higher percentage of RIC.

Table 1 – Patients' characteristics before induction chemotherapy.

Characteristic	*n* = 78	Percentage (%)
Age (years)		
Median	42	
Range	18–68	
Sex		
Male	43	55
Female	35	
Presentation		
Primary	56	72
Relapse	22	
Tumour localisation site		
Head/neck	4	5
Trunk	9	11
Upper extremity	2	2
Lower extremity	34	44
Retroperitoneal/abdomen	10	13
Girdle	19	25
Tumour size (mm)		
Median	110	
Range	10–250	
Histological grade[a]		
1	3	
2	25	
3	50	64
Histology		
Synovialosarcoma	14	18
Myxoid/round cell liposarcoma	13	17
Fibrosarcoma	12	15
Leiomyosarcoma	8	10
Other	31	40

[a] According to the FNCLCC grading system.

3.3. Time-to-progression and overall survival

After a median follow-up of 6.8 years (range 1.6–10.3 years), 49 patients were alive, all but 1 death were related to STS

Table 2 – Clinical response to chemotherapy.

Type of response°	*n* = 78	Percentage (%)
Complete response	4	5
Partial response	25	32
Stable disease	44	57
Progression of disease	5	6

°According to RECIST criteria.

Table 3 – Patients' characteristics after resection surgery.

Characteristic	n = 78	Percentage (%)
Type of initial surgery		
R0	64	82
R1 + R2	14	
Pathological response		
Residual identifiable cells (RIC) (%)		
Median	30	
Range	0–100	
Class 1: RIC[a] 0–9%	27	
Class 2: RIC 10–50%	26	
Class 3: RIC 51–100%	25	
Adjuvant radiotherapy		
Yes	72	92

[a] RIC = residual identifiable cells.

disease. Relapses occurred in 38 patients (47%), locally (2.5%), distant (45%) and both in 1 patient. The global 5-year DFS and OS were 48% and 64%, respectively (Fig. 1). Good HR patients displayed a better prognosis than poor HR patients. (Fig. 2).

3.4. *Immunohistochemistry (IHC)*

From the 78 patients, samples were available for Topo2A ($n = 60$), Topo2A ($n = 60$) and ERCC1 ($n = 59$) immunohistostaining (Fig. 3A). HER2 protein expression was negative in all samples ($n = 45$).

3.4.1. *Topo2A protein expression*

The median percentage of cells with a Topo2A positive staining was 27.5%. No correlation was found between Topo2A protein expression and clinical response while HR correlated to Topo2A expression ($p \leqslant 0.0004$): 73% of Topo2A high-expressing tumours achieved good HR compared to 33% of Topo2A low-expressing tumours ($p \leqslant 0.0019$) (Table 4) and high Topo2A protein status was associated with a low percentage of RIC [<30 %] ($r = 0.416$).

A low percentage of Topo2A positive staining found in grade 2 tumours ($p \leqslant 0.002$, OR 3.75 [1.41–9.94], $r = 0.404$) was also associated with higher prevalence of distant relapses ($p \leqslant 0.037$, OR 1.88 [1–3.54]). The annual risk of relapse decreased with Top2A staining intensity (risk of 17%, 15% and 8% for groups 1, 2 and 3, respectively). The median DFS in patients with low Topo2A was 2.7 years and was not reached in patients with high Topo2A ($p \leqslant 0.042$, HR = 0.46, 95% CI = [0.21–0.97]) (Fig. 3B). The median OS was not reached in both Top2A groups ($p \leqslant 0.48$, HR = 0.76, 95% CI = [0.32–1.82]).

3.4.2. *ERCC1 protein expression*

The median *H* score was 1, including 26 ERCC1-negative and 33 ERCC1-positive tumours (Table 4). Complete and partial response occurred more frequently in case of ERCC1 positivity

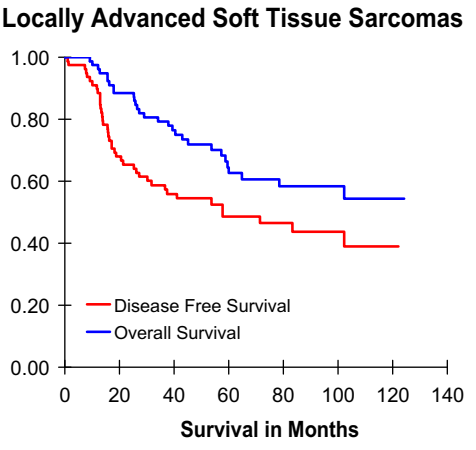


Fig. 1 – Disease-free and overall survivals of locally advanced soft tissue sarcomas. The 5-year global DFS and OS were 48% and 64%, respectively.

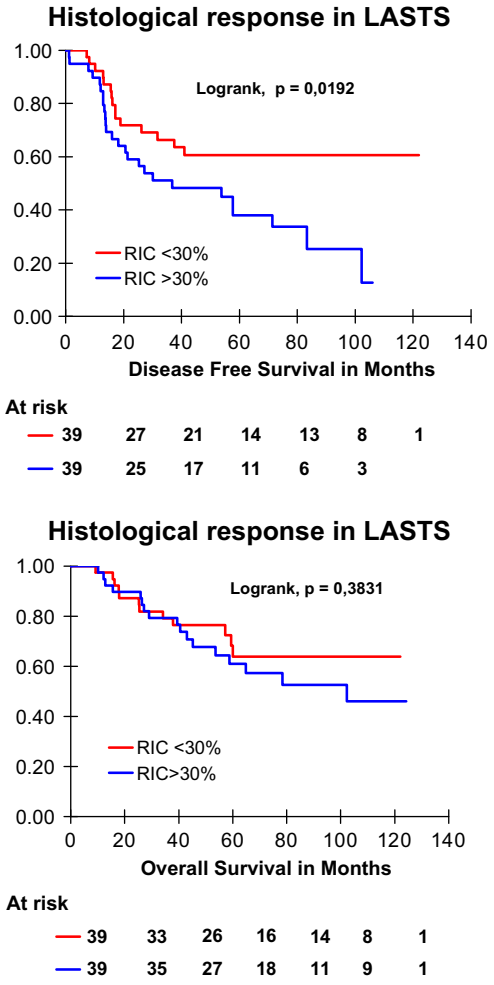


Fig. 2 – Disease-free and overall survivals of locally advanced soft tissue sarcomas (LASTS) patients according to histological response. Patients achieving a good HR (GHR) have a better prognosis than those having more than 30% of residual identifiable cells (RIC). The median DFS was 3.1 years in the group with >30% RIC but it had not been reached in the group with <30% RIC ($p \leqslant 0.021$) (histological response (HR) = 2.07, 95% confidence interval (CI) = [1.09–3.92]. The risk of relapse was twofold higher in patients who did not achieve a GHR. Median OS was 8.6 years in the group with >30% RIC and it had not been reached in the group with <30% RIC ($p \leqslant 0.33$) (HR 1.4, 95% CI = [0.68–3]).

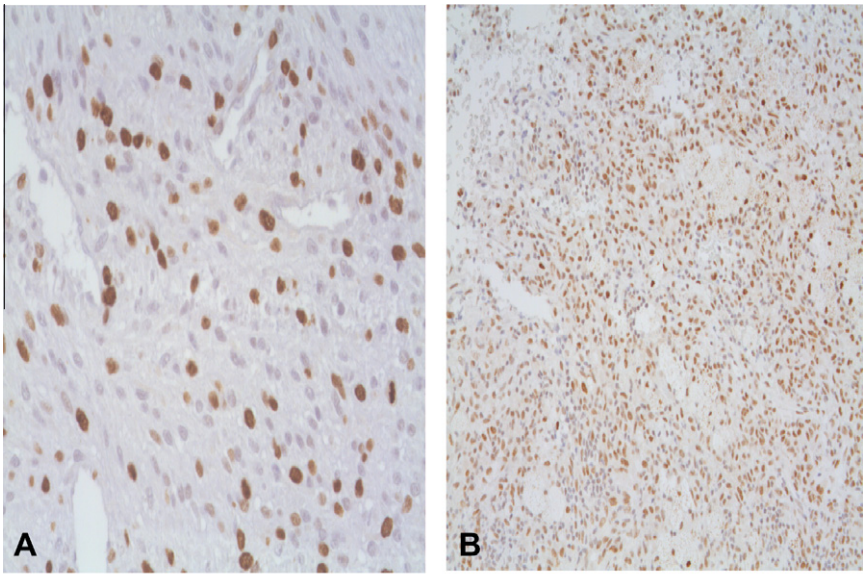


Fig. 3 – Expression of Topoisomerase II-alpha and excision repair cross-complementing group 1 (ERCC1). (A) Immunohistochemistry: A. Topoisomerase 2A nuclear positive sarcoma cells (G ×200). B. ERCC1 nuclear positive sarcoma cells (G ×50). (B) Disease-free and overall survivals in LASTS patients according to Topoisomerase II-alpha protein expression. (C) Disease-free and overall survivals of LASTS patients according to ERCC1 status. ERCC1 protein expression was not significantly correlated with DFS, even though the median DFS in patients whose tumours did not express ERCC1 was 3.16 years compared to 7 years in patients with ERCC1-expressing tumours ($p \leqslant 0.19$) (HR = 0.55, 95% CI = [0.26–1.15]). Median OS was not reached in ERCC1 high-expressing LATS as compared to 6.57 years in ERCC1-negative LASTS ($p \leqslant 0.058$) (HR = 0.43, 95% CI = [0.17–1.05]. (D) Disease-free and overall survivals of LASTS patients according to co-expression of Topoisomerase II-alpha and ERCC1 protein. Among the Topo2A high-expressing LATS, 76% (22/29) were positive for ERCC1 compared to 37% (11/30) in Topo2 low-expressing LATS ($p = 0.002$); the ERCC1 *H* score was correlated with the percentage of Topo2A-positive cells ($r = 0.464$). The study was not sufficiently powered to detect differences in DFS according to the co-expression of these proteins despite a significant difference (0.018) between LASTS patients high-expressing and those low-expressing these two proteins.

($p \leqslant 0.022$). ERCC1 positivity was not correlated to good HR: 61% of patients with ERCC1-positive tumours achieved a good HR as compared to 46% of ERCC1 low-expressing tumours ($p \leqslant 0.26$, $r = 0.042$).

ERCC1 protein expression was not significantly correlated with DFS (Fig. 3C), even though ERCC1-negative tumours showed median DFS and OS of 3.16 years and 6.57 years, respectively, compared to7 years for the DFS ($p \leqslant 0.19$) (HR = 0.55, 95% CI = [0.26–1.15]) in ERCC1-expressing tumours. Median OS was not reached in this group ($p \leqslant 0.058$) (HR = 0.43, 95% CI = [0.17–1.05]).

The ERCC1 *H* score was correlated with the percentage of Topo2A-positive cells ($r = 0.464$): 76% (22/29) of Topo2A high-expressing LASTS were positive for ERCC1 compared to 37% (11/30) in Topo2 low-expressing LASTS ($p = 0.002$). More tumours must be analysed to detect differences in DFS according to the co-expression of these proteins (Fig. 3Da) despite the OS difference being significant ($p = 0.018$) (Fig. 3Db).

3.5. *Multivariate analysis*

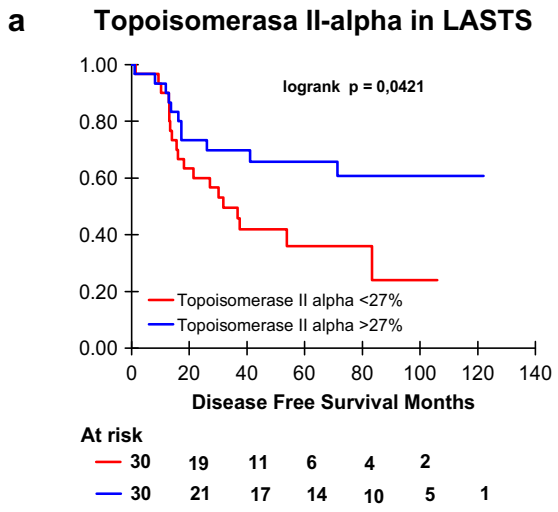
Gender, age >45 years, tumour size >10 cm, histological grade, initial tumour site and ERCC1 and Topo2A status were investigated for their predictive value for GHR using logistic regression analysis. The only independent variable was Topo2A ($p \leqslant 0.007$, OR 6.98 95% CI = [1.68–29.04]). LASTS containing a higher percentage of Topo2A-positive cells had almost a sevenfold greater probability of achieving a good HR after induction chemotherapy.

For the Cox proportional hazards model, 59 patients were included showing 28 events. Variables included in the model were tumour size (⩽10 cm versus >10 cm), histological grade (2 versus 3), Topo2A (⩽27% versus >27%) and ERCC1 *H* score (<1 versus ⩾1) (Table 4). Topo2A is an independent prognosis variable associated with PFS ($p \leqslant 0.017$) whereas grade correlates with both DFS ($p \leqslant 0.04$) and OS ($p \leqslant 0.004$).

4. Discussion

Prognosis of patients with LASTS remains dismal because of distant metastases even in case of adequate initial loco-regional treatments.[2] Neoadjuvant chemotherapy although a non-standard approach may facilitate an optimal initial R0 resection, one of the most achievable prognostic factor in resectable STS.[11] The number of reference centres that promote induction chemotherapy in critical situations (huge primary tumours or loco-regional relapses) increases.[3,5]

According to RECIST, only 5 patients (6%) of our series have progressed during induction chemotherapy leading thus to



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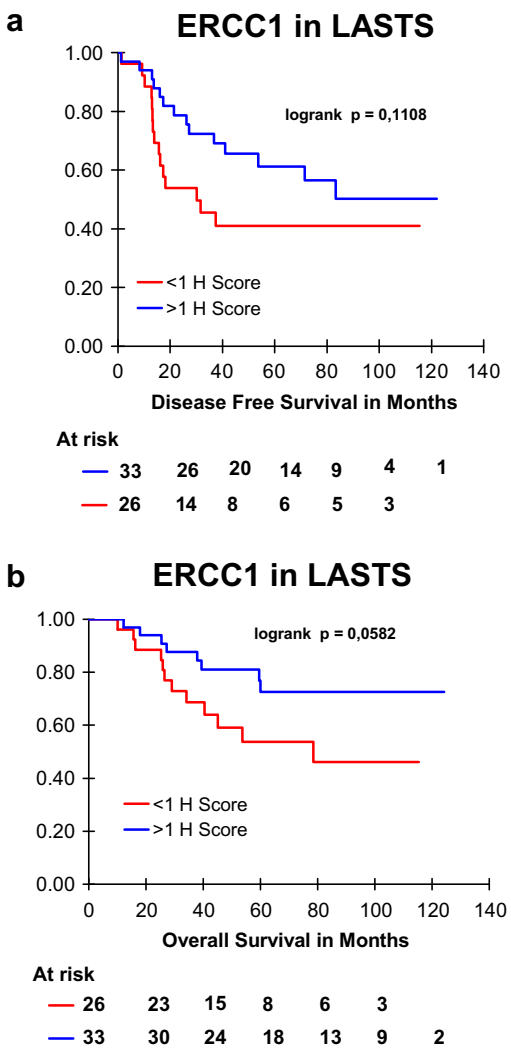

Fig 3. *(continued)*

favour for these patients radical surgery or second line therapies instead of complex conservative surgical procedures. All other patients experienced at least a stabilisation of their disease and 80% of patients achieved complete surgical resections (R0) in both primary and recurrences.

HR, a strong survival predictive factor for osteosarcomas and Ewing's tumours[4] has to be considered in STS. Although neoadjuvant therapy-induced necrosis ranges from 15% to 76%[3,5,12] (all histologies confounded) a few reports correlate it with clinical outcomes. For high-grade extremity sarcomas, a cutoff of 75% or 95% necrosis have been used to identify two cohorts of patients with significantly different outcomes (poor versus good HR)[3,13] while others, with the same cutoff, failed to demonstrate any correlation between HR and behaviour of patients.[14]

The median percentage of RIC was chosen as cutoff because necrosis may occur spontaneously and not only be due to induction treatment. In our cohort, patients with less than 30% of RIC in the resected sarcoma specimen after induction chemotherapy had a better DFS ($p \leqslant 0.021$) than those with more: This criterion was used to distinguish good and poor responders. HR appears to better predict chemosensitivity than clinical responses; indeed radiological response, traditionally measured by RECIST, is not a reliable factor for discriminating patients who are likely to benefit from neoadjuvant therapy and undergo better clinical outcomes after surgery.[15] Good HR were in fact observed in all patterns of responses, including one clinically-progressing patient, highlighting again the difficulties to accurately evaluate activity of cytotoxic drugs[16] or targeted therapies[17] in advanced STS and the need to combine functional (tissue response)[18] and morphological (size) imaging in this disease.

In contrast to radiological responses, a good HR is correlated with a lower incidence of relapses and metastases, and the risk of relapse increased with a higher percentage of RIC. Regarding STS histological subtypes, good HR occurred more frequently with poorly differentiated sarcomas compared to synovialosarcoma (only 22% of good HR). This could explain low impact of chemotherapy in the latter STS subtype both in localised and advanced settings despite the well known high incidence of tumour reduction after multidrug regimen according to RECIST.[19]

The challenging objective for LASTS is setting-up surrogate predictive biological markers for HR to avoid such

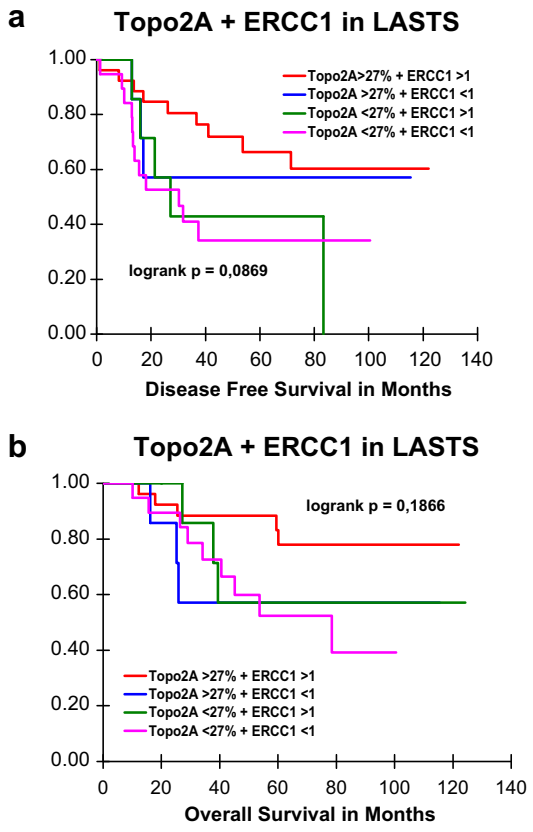

Fig 3. *(continued)*

non-standard therapeutic approaches in patients with initial chemoresistant tumours. In spite of the heterogeneity of STS, we speculate that chemo-responsiveness could be related to common genomic elements in all histological subtypes of STS. Tumour cell resistance to chemotherapy could reflect occurrence of a common biochemical bypass prompting tumour cells to evade toxic pathways. Although extensive in STS[20], search for predictive biomarkers yielded little information on pre-operative chemotherapy in LASTS except multidrug resistance (MDR)-positive tumour expression in poor responders[21] and a relationship between a decrease in ErbB-4 protein expression and patient outcomes.[22]

Topo2A overexpression, shown to be associated with a higher probability of response to doxorubicin in advanced breast cancer,[7] represents a tumour proliferation marker in STS of independent prognostic relevance.[23] Topo2A expression was correlated with the worst outcome in malignant peripheral-nerve sheath tumours[24] and synovialosarcomas.[25] As expected, the incidence of relapse was higher among Topo2A low-expressing LASTS and the annual risk of relapse increased as the percentage of Topo2A-positive cells diminished. Topo2A also discriminates patients with a better outcome: median DFS of 2.7 years in patients tumours with lower percentage of Topo2A had not been reached in LASTS with higher Topo2A ($p \leqslant 0.042$). With respect to OS, there was a trend of significance towards better median OS in LASTS with high Topo2A ($p \leqslant 0.48$).

Topo2A expression that inversely correlates with RIC in the resected specimen ($r = 0.416$) is an independent prognosis variable associated with PFS ($p \leqslant 0.017$) following a multivariate analysis. Therefore, Topo2 could be routinely analysed on initial biopsies as an early surrogate marker predictive factor for chemosensitivity. This histoprognostic grade correlates with both DFS ($p \leqslant 0.04$) and OS ($p \leqslant 0.004$) in a multivariate analysis. Since compared to grade 3, grade 2 STS was associated with a low percentage of Topo2A positive staining ($p \leqslant 0.002$), Topo2A may enable to identify in patients with grade 2 STS those who likely would benefit from doxorubicin induction chemotherapy (high expression) and those where loco-regional therapeutic options could be discussed (low expression) (Table 5).

Interestingly, Topo2A positivity was less frequent in synovialosarcoma. Patients' tumours harbouring SYT-SSX translocation-related sarcoma and low expression of Topo2A would require more alkylating agents or alternative targeted therapeutic approaches than anthracyclin-containing chemotherapy regimen.

Additional investigations must still be performed in Topo2A high-expressing STS who did not achieve a good HR in searching proteins such as those involved in the MDR process.[21] For instance, the Y-box binding protein-I (poor prognostic factor in synovial sarcomas) is recognised as associated with P-gp expression and Topo2A expression.[25] In addition, further analysis would determine whether Topo2A protein expression is related or not to *TOP2A* gene amplification or mutation, altered gene regulation and/or hypermethylation of the enzyme as factors contributing to abnormal protein activity in neoplastic LASTS cells.[26]

HER2 is expressed in osteosarcomas and in synovialosarcomas[26] but its role in the pathogenesis of STS appears to be minimal. *HER2* and *TOP2A* genes, both belonging to the 17q12–q21 amplicon are co-amplified and their protein products are co-expressed simultaneously in several solid tumour types.[7] This does not appear to be the case in LASTS. HER2 was found to be negative in the studied samples including 14 synovialosarcoma that expressed low Topo2A in the majority of cases.

Table 4 – (A) Topoisomerase II-alpha protein expression and good histological response and (B) excision repair cross-complementing group 1 (ERCC1) protein status and good histological response.

	Poor HR	Good HR[b]	Total
(A) Topo2A protein status			
Low[a]	20	10	30
High	8	22	30
Total	28	32	60
(B) ERCC1 protein			
Negative[c]	14	12	26
Positive	13	20	33
Total	27	32	59

[a] Topo2A low expression as defined by ⩽27.5% (median percentage of protein expression) cutoff.
[b] Good histological response (GHR) as defined by ⩽30% residual identifiable cells in the postoperative specimen.
[c] ERCC1 negative status as defined by <1 *H* score (median percentage of *H* score cutoff).

Table 5 – Multivariate analysis for overall and disease free survival.

Variable	Disease free survival			Overall survival		
	Histological response (HR)	95% confidence interval (CI)	*p*	HR	95% CI	*p*
Size (⩽10 cm versus >10 cm)	2.11	0.95–4.68	0.06	2.47	0.95–6.42	0.06
Tumour grade (2 versus 3)[a]	2.71	1.05–7.03	0.04	10.10	2.10–48	0.004
Good histological response (RIC <30% versus RIC ⩾30%)[b]	1.52	0.60–3.86	0.37	1.01	0.35–2.9	0.97
Topoisomerase II-alpha (⩽27% versus >27%)[c]	0.40	0.14–1.15	0.08	0.43	0.12–1.48	0.18
ERCC1 (*H* score <1 versus *H* score ⩾1)[d]	0.78	0.34–1.77	0.55	1.04	0.34–3.10	0.94

[a] According to the FNCLCC.
[b] RIC = residual identifiable cells.
[c] Median percentage of positive cells.
[d] Median *H* score value.

Excision repair cross-complementing group 1 (ERCC1) plays an important role in DNA nucleotide excision repair (NER). If patients harbouring different diseases with a deficient NER system (low ERCC1 levels)[9] benefit from cisplatin-containing chemotherapy, opposite results have been observed in LASTS where a high ERCC1 mRNA expression correlated positively with outcome of patients treated with trabectedin that requires an efficient NER system to be cytotoxic.[27]

In our series, ERCC1 expression does not correlate with HR but with PFS and OS, albeit not significantly. However and strikingly, there is a significant correlation between ERCC1 positivity and Topo2A over expression and the improved outcome of LASTS patients whose tumours co-expresses both markers (in comparison with those do not). Co-expression of Topo2A and ERCC1 has led to a promising high rate of tumour control.

The combination of an early predictive factor for chemosensitivity (Topo2A) and another one for survival (ERCC1) highlights the possibility to develop individualised therapeutic approaches in selected group of patients based on these biological markers. Despite heterogeneous STS subtypes, it might be possible to find a common biological signature that will help our decision making in marginally resectable STS. Using doxorubicin-containing induction chemotherapy should not be questioned in LASTS with high Topo2A expression while loco-regional approaches as isolated limb perfusion[28] or pre-operative radiation therapy could be preferable for Topo2A low-expressing tumours.[29] The role of ERCC1 in LASTS warrants further study. Evidence obtained from our retrospective analysis has now to be validated in prospective multi-institutional studies (CONTICANET program).

Conflict of interest statement

All authors disclose any financial and personal relationships with other people or organisations that could inappropriately influence their work.

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