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# Histopathological tumor response following neoadjuvant hyperthermic isolated limb perfusion in extremity soft tissue sarcomas: Evaluation of the EORTC-STBSG response score

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ABSTRACT

*Introduction:* This study aims to evaluate the applicability and prognostic value of the European Organization for Research and Treatment of Cancer-Soft Tissue and Bone Sarcoma Group (EORTC-STBSG) histopathological response score in extremity soft tissue sarcoma (ESTS) patients treated with neoadjuvant hyperthermic isolated limb perfusion (HILP) and delayed surgical resection.
*Methods:* Patients treated between 1991 and 2016 were included. The histopathological tumor response was established in accordance with the EORTC-STBSG response score. The distribution of patients was assorted according to the 5-tier histopathological response score for tumor grade, histological subtype and HILP regimen. Predictors for local recurrence free survival (LRFS) and overall survival (OS) were identified through Kaplan-Meier and Cox regression analyses.
*Results:* Ninety-one patients were included and their resection specimens were reanalyzed. Which resulted in 11 Grade A (12.1%), ten Grade B (11.0%), 15 Grade C (16.5%), 22 Grade D (24.2%) and 33 Grade E (36.3%) responses found among the series. The histopathological response was significantly influenced by the HILP regimen used, $p = 0.033$. Median follow-up was 65.0 (18.0–157.0) months. The histopathological response was not associated with LRFS nor OS. Resection margins, HILP regimen and adjuvant radiotherapy were associated with LRFS. Patients' age, tumor grade, tumor size and histological subtype were predictors for OS.
*Conclusions:* The EORTC-STBSG response score is applicable for determining the histopathological response to neoadjuvant ESTS treatment. However, this response does not seem to predict LRFS nor OS in locally advanced ESTS.


## Introduction

Soft tissue sarcomas (STS) are relatively rare and heterogeneous tumors, including over 50 histopathological subtypes [1]. Approximately 50–60% of the STS arise in the extremities [2]. In the Netherlands, 600–700 patients are diagnosed with a STS leading to 300 STS related deaths annually [3,4].

Extremity soft tissue sarcomas (ESTS) patients' survival is mainly determined by metastatic potential, whereas local tumor treatment is of lesser importance. Consequently, local tumor treatment has evolved from amputation to limb salvage surgery combined with radiotherapy [5,6]. At presentation, some ESTS are considered to be locally advanced. Since the overall survival of ESTS patients is not increased by amputation of the affected limb [5], neoadjuvant hyperthermic isolated limb perfusion (HILP), followed by surgical resection, has been used to prevent amputation in locally advanced ESTS in over 40 centers throughout Europe [7,8], resulting in a limb salvage rate of 80–90% [9–12].

Apart from neoadjuvant HILP, preoperative radiotherapy has been used in ESTS for decades. More recently, neoadjuvant

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chemotherapy has been tested in clinical trials in high-risk, but localized STS [13,14]. To evaluate the histopathological response to these neoadjuvant treatment strategies, a standardized approach for the pathological examination of pretreated sarcomas was proposed by the European Organization for Research and Treatment of Cancer-Soft Tissue and Bone Sarcoma Group (EORTC-STBSG) in 2016 [15]. This protocol includes a 5-tier response score based on the percentage of stainable, potentially viable tumor cells, clearly different from earlier methods in which the percentage of tumor necrosis was scored to determine the tumor response. Notably, thus far, data from the literature did not prove that the amount of tumor necrosis is prognostic in pretreated STS [15,16]. As tumor necrosis can be present in some STSs at diagnosis, it seems trustworthy to use the percentage of stainable cells in determining the histopathological response to neoadjuvant treatment. Recently, the first study applying the EORTC-STBSG response score found that it has no prognostic value with respect to recurrence free- and overall survival in a cohort of 100 extremity and trunk STS patients treated with radiotherapy prior to surgical resection [17].

This single tertiary sarcoma-center study aims to assess the applicability and the prognostic value of the EORTC-STBSG response score in locally advanced ESTS patients treated with neoadjuvant HILP followed by surgical resection of the residual tumor.

## Patients and methods

### *Patients*

The Institutional Review Board approved this study (case-number 2017-319). All consecutive patients over 18-years of age, with primary or recurrent, localized ESTS treated with neoadjuvant HILP followed by surgical resection, after 6–8 weeks, at the University Medical Center Groningen (UMCG) between 1991 and 2016 were analyzed. None of the patients were treated with neo-adjuvant chemotherapy. Patients' characteristics were obtained through medical record review. Patients for whom the required biopsy/tumor specimen was not available or not suitable for reanalyzes were excluded from the cohort.

### *Hyperthermic isolated limb perfusion*

The HILP technique used, is based on the technique developed by Creech et al. [18] and has previously been described in more detail [19]. Under general anesthesia the major artery and vein of the affected limb were isolated and cannulated, thereby, isolating the blood flow of the limb from the systemic circulation. The cannulas were connected to an extracorporeal circuit. Subsequently, a tourniquet was applied to minimize leakage of the cytostatic agents into the systemic circulation. At the beginning, the perfusate consisted of interferon-γ (IFN-γ), tumor-necrosis factor-α (TNF-α) (Beromun®, Boehringer-Ingelheim GmbH, Vienna, Austria) and melphalan (Alkeran®, GlaxoSmithKline Pharmaceuticals, Research Triangle Park, NC, USA). IFN-γ was soon abandoned, due to its ineffectiveness [7,9]. Potential leakage of the cytostatic agents into the systemic circulation was continuously monitored by a precordial scintillation detector and $I^{131}$-human serum albumin [20,21]. To perform the perfusion under controlled mild hyperthermia (38.5–40.0 °C), the limb was externally heated. Due to improvements in the HILP treatment, not all patients in this series were treated according the same HILP regimen. IFN-γ was abandoned, the TNF-α dose was reduced and the perfusion time was shortened [11]. Until 2001 the perfusion duration was 90 min whereas from 2001 till now the duration was 60 min. The 90 min regimen was divided in 30 min of TNF-α perfusion, followed by 60 min of melphalan perfusion. The 60 min regimen, started with 15 min of TNF-α perfusion, then the melphalan was added and after another 45 min the perfusion was ended. Nowadays, 2 mg TNF-α is used for femoral and iliac perfusions. Whereas 1 mg TNF-α is used for upper extremity and popliteal perfusions. These TNF-α doses are lower than the formerly used 3–4 mg TNF-α [11]. The melphalan dose was based on the limb volume, 10 mg/L for upper extremity and popliteal perfusions, and 13 mg/L for iliac and femoral perfusions. Following the perfusion, the limb was flushed with saline, 2 L for upper extremity and popliteal perfusions, and 6 L for iliac and femoral perfusions. Following the flushing of the limb, the limb was filled with 1 U red blood cell concentrate. Afterwards, the cannulas were removed, the vessels repaired and the heparin antagonized with protamine sulphate. A closed fasciotomy of the anterior compartment of the lower leg was performed to prevent a compartment syndrome [22,23]. The first 24 h following the procedure, the patient was closely observed in the medium care or intensive care unit.

### *Methods*

Prior to treatment, core-needle biopsies were performed for typing and grading of the tumors according to 'American Joint Committee on Cancer' and 'World Health Organization (WHO)' criteria [1,24]. Tumor margins were classified according to the 'Union for International Cancer Control' R classification [25] i.e. R0 for microscopically free tumor margins, R1 for microscopically compromised margins and R2 for macroscopically compromised margins. As previously reported, the histopathological examination of STSs, including the determination of the percentage tumor necrosis of the resection specimens has been standardized at the UMCG since 1991 [10,11,26]. In 2017, all resection specimens were re-analyzed by a pathologist with special interest and expertise in STS, who was blinded for clinical outcome, to classify the histopathological tumor response in accordance with the 5-tier, stainable tumor cell based, EORTC-STBSG response score; Grade A, no stainable tumor cells; Grade B, single stainable tumor cells or small clusters (overall below 1% of the whole specimen); Grade C, ≥1%-<10% stainable tumor cells; Grade D, ≥10%-<50% stainable tumor cells; Grade E, ≥50% stainable tumor cells [15].

The influence of tumor grade, histological subtype and HILP regimen on the histopathological response was investigated by assorting patients' distribution for these parameters according to the five response grades. Histopathological responders were defined as having <10% stainable tumors cells, combining response grade A, B and C. The remaining patients were considered histopathological non-responders with response grade D or E. Uni- and multivariate survival analyses were performed to identify associations between patient, tumor and treatment characteristics and 10-year local recurrence free survival (LRFS) or 10-year overall survival (OS).

### *Statistical analyses*

Data are presented as frequencies and percentages for discrete variables and median and inter quartile ranges (IQR) for continuous variables. None of the variables were normally distributed. The Mann-Whitney U and Kruskal-Wallis test were used to compare patients' distribution for tumor grade, histological subtype and HILP regimen according to their corresponding response scores. A p-value <0.05 was considered to indicate statistical significance. Oncological outcome was defined as time from date of HILP to event, either local recurrence or death. The Kaplan-Meier method and log-rank test were used for univariate survival analyses. Cox-regression was used to perform multivariate survival analyses. All potential predictors were included in a first multivariate cox-regression model. Backward selection was used, and predictors with a $p < 0.1$ were included in the final model. Hazard ratios (HR)

and 95% confidence intervals (CI) are presented. SPSS version 23.0 (IBM SPSS Statistics for Windows, Version 23.0 Armonk, NY: IBM Corp) was used.

## Results

Ninety-one patients, 48 male (52.7%), with a median age of 58.0 (44.0–65.0) years were included. Median tumor size was 9.0 (6.0–13.0) cm. Nearly 90% of the tumors were high grade and 83.5% of the tumors were located in the lower extremity. Eighty-one patients (89.0%) were treated for primary disease, the remaining 10 patients (11.0%) for recurrent disease. The predominant histological subtype was pleomorphic undifferentiated sarcoma not otherwise specified (Table 1). Not all patients underwent the same HILP regimen; 41 patients (45.1%) underwent the long and high dose HILP regimen, 12 patients (13.2%) underwent the short but high dose HILP regimen, 38 patients (41.8%) underwent the, now commonly accepted, short and low dose HILP regimen and 13 patients (14.3%) underwent a limb perfusion during the years that IFN-γ was included in the perfusate. Sixty patients (65.9%) underwent postoperative external beam radiotherapy (EBRT) following the HILP and surgical resection. Seventy patients (76.9%) underwent a R0 resection. The previously reported histopathological tumor responses were no change (NC), <50% necrosis, in 25 patients (27.5%); partial response (PR), 50–99% necrosis, in 50 patients (54.9%); and complete response (CR), 100% necrosis in 16 patients (17.6%). All 91 resection specimens were reanalyzed, and classified according to the EORTC response score. Eleven patients had no stainable tumor cells left in the resection specimen, Grade A (12.1%). Ten patients had <1% stainable tumor cells, Grade B (11.0%). Fifteen patients had ≥1%-<10% stainable tumor cells, Grade C (16.5%). Twenty-two patients had ≥10%-<50% stainable tumor cells, Grade D (24.2%) and 33 patients had ≥50% stainable tumor cells, Grade E (36.3%). Resulting in 36 responders (39.6%) and 55 non-responders (60.4%) (Table 2).

Table 3 presents patients' distribution for tumor grade, histological subtype and HILP regimen according to the five histopathological response grades. No significant differences in distribution were found for tumor grade and histological subtype among the EORTC grades, p = 0.104 and 0.111 respectively. A significant difference in distribution among the response grades was found for the various HILP regimens, p = 0.033.

**Table 1**
Patient and tumor characteristics.

| Characteristic | Total n = 91 (%) |
|---|---|
| Age, years (IQR) | 58.0 (44.0–65.0) |
| Gender | |
| Male | 48 (52.7) |
| Female | 43 (47.3) |
| Tumor size, cm (IQR) | 9.0 (6.0–13.0) |
| Tumor grade | |
| High | 80 (87.9) |
| Low | 11 (12.1) |
| Tumor location | |
| Lower extremity | 76 (83.5) |
| Upper extremity | 15 (16.5) |
| Histological subtype | |
| Pleomorphic undifferentiated/NOS | 25 (27.5) |
| Myxofibrosarcoma | 14 (15.4) |
| Myxoid liposarcoma | 14 (15.4) |
| Synovial sarcoma | 11 (12.1) |
| Leiomyosarcoma | 9 (9.9) |
| MPNST | 3 (3.3) |
| Pleomorphic rhabdomyosarcoma | 3 (3.3) |
| Pleomorphic liposarcoma | 3 (3.3) |
| Other | 9 (9.9) |
| Local presentation | |
| Primary | 81 (89.0) |
| Recurrent | 10 (11.0) |

Data presented as n (%) or median (IQR). Abbreviations: IQR = interquartile range; NOS = not otherwise specified; MPNST = malignant peripheral nerve sheath tumor.

**Table 2**
Treatment and tumor response characteristics.

| Characteristic | Total n = 91 (%) |
|---|---|
| HILP type | |
| Iliac | 36 (39.6) |
| Femoral | 13 (14.3) |
| Popliteal | 27 (29.7) |
| Axillar | 12 (13.2) |
| Brachial | 3 (3.3) |
| HILP drugs | |
| IFN-γ/TNF-α/Melphalan | 13 (14.3) |
| TNF-α/Melphalan | 78 (85.7) |
| HILP regimen | |
| Long (90 min) and high dose TNF-α | 41 (45.1) |
| Short (60 min) and high dose TNF-α | 12 (13.2) |
| Short (60 min) and low dose TNF-α | 38 (41.8) |
| Resection quality | |
| R0 | 70 (76.9) |
| R1 | 18 (19.8) |
| R2 | 3 (3.3) |
| Adjuvant EBRT | |
| No | 31 (34.1) |
| Yes | 60 (65.9) |
| Tumor necrosis, historical | |
| NC; <50% | 25 (27.5) |
| PR; 50–99% | 50 (54.9) |
| CR; 100% | 16 (17.6) |
| EORTC STS response score | |
| Grade A | 11 (12.1) |
| Grade B | 10 (11.0) |
| Grade C | 15 (16.5) |
| Grade D | 22 (24.2) |
| Grade E | 33 (36.3) |
| Histopathological responder | |
| No | 55 (60.4) |
| Yes | 36 (39.6) |

Data presented as n (%). Abbreviations: HILP = hyperthermic isolated limb perfusion; IFN-γ = interferon-γ; TNF-α = tumor necrosis factor-α; EBRT = external beam radiotherapy; NC = no change; PR = partial response; CR = complete response. EORTC STS response score: Grade A, no stainable tumor cells; Grade B, single stainable tumor cells or small clusters (overall below 1% of the whole specimen); Grade C, ≥1%–<10% stainable tumor cells; Grade D, ≥10%–<50% stainable tumor cells; Grade E, ≥50% stainable tumor cells [15]. Histopathological responders having <10% stainable tumor cells.

### *Follow-up*

Median follow-up was 65.0 (18.0–157.0) months for the entire cohort. Ten patients (11.0%) developed a local recurrence and 47 patients (51.6%) developed distant metastases. Ultimately, 43 patients (47.3%) died of disease and 8 patients (8.8%) died of other causes. At end of follow-up 40 patients (44.0%) were alive. Of which 36 patients (90.0%) had no evidence of disease, while 4 patients (10%) were alive with disease.

Univariate survival analyses displayed a significant influence of the HILP regimen, resection quality and adjuvant EBRT on 10-year LRFS. Showing a worse 10-year LRFS for patients treated with the short + high dose TNF-α HILP regimen, as well as for patients with compromised resection margins. Furthermore, postoperative irradiated patients had a 10-year LRFS of 89.5% compared to 65.2% for patients who did not undergo adjuvant EBRT, p = 0.004. No significant association between the histopathological tumor response and 10-year LRFS was found. Due to the limited amount of local recurrences, no multivariate analyses for LRFS was performed.

**Table 3**
Tumor response following neoadjuvant HILP according to tumor grade, histological subtype and HILP regimen.

| Characteristic | Total | EORTC Grade | | | | | p-value |
|---|---|---|---|---|---|---|---|
| | n = 91 | Grade A (n = 11) | Grade B (n = 10) | Grade C (n = 15) | Grade D (n = 22) | Grade E (n = 33) | |
| Tumor grade | | | | | | | *p = 0.104*[a] |
| High | 80 (87.9) | 9 (91.8) | 8 (80.0) | 12 (80.0) | 20 (90.9) | 31 (93.9) | |
| Low | 11 (12.1) | 2 (18.2) | 2 (20.0) | 3 (20.0) | 2 (9.1) | 2 (6.1) | |
| Histological subtype | | | | | | | *p = 0.111*[b] |
| Pleomorphic undifferentiated/NOS | 25 (27.5) | 6 (54.5) | 3 (30.0) | 3 (20.0) | 5 (22.7) | 8 (24.2) | |
| Myxofibrosarcoma | 14 (15.4) | – | 1 (10.0) | 1 (6.7) | 4 (18.2) | 8 (24.2) | |
| Myxoid liposarcoma | 14 (15.4) | 1 (9.1) | 2 (20.0) | 4 (26.7) | 5 (22.7) | 2 (6.1) | |
| Synovial sarcoma | 11 (12.1) | – | 2 (20.0) | 1 (6.7) | 3 (13.6) | 5 (15.2) | |
| Leiomyosarcoma | 9 (9.9) | 2 (18.2) | 1 (10.0) | 3 (20.0) | 1 (4.5) | 2 (6.1) | |
| MPNST | 3 (3.3) | – | – | 2 (13.3) | – | 1 (3.0) | |
| Pleomorphic rhabdomyosarcoma | 3 (3.3) | 1 (9.1) | – | – | – | 2 (6.1) | |
| Pleomorphic liposarcoma | 3 (3.3) | – | – | – | – | 3 (9.1) | |
| Other | 9 (9.9) | 1 (9.1) | 1 (10.0) | 1 (6.7) | 4 (18.2) | 2 (6.1) | |
| HILP regimen | | | | | | | *p = 0.033*[b] |
| Long (90 min) and high dose TNF-α | 41 (45.1) | 8 (72.7) | 6 (60.0) | 6 (40.0) | 9 (40.9) | 12 (36.4) | |
| Short (60 min) and high dose TNF-α | 12 (13.2) | – | 3 (30.0) | 3 (20.0) | 4 (18.2) | 2 (6.1) | |
| Short (60 min) and low dose TNF-α | 38 (41.8) | 3 (27.3) | 1 (10.0) | 6 (40.0) | 9 (40.9) | 19 (57.6) | |

Data presented as n (%). Abbreviations: EORTC = European Organization for Research and Treatment of Cancer; NOS = not otherwise specified; MPNST = malignant peripheral nerve sheath tumor; HILP = hyperthermic isolated limb perfusion; TNF-α = tumor-necrosis factor-α.
[a] Mann-Whitney *U* test.
[b] Kruskal-Wallis test.

Patients' age at start of treatment, tumor grade, histological subtype and adjuvant EBRT were significantly associated with 10-year OS in univariate analyses (Table 4). Multivariate cox-regression analyses identified patients' age 1.04 (1.01–1.06), p = 0.003; tumor size 1.09 (1.03–1.15), p = 0.001; high tumor grade 4.52 (1.12–18.23), p = 0.034; and histological subtype, p = 0.011 to be predictors for 10-year OS (Table 5). Leiomyosarcoma and MPNST were associated with a significantly worse 10-year OS.

## Discussion

This study shows that the EORTC-STBSG response score can be applied to determine the histopathological tumor response following neoadjuvant HILP and delayed surgical resection in locally advanced ESTS. A significant difference in the percentage stainable tumor cells was found for the various HILP regimens used during the study period. However, no association between the histopathological tumor response, i.e. tumor necrosis or stainable tumor cells, and LRFS or OS was found.

STS are heterogeneous tumors and the neoadjuvant treatment-induced tumor changes can differ throughout the tumor. Furthermore, STS tend to have a necrotic tumor center at presentation due to rapid tumor growth. At histopathological examination after resection it is impossible to determine the cause of necrosis (pre-existent or treatment-induced). Earlier studies showed that the percentage of tumor necrosis following neoadjuvant treatment is not prognostic for oncological outcome in ESTS [15,16]. Therefore the EORTC response score may have greater potential for the determination of the therapy effect compared to the determination of the percentage tumor necrosis. However, as our results show, the EORTC response score does not seem to influence the LRFS or OS.

In bone sarcomas, especially osteosarcomas, the use of tumor necrosis and later the proportion of vital tumor cells has been established, and was found to be prognostic [27–29]. Subsequently, histopathological responders, <10% vital tumor cells, and non-responders in osteosarcomas were identified by the WHO [1]. The standardized protocol for the pathological examination of pre-treated STS as proposed by the EORTC-STBSG includes a 5-tier STS response score to interpret the efficacy of the various neoadjuvant treatment strategies used in STS nowadays [15]. The current study could not establish an association between this STS response score and LRFS or OS. Subsequently patients were divided into two groups, being histopathological responders and non-responders to create larger groups for statistical analyses. The cut-off value used was based on the cut-off value currently used to determine response to chemotherapy for osteosarcomas. Histopathological responders were defined as having residual tumors containing <10% stainable tumor cells. However, as Table 4 shows being a histopathological responder did not influence 10-year LRFS nor OS.

The first study applying the EORTC-STBSG response score, showed no prognostic value considering recurrence free- and overall survival in a cohort of 100 extremity and trunk STS patients treated with radiotherapy prior to surgical resection of the residual tumor [17]. Till date, there is no data addressing the prognostic value of the EORTC response score following chemotherapy in STS. As the use of (neo)adjuvant chemotherapy is controversial and under ongoing investigation in localized STS [14], it might be of interest to include the EORTC-STBSG response score as parameter in current and future studies, especially since the histopathological tumor response of the primary tumor might provide additional information regarding the chemosensitivity of potential metastases developing during follow-up in these patients.

The current study has some limitations. The retrospective nature affects data collection and selection of patients. Not all patients in this cohort underwent the same HILP regimen. Over time IFN-γ was abandoned due to its ineffectiveness, the TNF-α dose was lowered and the perfusion duration was shortened. These improvements in HILP treatment were found to be safe and effective in terms of long-term patient outcome [7,9,11]. However, as established in the current series these changes in HILP regimen significantly influence the histopathological response when classified according to the EORTC-STBSG score.

The current study shows an univariate association between the various HILP regimens, resection margins and adjuvant EBRT, and 10-year LRFS. The significant effect of the HILP regimen on LRFS was unexpected, and seems to be explained by a worse LRFS for patients who underwent the short and high dose regimen. We cannot fully explain this worse LRFS for these patients. However, this regimen is no longer in use as the shorter and reduced dose regimen was shown to be oncologically safe in 2011 [11].

**Table 4**
Univariate analyses of the association between patient, tumor and treatment characteristics and 10-year LRFS and OS.

| Characteristic | n | 10-year LRFS | | 10-year OS | |
|---|---|---|---|---|---|
| | | (%) | p-value | (%) | p-value |
| All patients | 91 | 81.6 | NA | 45.7 | NA |
| Age, years | | | 0.507 | | 0.003 |
| <45 | 23 | 83.7 | | 73.7 | |
| 45–54 | 17 | 68.6 | | 45.4 | |
| 55–65 | 30 | 91.6 | | 43.3 | |
| ≥ 65 | 21 | 79.0 | | 15.9 | |
| Gender | | | 0.178 | | 0.733 |
| Male | 48 | 90.9 | | 43.8 | |
| Female | 43 | 73.0 | | 48.2 | |
| Tumor size (cm; 4 missing) | | | 0.944 | | 0.442 |
| <5 | 16 | 79.1 | | 50.0 | |
| ≥ 5 | 71 | 82.8 | | 44.5 | |
| Tumor grade | | | 0.529 | | 0.050 |
| High | 80 | 80.9 | | 42.3 | |
| Low | 11 | 85.7 | | 71.6 | |
| Tumor location | | | 0.154 | | 0.617 |
| Lower extremity | 76 | 85.0 | | 46.9 | |
| Upper extremity | 15 | 60.9 | | 40.0 | |
| Histological subtype | | | 0.829 | | 0.011 |
| Pleomorphic undifferentiated/NOS | 25 | 84.3 | | 32.0 | |
| Myxofibrosarcoma | 14 | 80.2 | | 42.9 | |
| Myxoid liposarcoma | 14 | 88.9 | | 71.4 | |
| Synovial sarcoma | 11 | 83.3 | | 72.7 | |
| Leiomyosarcoma | 9 | 43.8 | | 22.2 | |
| MPNST | 3 | NA* | | 0.0 | |
| Pleomorphic rhabdomyosarcoma | 3 | NA* | | 66.7 | |
| Pleomorphic liposarcoma | 3 | NA* | | 33.3 | |
| Other | 9 | 80.0 | | 53.3 | |
| Local presentation | | | 0.116 | | 0.477 |
| Primary | 81 | 84.7 | | 43.9 | |
| Recurrent | 10 | 63.5 | | 60.0 | |
| HILP type | | | 0.320 | | 0.085 |
| Iliac | 36 | 72.7 | | 41.3 | |
| Femoral | 13 | NA* | | 44.9 | |
| Popliteal | 27 | 92.0 | | 55.6 | |
| Axillar | 12 | 60.2 | | 50.0 | |
| Brachial | 3 | NA* | | 0.0 | |
| HILP drugs | | | 0.653 | | 0.903 |
| IFN-γ/TNF-α/Melphalan | 13 | 76.4 | | 46.2 | |
| TNF-α/Melphalan | 78 | 82.7 | | 45.6 | |
| HILP regimen | | | 0.008 | | 0.634 |
| Long (90 min) + high dose TNF- α | 41 | 84.2 | | 41.5 | |
| Short (60 min) + high dose TNF-α | 12 | 48.9 | | 50.0 | |
| Short (60 min) + low dose TNF-α | 38 | 97.2 | | 49.3 | |
| Resection quality | | | 0.006 | | 0.704 |
| R0 | 70 | 88.0 | | 45.5 | |
| R1 | 18 | 59.9 | | 50.0 | |
| R2 | 3 | 66.7 | | 33.3 | |
| Adjuvant EBRT | | | 0.004 | | 0.047 |
| No | 31 | 65.2 | | 34.9 | |
| Yes | 60 | 89.5 | | 51.5 | |
| Tumor necrosis, historical | | | 0.931 | | 0.928 |
| NC; <50% necrosis | 25 | 81.7 | | 50.9 | |
| PR; 50–99% necrosis | 50 | 84.2 | | 44.0 | |
| CR; 100% necrosis | 16 | 76.2 | | 43.8 | |
| EORTC STS response score | | | 0.514 | | 0.260 |
| Grade A | 11 | 85.7 | | 45.5 | |
| Grade B | 10 | 83.3 | | 60.0 | |
| Grade C | 15 | NA* | | 26.7 | |
| Grade D | 22 | 81.8 | | 58.7 | |
| Grade E | 33 | 72.6 | | 42.4 | |
| Histopathological responder | | | 0.156 | | 0.729 |
| No | 55 | 77.5 | | 48.8 | |
| Yes | 36 | 87.8 | | 41.7 | |

Data presented as actuarial survival percentages, log-rank test was used for comparison of characteristics. *Not applicable, all cases were censored. Abbreviations: LRFS = local recurrence free survival; OS = overall survival; NA = not applicable; HILP = hyperthermic isolated limb perfusion; IFN-γ = interferon-γ; TNF-α = tumor-necrosis factor-α; NOS = not otherwise specified; MPNST = malignant peripheral nerve sheath tumor; NC = no change; PR = partial response; CR = complete response; EORTC = European Organization for Research and Treatment of Cancer; STS = soft tissue sarcoma.

**Table 5**
Multivariate cox-regression analyses of the association between patient, tumor and treatment characteristics and 10-year OS.

| Characteristic | Overall survival | |
|---|---|---|
| | HR (95% CI) | p-value |
| Age, years | 1.04 (1.01–1.06) | 0.003 |
| Tumor size (cm) | 1.09 (1.03–1.15) | 0.001 |
| Tumor grade | | 0.034 |
| Low | 1 | |
| High | 4.52 (1.12–18.23) | |
| Histological subtype | | 0.011 |
| Myxoid liposarcoma | 1 | |
| Leiomyosarcoma | 5.86 (1.47–23.34) | |
| Myxofibrosarcoma | 1.52 (0.36–6.39) | |
| Synovial sarcoma | 1.54 (0.29–8.23) | |
| MPNST | 10.66 (1.92–59.37) | |
| Pleomorphic undifferentiated/NOS | 2.76 (0.71–10.69) | |
| Pleomorphic rhabdomyosarcoma | 0.65 (0.06–6.63) | |
| Pleomorphic liposarcoma | 0.75 (0.11–5.05) | |
| Other | 1.25 (0.26–6.08) | |

Abbreviations: OS = overall survival; HR = hazard ratio; CI = confidence interval; NOS = not otherwise specified.

In corroboration with earlier studies, we found that the 10-year OS is predicted by patients' age, tumor size, tumor grade and histological subtype through multivariate analyses in the current series [1,30–33]. However, there are studies showing that local recurrence development is a predictor for distant metastases and (disease-specific) death as well [34–36]. Due to small sample size and low event rate i.e. local recurrence rate, we were not able to perform multivariate analyses for LRFS. Besides, nearly 66% of the patients in this cohort received postoperative EBRT following the HILP and surgical resection, and although the adjuvant EBRT does not influence the histopathological response, it is well-accepted that postoperative EBRT following HILP and surgical resection lowers the local recurrence risk [26]. Since postoperative EBRT lowers the local recurrence risk, the tumor margin combined with the tumor response at the closest surgical margin might be of prognostic value for local recurrence development. Studies addressing the influence of the histopathological response at the closest surgical margin combined with the role of postoperative EBRT in these cases are necessary.

In conclusion, we corroborated earlier studies, showing that the histopathological tumor response, scored by the relative amount of tumor necrosis or stainable tumor cells, has no prognostic value considering LRFS and OS in pretreated STS. Therefore, the histopathological response should not be used in making treatment decisions at this point. Nevertheless, it is important to standardize the pathological examination of pretreated STS and to conform to the use of the EORTC-STBSG response score. In pretreated STS the use of stainable tumor cells seems rational and trustworthy, and further prospective research considering its prognostic value for oncological outcome is warranted.

## Conclusions

In STS management, the proposed standardization of histopathological examination of pretreated STS by the EORTC-STBSG is a step forwards. However, in our series the histopathological response (either stainable tumor cells or tumor necrosis) of these tumors does not seem to have prognostic value considering LRFS and OS and therefore it should not be used in making treatment decisions at this point. Further prospective studies addressing the prognostic value

of the histopathological response, preferably including vital tumor cells, in pretreated STS are necessary.

## Conflict of interest statement

Disclosure: The authors have no conflicts of commercial interest to declare.

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