Clinical and Biological Patterns in Soft Tissue Sarcoma

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Clinical and Biological Patterns in Soft Tissue Sarcoma

Emelie Styring

DOCTORAL DISSERTATION
by due permission of the Faculty of Medicine, Lund University, Sweden.
To be defended at Lecture hall C1, Blocket, SUS Lund, May 17th 2013 at 10 am

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Clinical and Biological Patterns in Soft Tissue Sarcoma

Abstract
Soft tissue sarcomas (STSs) are rare malignant tumors, of which 3/4 are high-grade and 1/3 metastasize. For optimal management, STSs should be treated at multidisciplinary sarcoma centers.

Study I demonstrated that simple referral guidelines, an open-access outpatient clinic and repeated educative measures to raise sarcoma awareness result in successful referral of untouched STSs to sarcoma center. Further, for each malignant tumor, 3 benign tumors were referred.

Study II showed that though small STSs in general have a good prognosis, tumors with either of the risk factors necrosis or intratumoral vascular invasion had a 3-fold increased risk and STSs with both risk factors had an 11-fold increased risk of metastases.

Study III describes changing clinical presentation of secondary angiosarcoma after breast cancer treatment; from late tumors in edematous arms, after median 11 years, to early tumors in the irradiated fields after median 7 years.

Study IV report that secondary angiosarcomas on the thoracic wall are difficult to treat; the recurrence rate is high also after surgery with R0 margins and the prognosis dismal.

Study V addressed differences in gene expression profiles between primary and secondary angiosarcomas. In secondary angiosarcomas RET, KIT and FLT4 within the receptor protein tyrosine kinase pathway, are significantly up-regulated.

In summary, these studies show that high referral rates of STSs to a sarcoma center are possible to achieve, that small STSs with necrosis and vascular invasion had high risk of metastases, that the clinical presentation of secondary angiosarcomas has changed, that these tumors have high recurrence rates, and that up-regulation of the receptor protein tyrosine kinase pathway is common.

Key words: Referral pattern, referral guidelines, risk factors, metastasis, vascular invasion, necrosis, secondary angiosarcoma, radiation-associated sarcoma, irradiated, RET, KIT, MYC.

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STS, soft tissue sarcoma; sAS, secondary angiosarcoma; pAS, primary angiosarcoma.
Populärvetenskaplig sammanfattning


Eftersom godartade knölar är mer än 200 gånger vanligare än mjukdelssarkom krävs riktlinjer för när mjukdelssarkom ska misstänkas och när en patient ska remitteras. Lunds sarkomcentrum rekommenderar att alla tumörer som är ≥5 cm och/eller är djupt belägna (sitter i eller mellan muskler) ska remitteras in för utredning innan någon operativ åtgärd görs.

Studie I visar att 97/100 patienter diagnosticerade med mjukdelssarkom inom södra sjukvårdsregionen (från 1 januari 2002) remitterades till sarkomcentret i Lund. Alla djupt belägna och 28/42 ytliga mjukdelssarkom remitterades in orörda, det vill säga innan någon operation. Som en jämförelse remitteras mellan 1/5 och hälften av alla mjukdelssarkom remitteras till sarkomspecialister med orörda tumörer i ånga andra länder. Av alla patienter som remitterats till sarkomcentret i Lund under två år, oavsett

1 Ben, brosk, bindväv, muskler och fettväv räknas till stödjevävnaderna.
2 Extremitetsbevarande kirurgi innebär att man opererar bort mjukdelssarkomet utan att amputera armen eller benet.
slutdiagnos, hade 113/464 patienter elakartade tumörer, varav 72 var mjukdelssarkom. Av de 351 godartade tumörerna behandlades 122 på sarkomcentret. Studien visar att det är möjligt att uppnå remittering innan kirurgi av en hög andel mjukdelssarkom. Konsekvenserna i form av utredning och eventuell behandling av godartade tumörer är hanterbara; för varje elakartad tumör remitterades 3 tumörer som efter utredning visade sig vara godartade.


Behandlingen av sekundära angiosarkom är svår. Studie IV visar att även om tumören opererats bort med goda marginer återkom den ofta lokalt. Risken att tumören sprider sig, metastaseras, var också stor och många patienter till följd av sin tumörsjukdom. Vi fann emellertid att 4 av 5 patienter som opererats med mer omfattande kirurgi, inkluderande borttagande av all strålbehandlad vävnad (hud, underhudsfat och delar av muskulaturen), förtövande lever utan tecken på sjukdom. Det kan tala för att mer omfattande kirurgi skulle kunna förbättra överlevnaden.

I studie V jämfördes primära (utan känd utlösande orsak) och sekundära angiosarkom på genetisk nivå. De två tumörförmerna kan inte skiljas åt vid granskning i mikroskop men de har olika så kallade genetiska profiler. Sekundära angiosarkom uttrycker mer av gener som påverkar cellers tillväxt och utveckling till specifika vävnadstyper, till exempel så kallade tyrosin-kinas-receptorer.
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Introduction

Soft tissue sarcomas (STSs) are rare, malignant tumors that develop from mesenchymal cells. They constitute 1% of all malignancies and can develop at any age with a peak incidence in the 6th-8th decades of life. Most STSs are located in the extremities or the trunk wall. The clinical presentation is often indolent and an STS is typically noticed as a painless lump. Benign lumps in the extremities or the trunk wall are common and outnumber STSs by 200:1.1 Most physicians thus encounter many benign lumps (e.g., lipomas, hemangiomas, fibromas and neurilemmomas) but only few, or no, STSs during their clinical careers. It is thus understandable that most patients and physicians have a low suspicion of malignancy when a patient presents with a lump.

In spite of the indolent presentation, three-quarters of the STSs are high-grade tumors and one third of the patients will die from their tumor.2,3 Historically, amputation was the mainstay of treatment for extremity STS. Following increased centralization of sarcoma care during the 1970’s and 1980’s, the rate of limb-sparing surgery increased.4 Increased use of adjuvant radiotherapy resulted in local control also after marginal surgical margins.5,6 The role of adjuvant chemotherapy is uncertain and prevention and treatment of metastatic disease has proven difficult.5,4

This thesis aims to:
- evaluate the simple referral guidelines applied in the southern Sweden health care region with respect to the referral rate of untouched STSs and the ratio of benign to malignant tumors referred,
- identify risk factors for metastasis for improved prognostication of small STSs,
- characterize the clinical presentation of secondary angiosarcomas after breast cancer in a population-based series,
- analyze treatment of secondary angiosarcomas of the thoracic wall with respect to surgical approach and outcome,
- compare the genetic profile of primary and secondary angiosarcomas to identify key genetic features.
STS referral

If an STS has been shelled-out (whoops-procedure), most often outside of a sarcoma center, remaining tumor tissue is often found upon re-excision. Unplanned surgery may also compromise the possibility to perform limb-sparing surgery. Therefore, prompt referral of patients with untouched suspected lumps to sarcoma centers is crucial. To raise sarcoma awareness and to aid physicians to optimally manage suspected tumors, referral guidelines have been issued by sarcoma organizations and the health care authorities in several countries. The Lund Sarcoma Center, established 1970, has an open-access outpatient clinic accepting referrals from any physician, or directly from a patient, without requiring any pre-referral diagnostic investigations of a suspicious lump. The referral guidelines are kept simple; superficial tumors ≥5 cm and/or deep-seated tumors irrespective of size should be referred before any surgical intervention. Internationally, similar guidelines are adopted by many sarcoma organizations, although some include additional criteria, i.e. pain, tumor growth or tumor recurrence after previous excision. However, the referral situation for STSs remains problematic; some report rates of untouched STSs referred to sarcoma specialists of only between 18 and 57%. Also, data is insufficient as to how many STS patients are not referred at all.

The National Swedish Cancer Register has 98% coverage of all malignancies diagnosed in Sweden. The Lund Sarcoma Center’s register is regularly checked against the National Swedish Cancer Registry and missing cases are entered after confirmation of a sarcoma diagnosis. This provides a possibility for population-based studies of STSs. It has also led to reliable evaluations of the referral patterns in the southern Sweden health care region. In study I, we investigated the referral pattern of STSs in the southern Sweden health care region (1.5 million adults) based on the simple referral guidelines used at the Sarcoma Center. We also assessed the ratio of benign to malignant diagnoses among patients referred to the open-access outpatient clinic. The rate of untouched STSs referred was correlated to the ratio of benign/malignant tumors referred.

Treatment of STS

STSs constitute a heterogeneous group of tumors, many of which are high-grade and prone to metastasize and/or recur locally. Nearly one-third of all patients succumb to their STSs, mainly due to pulmonary metastases. The mainstay of STS treatment is surgical resection of the tumor with wide margins. To improve local control adjuvant radiotherapy is recommended for large and/or deep-seated STSs. Radiotherapy to the tumor area reduced local recurrences but has not been shown to decrease the risk for metastases or to improve overall survival. Chemotherapy can be used in an effort to achieve systemic control. Today, most regimens are based on doxorubicin and ifosfamide. Considering the uncertain benefit of the treatment and the toxicity, both cardiac and neurological, linked to the treatment, most protocols
recommend that chemotherapy is used for selected patients whose tumors exhibit high-risk features.24,25,4,6,3

**Prognostic factors in STS**

Several risk factors for metastatic disease, most importantly histological malignancy grade and tumor size, have been identified and combined into different grading and prognostication systems.26-30 Tumor necrosis may be assessed as part of tumor malignancy grading or as an independent risk factor in a prognostic system.31,26,27,32,29,30 Further prognostic information is obtained from presence of intratumoral vascular invasion.33-37,32,38,39 The latter is, however, not included in any of the three major prognostic and grading systems, e.g. the MSKCC nomogram, the American AJCC/UICC system, and the French FNCLCC system.26-30

A prognostic model based on size, vascular invasion, and necrosis, the SIN model, has been developed and validated in Scandinavia.32,38 The prognostication model was later refined by the inclusion of peripheral tumor growth pattern classified as pushing or infiltrative.39 The SING model (size ≥ 8 cm, intratumoral vascular invasion [present or not], necrosis [present or not] and peripheral growth pattern [pushing or infiltrative]) is now used in Scandinavia to identify patients with high-risk tumors. Vascular invasion alone and/or presence of at least 2 of the other variables define high-risk tumors.40,32,38,41,39 The SING model compares favorably to the MSKCC nomogram and the AJCC/UICC system for identification of tumors prone to metastasize.38,42,39

Small STSs in general are considered to have a good prognosis with a rate of metastasis of <10 %. Surgery alone is often considered sufficient in this group43,44,13,45,46,6 and small STSs are often excluded from chemotherapy trials.43 When applying the SING model, some small (≤5 cm) STSs may be considered high-risk tumors and the patient may accordingly be recommended adjuvant treatment. However, none of the SIN/SING-studies specifically addresses small STSs. Due to the uncertain benefit from chemotherapy, the severe side effects and the generally good prognosis for small STSs concerns for overtreatment were raised. Therefore, we analyzed the prognostic influence of risk factors in a population-based series of small STSs (study II).

**Secondary angiosarcomas**

Angiosarcomas (AS) constitute 1% of all STSs. They are highly infiltrative tumors that are prone to recur, both locally and as distant metastases. AS have limited sensitivity to chemotherapy.47-50 AS typically develop without known cause, i.e. as primary angiosarcoma (pAS). The most common presentation is on the scalp in an elderly patient but pAS may develop in any organ.51,50 AS that develop after exposure to certain chemicals, following longstanding lymphedema or after radiotherapy are referred to as secondary angiosarcomas (sAS).52-58 The first known sAS cases were presented as parts of a series of patients developing sarcomas after various traumas.59 Later, sAS was described in women with longstanding, severe lymphedema in the upper extremity after breast cancer
In the 1980’s, sAS arising in previously irradiated field of the thoracic wall after breast cancer treatment were reported. Today, the latter presentation is the most common form of sAS with a standardized incidence ratio (number of observed cases/number of expected cases) of 6. Although the relative risk for sAS is increased following radiotherapy or lymphedema after breast cancer treatment, the absolute risk is low. The risk for developing any secondary STS is $1.3 / 10^4$ person years which should be compared the risk of $0.8 / 10^4$ person years for developing a primary STS in a control population. The cumulative incidence of sAS in women treated with radiotherapy for breast cancer has been reported to be $9 / 10^4$ cases at 15 years after breast cancer diagnosis.

To characterize the clinical presentation and disease pattern of sAS over time we identified all cases of sAS after breast cancer treatment diagnosed in our region between 1958 and 2008 and in correlation to the type of breast cancer treatment (study III).

Treatment of secondary angiosarcomas

Treatment of sAS is challenging. The tumors grow infiltratively and macroscopic delineation is difficult. Also, the tissue planes are less distinct due to previous irradiation. Extensive resections are needed and often require soft tissue reconstruction. In spite of macroscopically free margins at surgery there is often microscopic tumor growth in the resection margin. Even in cases where an R0 margin is achieved the local recurrence rate is high. Radiotherapy can be beneficial but the possibility to use it is limited due to the previous irradiation of the tumor area. Further, the adjuvant systemic drugs available today have uncertain effect on sAS. The 5-year survival rates are reported to be as low as 15%. To evaluate the surgical treatment and outcome in a population-based series of sAS patients we analyzed all sAS patients (1990-2004) operated with curative intent at the Lund Sarcoma Center and the sarcoma center at the University Medical Center of Groningen, the Netherlands (study IV).

Genetic characteristics of secondary angiosarcomas

Knowledge about the biology of sAS is scarce due to the rarity of the disease. Studies have applied different modalities and various types of control tumor samples. However, recurrent features of sAS have been found. In comparison to pAS and atypical vascular lesions an up-regulation of MYC is described in sAS. MYC encodes a transcription factor of genes controlling e.g. cell differentiation, cell growth, the cell cycle and angiogenesis. Another interesting feature, of both pAS and sAS located in the breast region, is the activating KDR mutation. To identify key molecular mechanisms in sAS and to validate those described by others, we analyzed the gene expression profiles of sAS with comparison to pAS (study V). Key findings were thereafter related to genetic abnormalities in other radiation-associated malignancies.
Patients and methods

Ethical approval for all studies in this thesis has been obtained from the Lund University ethics committee.

Study I

Two patient cohorts were included (Figure 1). Cohort 1 consisted of 100 consecutive adult patients (≥18 years old) diagnosed with a primary STS of the extremities or the trunk wall between 2002 and 2006 in the southern Sweden health care region. The patients were identified using the population-based National Swedish Cancer Registry. Clinical data were collected from patients’ charts at primary health care centers, local hospitals, and at the Sarcoma Center. Using prospectively recorded data, adherence to the referral guidelines was analyzed as were referral times and patterns. Referral time was defined as number of days from the first visit to a physician until referral to the Sarcoma Center. The referrals were evaluated according to if they followed our guidelines, i.e. direct referral to the Sarcoma Center of tumors ≥5 cm and/or deep-seated, without prior surgical intervention.

Cohort 2 included all new patients referred (n=464) to the Sarcoma Center’s open-access outpatient clinic during 2 consecutive years (2004 and 2005) because of a suspected malignant soft tissue-tumor. The patients were analyzed with respect to diagnostic work-up (cytology and/or imaging), benign or malignant final diagnosis, and treatments.

In cohort 1, 51 STSs were referred to the Sarcoma Center during 2004 and 2005 and they were thus also included in cohort 2.

Statistical evaluation was performed using the 2-sample Wilcoxon rank sum test in STATA 11 (StataCorp LP, College Station, TX, USA).
Study II

All adult (>18 years) patients in the southern Sweden health care region diagnosed with an STS of the extremities or the trunk wall between 1986 and 2010 were identified in the population-based Lund Sarcoma Center register. 848 patients were registered of which 243 (29%) had an STS ≤5 cm. Dermatofibrosarcoma protuberans, fibromatosis, strictly cutaneous tumors or atypical lipomatous tumors were not included in this series. A minority of the patients had been included in previous risk-factor analysis studies from our center. 32,38,39

Of the 243 small STSs, we excluded 4 patients with rhabdomyosarcoma, extraskeletal osteosarcoma or EWING/PNET, 8 patients with metastatic disease at diagnosis, 1 patient who died from another cause prior to surgery and 1 patient who was lost to follow-up. Hence, 229 patients were studied with metastatic disease at 5 years as the primary end point (Figure 1). The median follow up time was 5.7 years (2 months-19 years).

Necrosis and intratumoral vascular invasion were defined as present or not as previously described. 32,38,39  Histologic malignancy grading was based on the Scandinavian 4-tiered scale with grade 3-4 tumors being high-grade. 74  Peripheral tumor growth pattern could not be evaluated in this study due to insufficient recording.

Time to metastasis was analyzed using the univariate log-rank test and was visualized using Kaplan-Meier estimates for each risk factor. Multivariate cox regression analysis was used to estimate hazard ratios (HR) for risk factors with statistically significant impact (p≤0.05) in univariate analysis. Size was defined as a semi-continuous variable whereas depth, necrosis, and vascular invasion were analyzed as dichotomous variables. All calculations were performed using STATA 12.

Figure 1. Patient cohorts analyzed in studies I-II (yellow). Angiosarcoma patients analyzed in study III-V (green).
Study III

sAS following breast cancer treatment was defined as an AS that developed within the irradiated field or in a field of long-standing, severe lymphedema after a latency period of at least 3 years in accordance with the definition of radiation-associated sarcomas proposed by Cahan et al.\textsuperscript{75} and Arlen et al.\textsuperscript{76} Women with AS (or synonymous diagnoses) localized in the upper extremity, the upper torso or at an unspecified location were identified using the national Swedish Cancer Registry (established in 1958) and the regional pathology register (established in 1960). For 67 of the 69 identified cases data were available and 31 fulfilled the criteria for sAS after breast cancer treatment (Figure 1). The patients’ charts were reviewed and data on breast cancer treatment and the clinical presentation of the sAS were collected. All calculations were performed using STATA 11.

Study IV

Patients with radiation-associated sAS of the thoracic wall operated with curative intent between 1990 and 2004 were analyzed with respect to surgical and long-term outcome (Figure 1). 15 patients included in study III were eligible for inclusion as were 16 patients identified in the northern Dutch Health Care Region (1.8 million inhabitants) using the nationwide network and registry of histopathology and cytopathology in the Netherlands (PALGA) (established 1971). The study was performed as a collaboration with the University Medical Center of Groningen, the Netherlands.

Data were retrieved from clinical charts and from pathological reports. Surgical margins were defined as R0 (microscopically free) or R1 (microscopically intralesional). Time to local recurrence or distant metastasis was defined as time from treatment to radiological or pathological confirmation of local or distant recurrence. Disease-free survival was defined as time from start of treatment to local recurrence, distant metastasis or last follow-up. Disease-specific survival was defined as time from diagnosis to death due to sAS or latest follow-up. The disease-free and disease-specific survival was analyzed using the Kaplan-Meier method. All calculations were performed using SPSS 18 (SPSS, Chicago, IL, USA).
Study V

Paraffin-embedded tumor tissue from 26 patients with pAS was compared to that from 29 sAS patients (diagnosed between 1976 and 2009; Figure 1). All pAS patients were identified using the same registers as in studies III and IV. Before inclusion, the pAS patients were confirmed not to have any predisposing conditions (i.e. previous irradiation of or longstanding lymphedema in the tumor area). Biological replicates, i.e. multiple samples from different parts of a tumor, were available for 2 pAS and 5 sAS patients rendering 28 pAS and 34 sAS tumor samples for analysis.

From the 62 formalin-fixed paraffin-embedded tumor blocks representative, non-necrotic tumor areas were identified and were macro-dissected to minimize the inclusion of non-sAS tissue in the gene expression analysis. Thereafter, RNA was extracted from three 10-μm sections using the High Pure RNA Paraffin Kit (Roche, Castle Hill, Australia) according to the manufacturer’s instructions. RNA concentrations were determined using a NanoDrop Spectrophotometer (NanoDrop Technologies, Wilmington, DE) and samples yielding sufficient amounts of RNA were selected.

**Figure 2.** The cDNA-mediated Annealing, Selection, Extension, and Ligation (DASL) assay. RNA extraction (not shown) from paraffin-embedded formalin-fixed tumor tissue yields RNA for conversion into c-DNA using reverse transcriptase. The probes are annealed, extended and ligated before being amplified using PCR. The PCR products were labeled with fluorescent markers and captured by hybridization on the BeadChip. The fluorescent signal intensity for each bead were recorded and used for further analysis. Reproduced by courtesy of Christina Therkildsen.
Gene expression analysis was performed at the SCIBLU Genomics Center, Lund University, Sweden. The Illumina Bead-array (HumanWG-6 v4 Expression Beadchip, Illumina) system was used according to the manufacturer's instructions (Figure 2). Expression data were uploaded and processed in the GenomeStudio software (Illumina, San Diego, CA). Data were normalized using background correction, cubic spline normalization method and plate scaling. RefSeq features with a detection p-value of ≤0.01 in at least 80% of the samples were included, leaving 14382 features for further analysis. The data were uploaded into MeV 4.7.4 where they were log 2-transformed and median-centered across assays. Unsupervised hierarchical clustering was performed using the Pearson Correlation and average linkage clustering. Technical replicates were used to control for BeadChip-related effects. Each technical replicate paired tightly and one expression signature of each replicate was included in the final analysis. Significance Analysis for Microarrays (SAM) was used to identify differentially expressed genes in pAS and sAS with a False Discovery Rate (FDR) of 0%. Biological pathways and gene ontology (GO) terms were identified using the web-based Database for Annotation, Visualization and Integrated Discovery 6.7 (DAVID) software with a FDR ≤5%.

Figure 3. Illustration of the immunohistochemical method used to assess protein (green), e.g. KDR, expression levels. A primary, monoclonal antibody (red) specifically aimed at epitopes expressed on the protein is used. Secondary antibodies (blue), attached to a dextran backbone (black) labeled with multiple horseradish peroxidase molecules (yellow). Reproduced by courtesy of Ana Carneiro.

Immunohistochemical expression of KDR was assessed on fresh 4-μm sections (Cell Signaling, KDR, clone 55B11; diluted 1:100). The dilution was decided based on test-staining of AS tumors with high, low or neutral gene expression levels. The detection was performed using Dako’s EnVision system with a secondary antibody attached to a dextran backbone labeled with multiple horseradish peroxidase molecules (Figure 3). Thereafter, the slides were counterstained with hematoxylin. The KDR protein expression was determined in the area with the highest staining intensity and was graded in a 3-tier scale where 1+ represented <20% positive cells; 2+, ≥20% but <75% positive cells and 3+, ≥75% positive cells as suggested by Antonescu et al.
Results and discussion

Study I

Referral pattern

The Lund Sarcoma Center’s guidelines recommend direct referral of all patients with superficial soft tissue tumors ≥5 cm and all deep-seated tumors without any pre-referral diagnostics. Previous studies of the referral pattern in the southern Sweden health care region have demonstrated an increasing proportion of untouched tumors referred.1,7 In the current study, 97/100 consecutive STSs patients in cohort I were referred to the Sarcoma Center. All 58 deep-seated STSs and two-thirds (28/42) of the superficial STSs were referred before any surgical procedure. Of the 14 patients with superficial tumors operated outside the Sarcoma Center 6 had tumors >5 cm. Of these, 2 elderly patients with comorbidities were never referred. All but 1 of the 8 patients operated on for small, superficial tumors were referred after surgery. Notably, no open biopsies were performed outside of the Sarcoma Center and no patient was referred first after a local recurrence.

Figure 4. The referral pattern over time in the southern Sweden health care region showing the percentage (y-axis) of patients who were referred with the tumor untouched, after surgery or not referred at all. The figure is based on data from Rydholm1, Gustafson et al2 and study I.
The Lund Sarcoma Center was established more than 40 years ago. During the first years, only 40% of the STS patients were referred before surgery and one-quarter of the patients were not referred at all. Therefore simple referral guidelines, later adopted by the SSG, were issued and work to raise sarcoma awareness resulted in improved referral rates (Figure 4). An important part in raising STS awareness has been the continuous education of medical students, physiotherapy students and residents in orthopedics. The key message has been kept simple; sarcomas exist and all deep-seated lumps regardless of size and all superficial lumps ≥5 cm should be referred for diagnosis. Also, feedback is sent to physicians involved in the initial evaluation of a patient with an STS.

The median age at diagnosis (74 years) and the share of superficial tumors (2/5) was higher in this population-based series than in many center-based series. Studies of STS patients treated at sarcoma centers and those treated at local hospitals have revealed an underrepresentation of older patients and patients with small tumors at the specialized institutions. Thus the higher median age and larger rate of superficial tumors in our series probably reflect its population-based nature.

**Pre-referral investigations**

Although we do not require pre-referral diagnostic work-up, 64 patients had undergone imaging investigations and 38 had had cytological evaluations outside of the Sarcoma Center. Many pre-referral investigations had to be repeated at the Sarcoma Center due to suboptimal imaging techniques (19/64) or insufficient cytologic material for diagnosis (27/38). 41/64 patients evaluated with magnetic resonance imaging (MRI) before referral had small superficial tumors, i.e. tumors where MRI is not always necessary. The median time to referral to the Sarcoma Center was longer for patients undergoing pre-referral imaging investigations, 50 compared to 25 days \(p=0.1\). In 27/38 patients referred after fine needle aspiration or core biopsy the procedure had to be repeated at the Sarcoma Center due to insufficient material. Also Mankin et al reported problems with non-center biopsies in his 2 surveys conducted 1982 and 1992; they were non-diagnostic at higher rates than biopsies performed at sarcoma centers and in some cases hindered limb-sparing surgeries to be performed resulting in amputations. No open biopsies were performed outside of the Sarcoma Center in this series, which is remarkable compared to the situation in many other countries. Further, the need for repeated radiographic and cytological evaluation at the Sarcoma Center suggests that requirements of pre-referral diagnostic work up may delay referral and may not be optimal use of health care resources.

**First health care professional contact**

Three-quarters (75/100) of the STS patients in this series initially contacted their general practitioner. Similar rates have been reported for STS patients in other countries and also apply to patients with other types of tumors. In our series, 60 of these 75 patients had tumors that were either deep-seated or superficial and large and thus fulfilled the criteria for referral. However, only 20 patients were directly referred. The 25 patients who sought
medical care through other routes were directly referred in 8 cases though 20 had tumors that fulfilled the referral criteria. Also in the United Kingdom, only a minority of the patients presenting with a tumor that fulfilled the referral criteria are referred directly to a sarcoma center.\textsuperscript{16}

Referral lead times

The analysis of referral times was based on complete data from 78 patients. The median time to referral was 50 days. For the 28 patients directly referred, the median time was 30 days, compared to 64 days for the 50 patients initially referred to a local hospital (p=0.007). Reported delays in referral of STSs in other countries and for patients with other tumor types is often longer.\textsuperscript{82,85-87,16}

Once referred, similar times to treatment at the Sarcoma Center were found irrespective of referral pathway (Figure 5). Also regarding tumor size or depth the two groups were similar. For the 60 patients, for whom patient’s delay was recorded, the median delay was 2 months, which is longer than the time reported by Johnson et al\textsuperscript{88}, but similar to that reported by others.\textsuperscript{82,88,16}

\textbf{Figure 5.} Median lead times (days) for patients referred directly to the Sarcoma Center compared to those initially referred to a local hospital. Once referred, the time to the first visit at the Sarcoma Center and to start of treatment were similar.

Non-STS referrals

To determine if the simple referral guidelines, the open-access outpatient clinic and the high referral rates of untouched STSs were associated with a manageable rate of benign to malignant tumors, all patients examined at the Sarcoma Center outpatient clinic were analyzed (cohort II). Of the referred patients 113/464 were diagnosed with a malignant tumor. Of these, 72 had an STS, 29 a carcinoma and 10 a lymphoma. The remaining 351 patients had various benign soft tissue tumors. All STSs were treated at the Sarcoma Center, as were 122/351 benign tumors for which the treatment of a sarcoma surgeon was considered beneficial. 66 of the benign tumors treated at the Sarcoma Center were
deep-seated. Of the remaining 229 patients with benign tumors, 4 received medical treatments for desmoid tumors, 57 were referred to their local hospital for surgery while 168 patients did not require any treatment after confirmation of a benign diagnosis. For each malignant tumor referred, 3 benign tumors were evaluated. Similar rates have been described in other studies although different conclusions have been made. Malik et al found that one quarter of the patients referred under the “2-week” referral rule had a malignant tumor. They argued that this ratio was too low and that accepting such referrals could lead to disadvantages for other patients with malignant tumors. However, lower detection rates are accepted in screening programs for other tumors. For instance, 95% of women with suspicious findings on mammography screenings do not have breast cancer. With this in mind and considering the improved local control and lower rate of amputations in patients receiving primary treatment at sarcoma centers we consider the ratio of 1:3 highly justifiable.

In summary, we have shown that high referral rates of untouched STSs can be achieved and that the consequent ratio of malignant to benign tumors referred is manageable. Further efforts to increase awareness and decrease delays in sarcoma management are needed since tumor size is an important prognostic factor, and the only factor which can be influenced. Considering that a majority of patients first contacts their general practitioner efforts to improve the referral pattern for patients with STS should probably be directed at this group of physicians.

Study II

Different models exist for grading and prognostication of STSs, but there is a general consensus that small (≤5 cm) STSs, as a group, have a favorable prognosis. Surgery alone is often considered sufficient. To evaluate risk factors for metastases specifically in this subset we evaluated a population-based series of small STSs.

Small STS epidemiology

Of the 848 patients registered in the Lund Sarcoma Center register, 243 (29%) had small (≤5 cm) STSs, which translates into an annual incidence of 9 per million inhabitants. The thigh was the most common location and one-third of the tumors were deep-seated. 195 tumors (80%) were high-grade. Pleomorphic sarcoma and leiomyosarcoma were the most frequent histotypes.

13% of the small STSs metastasized: 8/243 patients had metastatic disease at diagnosis and 24/243 developed metastatic disease during the 5-year follow-up. The median time to distant metastasis was 20 months (2 months-9 years). The reported incidence of metastasis in series of small STSs is ~10%. In our series, only histopathologically high-grade tumors metastasized. The 48 low-grade tumors were therefore not included in
the risk factor analysis. Of the 181 high-grade tumors with localized disease at diagnosis, complete data were available from 170 cases.

Table 1. Risk for metastases in 170 small high-grade STSs.

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>HR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Univariate analysis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Necrosis</td>
<td>4.8</td>
<td>2.0-12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vascular invasion</td>
<td>4.5</td>
<td>1.9-11</td>
<td>0.002</td>
</tr>
<tr>
<td>Local recurrence</td>
<td>2.6</td>
<td>1.1-5.9</td>
<td>0.03</td>
</tr>
<tr>
<td>Size*</td>
<td>1.2</td>
<td>0.8-1.7</td>
<td>0.3</td>
</tr>
<tr>
<td>Deep-seated</td>
<td>1.1</td>
<td>0.5-2.4</td>
<td>0.9</td>
</tr>
<tr>
<td>Surgical margin**</td>
<td>0.7</td>
<td>0.3-1.4</td>
<td>0.3</td>
</tr>
<tr>
<td><strong>Multivariate analysis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Either necrosis or vascular invasion</td>
<td>2.7</td>
<td>1.0-7.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Both necrosis and vascular invasion</td>
<td>11</td>
<td>4.0-31</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Semi-continuous variable, 1-5 cm.
**Intralesional vs marginal vs marginal with RT and wide margin.

Figure 6. The rate of metastasis-free survival at 5 years for patients with small STS in relation to the presence of neither, 1 or both necrosis and vascular invasion.

Risk factor analysis

Tumor size, depth, surgical margin, age and sex were not predictive for the development of metastasis. Necrosis and vascular invasion provided independent prognostic information for metastatic disease. In univariate analysis the HR for necrosis was 4.8 and for vascular invasion 4.5 (Table 1). These HRs are similar to those previously described in unselected STS series.\textsuperscript{38,24,39} In multivariate analysis presence of 1 of these factors predicted development of metastasis with a HR of 2.7 (95% CI 1-7) and presence of both factors corresponded to a HR of 11 (95% CI 4-31; Figure 6; Table 1).
Local recurrence

Local tumor recurrence developed in 42/229 patients, 9 of whom also developed metastatic disease. Surgical margin was the only factor that predicted local recurrence whereas size, depth, vascular invasion, necrosis and malignancy grade did not (data not shown).

Small high-risk STS

As described by others, this study shows that most small STSs have a good prognosis.\textsuperscript{44-46} However, there are cases that do metastasize. Also in breast cancer, size is an important risk factor for metastases. Although most small breast cancers have a good prognosis a subset has more aggressive biology.\textsuperscript{91} We believe this applies also to small STSs and this study shows that this subset of small STSs with high risk of metastases can be identified through analysis of vascular invasion and necrosis. The presence of either one of these risk factors was associated with a 3-fold increased risk for metastasis within 5 years. If a tumor had both risk factors the risk for metastases was increased 11-fold. Small STSs of low histologic malignancy-grade and high-grade tumors with neither risk factor had low risk for metastases (0 and 7%, respectively). These data therefore support that adjuvant treatment should not be administered to this group. In contrast, small high-grade STSs with vascular invasion and tumor necrosis have a high risk of metastasis and should be probably be treated as any high-risk tumor.

Study III

Changing clinical presentation of sAS

When combining data from different registers, we identified all sAS patients after breast cancer treatment diagnosed in our region between 1958 and 2008. 31 patients were identified and analyzed. 14 of these patients had been treated for breast cancer between 1949 and 1988 with mastectomy and resection of the axillary lymph nodes and adjuvant radiotherapy. These 14 cases developed sAS in the upper extremity, following severe, long-standing lymphedema (Stewart-Treves syndrome) after median 11 (3-25) years. In 10 patients the sAS presented as a solitary tumor, whereas 4 patients reported multiple, small tumors as the first symptom. A second group consisted of 17 patients treated for breast cancer between 1980 and 2005. Of these, 16 were treated with segmental resection for breast cancer and 1 with modified mastectomy. All 17 had undergone postoperative adjuvant radiotherapy. They had mild or no reported lymphedema in the upper extremity. The sAS developed within the irradiated fields on the thoracic wall after median 7.3 (3-15) years. The sAS presented as a solitary tumor in 8 patients and as multiple smaller nodules in 8 patients. In the last case the initial sAS symptom could not be identified. The age at sAS diagnosis, time to sAS
after breast cancer treatment and sAS tumor symptom (solid tumor or multiple smaller nodules) are similar to that reported in the literature.\textsuperscript{66,57}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{distribution.png}
\caption{Distribution of breast cancers (circles) and sAS (squares) over time. Breast cancer and sAS are linked together for each patient. The steeper slopes reflect shorter time to sAS in the cases developing within the irradiated field (red) compared to the Stewart-Treves cases (green).}
\end{figure}

The median age at breast cancer diagnosis and sAS diagnosis was similar in both groups. However, when comparing the time from breast cancer to sAS using the log-rank test it was significantly shorter for women developing sAS within the irradiated field, median 7.3 years compared to 11 for those with Stewart-Treves syndrome (Figure 7; p=0.01). This decrease in time from breast cancer treatment to development of sAS is consistent with previous reports.\textsuperscript{52,66,57,92,93}

\textit{Changing breast cancer treatment}

In the 1970’s and 1980’s the treatment principles for early-stage breast cancer changed; from mastectomy to breast-conserving surgery often combined with adjuvant radiotherapy.\textsuperscript{94-96} The treatment was planned in multidisciplinary teams and included altered surgical approaches, new systemic treatments and radiotherapy. As a result, the breast cancer survival rates improved. The increasing incidence of breast cancer in combination with the increased survival rates, imply that a growing number of women are at risk for late side effects.

All patients who developed a sAS had had radiotherapy as part of the breast cancer treatment, albeit with different modalities. After converting the administered doses to Gray no statistically significant difference in received dose was found between the 2 sAS groups. At which dose radiotherapy can induce carcinogenesis is unknown, but a minimum of 10 Gray has been proposed.\textsuperscript{63,93} All patients in this series were treated with at least 30 Gray (see Table 1, Paper III). Due to increased incidence of breast cancer, major changes in breast cancer treatment, improved survival rates and the uncertainty of who was at risk, we were unable to estimate the incidence of sAS.
**sAS survival**

Both sAS groups had poor outcome. Median follow-up for patients in the Stewart-Treves group was 19 (0-65) months compared to 12 (2-88) months for the radiation-associated group. In the former group all women are dead, in 10/14 cases due to sAS. In the latter group 7/17 died from sAS and 4 from other reasons. Of the 6 women alive at the end of the study, 1 died of generalized sAS 2 years after diagnosis while 5 are alive without evidence of disease at 4.5, 5, 7, 8 and 11 years respectively as found in a follow up 2 years later.

Although the different clinical presentations of sAS are already known this is, to our knowledge, the first time a population-based cohort of sAS patients has been described and the changing clinical presentation correlated to type of breast cancer treatment.

**Study IV**

Considering the dismal prognosis for patients with sAS, we evaluated the outcome for patients surgically treated with curative intent at 2 sarcoma centers. In collaboration with the University Medical Center of Groningen, the Netherlands, we identified 35 cases of sAS in the irradiated field on the thoracic wall. 13 presented with a discoloration, 14 with a solid tumor and 8 with both symptoms. The median time from breast cancer treatment to sAS was 7 (3-25) years. The median age at sAS diagnosis was 67 (47-89) years. In 4 patients the sAS was locally too advanced, or generalized, to attempt curative surgery. Thus 31 patients were analyzed with respect to surgical treatment and outcome.

*Primary treatment of sAS on the thoracic wall*

24 patients had a mastectomy and 7 had a local excision. The 7 local excisions were either performed outside of the sarcoma centers or in patients who previously had a mastectomy as part of their breast cancer treatment. Due to R1 margins, a second surgery was performed for the primary tumor in 7 cases. Free margins (R0) were achieved in 14/24 patients treated with mastectomy and in 2/7 patients treated with local excision. In 3 patients the mastectomy was part of an excision of all irradiated skin and extrathoracic soft tissue, resulting in R0 resection in 2 cases. Soft tissue reconstruction was required in 16 patients. 1 patient had adjuvant chemotherapy and 1 patient had adjuvant radiotherapy.

*Tumor recurrence*

19/31 patients developed a local recurrence after median of 6 (1-89) months. Of the 23 patients operated with R0 margins, 14 had a local recurrence compared to 5/8 patients with a R1 margin. In 11 patients, local recurrences were treated surgically with R0 margins in 10 patients. In addition to surgery, 1 patient received adjuvant radiotherapy.
and 1 had adjuvant chemotherapy at this stage. 8 patients were not operated on due to locally advanced disease (n=2), concurrent metastases (n=5) or unknown reasons (n=1). As expected, patients whose local recurrences were resectable had better survival (median 34 (6-84) months) than those not operable (median 6 (5-24) months).

In a series presented by Torres et al, nearly half of the patients operated with R0 margins has local recurrences. In a study from MSKCC, the 5-year survival rate for patients with mixed radiation-induced sarcomas (n=123) operated with curative intent was ~40%. Although 90% of the patients in the study underwent surgery with curative intent, R0 margins were only obtained in half of the cases. Cha et al concluded that defining anatomic and tumor planes may be more difficult in irradiated tissue emphasizing the importance of an aggressive approach.

In our series, 13 patients developed metastases after median 17 (2-50) months. 9 of these also had local recurrences. The 4 patients with regional metastases underwent surgical resection of the involved lymph nodes and survived median 20 (8-29) months after this surgery. The patients with distant metastases had no further surgical treatment; they survived median 5 (1-24) months. In addition to surgery, 6 patients received chemotherapy and 6 patients were treated with radiotherapy.

**Survival**

After a median follow-up of 27 (1-151) months, 21/31 patients had died. 17 died from sAS, 3 from other diseases, and 1 for unknown reasons. The median disease-free and disease-specific survival were 16 and 37 months, respectively. Of the 10 patients still alive, 9 had no evidence of disease after median 53 (10-108) months. The 10th patient had persistent local disease after 7 years. Of the 9 patients with no evidence of disease, 1 had been operated for lymph node metastasis 3 years after diagnosis. Survival rate in this series, 30%, is thus higher than the 15% 5-year survival reported in most studies.

**Excision of all irradiated skin and extrathoracic soft tissue**

At the Lund Sarcoma Center wide excision of the whole irradiated field, i.e. all irradiated skin and extrathoracic tissue, has been performed as sAS treatment in 5 patients. In 3 patients, it was the primary surgical treatment and resulted in R0 resection in 2 cases. The patient operated with R1 margins was diagnosed with generalized disease shortly after the surgery and died of disease 7 months later. The 2 patients operated with R0 margins are alive with no disease after 5 and 8 years, respectively.

In 2 patients, the approach was used to treat local recurrences. In 1 case, it was combined with chemotherapy to treat the first local recurrence. This patient developed 1 more local recurrence which was excised and adjuvant radiotherapy administered. This patient is disease free since 5.5 years (12 years after the initial diagnosis of sAS). The 2nd case was only referred after multiple local recurrences. The excision resulted in R0 margins but the patient developed regional metastases 9 months later. These were resected and the patient
received adjuvant chemo- and radiotherapy. This patient is alive without evidence of disease since 4.5 years (8 years after the initial diagnosis of sAS).

All irradiated skin and extrathoracic soft tissue was resected. The surgery was planned based on the radiotherapy field films and tattooed radiotherapy coordinates when present. The depth of the excision was determined based on pre-operative MRI scans (Figure 8). Pedicled muscle flaps and skin have been used in combination with split-thickness skin grafts to reconstruct the soft tissues (Figure 9).

Long-term survival has been described after extensive surgical approaches in patients with sAS. The method described in this series emphasizes the need for wide resection margins including all irradiated skin and muscle tissue. Based on our results, we recommend that patients with sAS within previously irradiated fields on the thoracic wall should be evaluated for extensive surgery with curative intent for both primary and recurrent disease since this type of surgery may improve survival.
Study V

*Genetic profiles of pAS and sAS*

To assess the genetic characteristics of sAS, we performed whole-genome expression profiling of 34 sAS and 28 pAS tumor samples. Unsupervised hierarchical cluster analysis did not yield clear separation of pAS and sAS (see Supplementary Figure 1, Paper V) but distinct gene expression profiles were found (Figure 10). SAM analysis identified 54 up-regulated genes in sAS including RET, KIT, MYC and FLT4, whereas CDKN2C was significantly down-regulated. DAVID analysis of the 54 up-regulated genes identified up-regulation of the receptor protein tyrosine kinase pathway (EC number 2.7.10.1; FDR 4.4%, p<0.01, Figure 11), which was overrepresented by e.g. RET, KIT and FLT4.

![Figure 10.](image)

In pAS, SAM analysis identified 49 up-regulated genes including NTSR-1, ANKRD1 and CDKN2C. No significant pathways were identified by DAVID analysis, though 7 GO-terms, including cytokine receptor activity, regulation of cell size, the cell membrane and cell adhesion, were enriched.
**Deregulated RET signaling**

pAS and sAS are histopathologically indistinguishable but their genetic profiles differed by 103 significantly deregulated genes in our series (Figure 10). Our study is the first to link altered RET signaling to sAS, which showed up-regulation of RET and down-regulation of CDKN2C (Table 2). The RET proto-oncogene encodes for a tyrosine kinase receptor that transduces cell growth and differentiation signals. Activating mutations and over-expression of RET have been reported in thyroid cancer with particularly high frequencies in radiation-associated tumors.99,100 CDKN2C represents a downstream target of RET signaling and does, in combination with p27, maintain growth arrest. In multiple endocrine neoplasia type 2 (MEN2) and medullary thyroid cancer N-MYC induction has been identified as a key tumorigenic step associated with down-regulation of CDKN2C and p27. Functional studies also support synergistic effects from RET activation and CDKN2C inactivation.101-104 Also MYC and FLT4 were among the top up-regulated genes in secondary angiosarcomas, which is in line with observations from other studies. 73,67 Co-amplification of MYC and FLT4 has been described and the MYC genome suggested to be particularly prone to radiation-induced damage.67

\[\text{Figure 11.} \] The receptor protein kinase pathway and related genes. KIT, RET and MYC were up-regulated in sAS as indicated (↑) while CDKN2C was down-regulated (↓) in relation to the expression-levels seen in pAS.

N-MYC represents a down-stream target of RET-activation and a key step in CDKN2C down-regulation. Our findings of RET, MYC and FLT4 up-regulation and CDKN2C down-regulation thus fit well with the tumorigenic mechanisms suggested in other types of radiation-induced tumors.
Table 2. Characterization of deregulated key genes in sAS.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Definition</th>
<th>Chromosome</th>
<th>Fold Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>MYC</td>
<td>V-myc myelocytomatosis viral oncogene homolog</td>
<td>8</td>
<td>3.7</td>
</tr>
<tr>
<td>RET</td>
<td>Ret proto-oncogene (RET-ELE1, MEN2A, MEN2B, RET51)</td>
<td>10</td>
<td>3.0</td>
</tr>
<tr>
<td>KIT</td>
<td>V-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog (CD117, C-KIT)</td>
<td>4</td>
<td>2.1</td>
</tr>
<tr>
<td>FLT4</td>
<td>Fms-related tyrosine kinase 4 (FLT4)</td>
<td>5</td>
<td>2.0</td>
</tr>
<tr>
<td>CDKN2C</td>
<td>Cyclin-dependent kinase inhibitor 2C (INK4C, p18)</td>
<td>1</td>
<td>0.5</td>
</tr>
</tbody>
</table>

KDR expression

Up-regulation of vascular-specific receptor tyrosine kinases, e.g. TIE1, KDR, and FLT1 has been reported in sAS. KDR regulates endothelial cell survival, proliferation, migration and vascular formation during embryonic development and tumorigenesis. Activating mutations in KDR have been demonstrated in 10% of AS and have been linked to tumor localization in the breast. Intense immunohistochemical expression of KDR was demonstrated in 36/39 sAS in our series (p=0.007). This difference was also found when comparing the gene expression levels between pAS and sAS using Wilcoxon rank sum test (p=0.01) but on SAM analysis KDR was not identified as a significantly deregulated gene between the two tumor types. This discrepancy may reflect analytic variability or posttranscriptional modifications affecting mRNA stability.

Transcriptome signature and targeted therapies

Hadj-Hamou et al recently reported a transcriptome signature for sAS suggesting that radiation-associated AS develop from radiation-stimulated lymphatic vessel endothelial cells. Our assay included 29 of these 53 genes, but did not allow independent clustering between pAS and sAS (data not shown).

AS are generally resistant to chemotherapy and represent a tumor subset suitable for novel therapeutic approaches, including use of targeted drugs. Identification of RET-signaling as a key feature in secondary angiosarcomas suggests that this subgroup may be relevant for treatment with RET-kinase-inhibitors. No specific inhibitor directed only at RET is available but several multi-kinase-inhibitors such as sorafenib, vandetanib and sunitinib, have significant activity against RET. Use of these small-molecule inhibitors also result in inhibition of RET-kinase and tumor growth arrest in experimental models. In phase I-II trials limited effect has been found in STSs. However, the AS subset showed a response rate of 14% which motivates further studies. Considering the dismal prognosis for angiosarcomas and no prior selection of secondary RET-driven tumors, our observation suggest that further investigation of RET-inhibiting therapies in radiation-induced sAS is motivated.
Conclusions

High referral rates of untouched STSs to sarcoma centers can be obtained using simple referral guidelines and an open-access outpatient clinic in combination with repeated education to raise sarcoma awareness. The excess referral of 3 benign tumors for each malignant tumor is manageable and motivated by the high rates of untouched STSs referred.

Small STSs represent one-quarter of all STSs. Most have a favorable prognosis, but small STSs with high-risk for metastases can be identified through analysis of necrosis and intratumoral vascular invasion; presence of either risk factor is associated with a 3-fold risk increase while tumors with presence of both had an 11-fold risk. Tumors with neither risk factor had low risk for metastases.

Secondary angiosarcomas after breast cancer treatment show a changing clinical presentation over time, from late tumors in edematous arms, after median 11 years, to early tumors in the irradiated fields after median 7 years. The change parallels altered principles for breast cancer treatment.

Secondary angiosarcomas that develop in previously irradiated fields on the thoracic wall are difficult to treat. Even when R0 margins are achieved the recurrence rate is high and the prognosis dismal. Extensive surgery, preferably including resection of all irradiated skin and extrathoracic soft tissue, may lead to long-term survival.

Although histopathologically indistinguishable, primary and secondary angiosarcomas show distinct genetic profiles that differ by 103 de-regulated genes. The pathways involved include the receptor protein tyrosine kinase pathway. Key up-regulated genes in secondary angiosarcomas include RET, KIT and FLT4. Our findings suggest that drugs aiming the RET signaling pathway may be relevant to evaluate in secondary angiosarcomas.
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