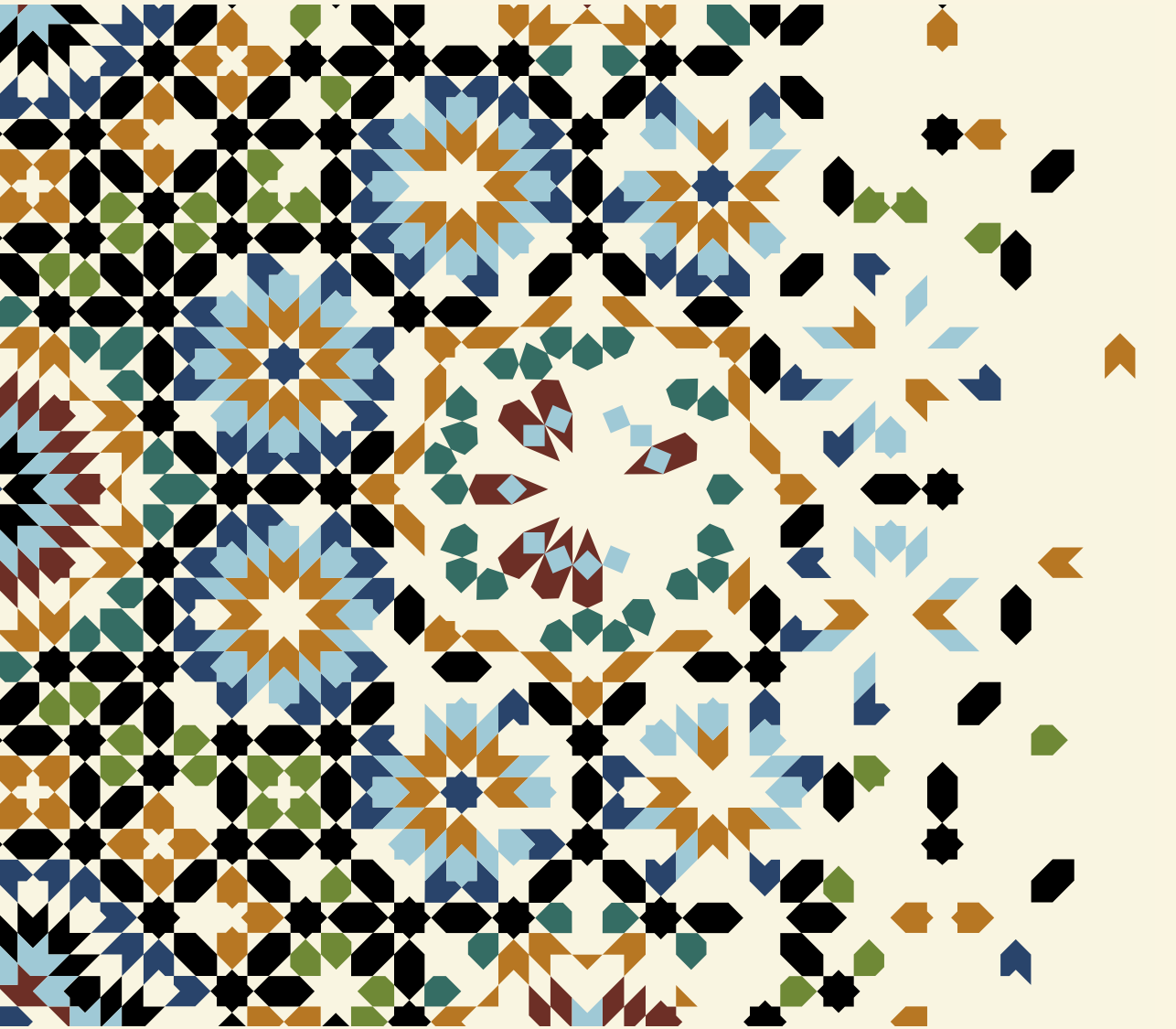


Towards a Subtype-Specific and Personalized Approach of Soft Tissue Sarcomas

FILLING IN THE GAPS OF THE MOSAIC



Melissa Vos



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Towards a Subtype-Specific and Personalized Approach of Soft Tissue Sarcomas

FILLING IN THE GAPS OF THE MOSAIC

Op weg naar een subtype-specifieke en gepersonaliseerde
benadering van wekedelen sarcomen
Het aanvullen van de gaten in het mozaïek

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de rector magnificus

prof. dr. F.A. van der Duijn Schouten

en volgens het besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op

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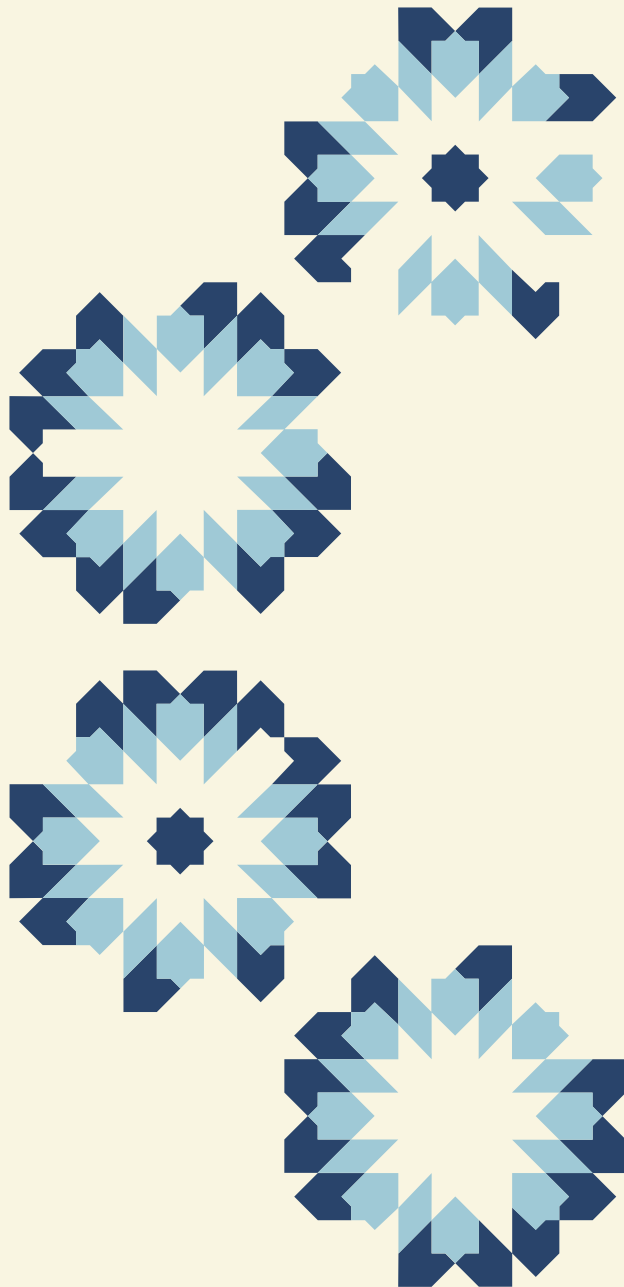
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CHAPTER 1

GENERAL INTRODUCTION AND OUTLINE OF THIS THESIS

General introduction

Clinical features

1

Soft tissue tumors are one of the most commonly observed tumors, which is mostly due to the high incidence of benign soft tissue tumors [1]. On the contrary, malignant or locally aggressive soft tissue tumors, also called soft tissue sarcomas (STS), are rare and account only for approximately 1% of all adult cancers, which is equivalent to 650-700 new patients annually in the Netherlands [2, 3]. STS is a heterogeneous disease of mesenchymal origin, consisting of over 50 different subtypes with each subtype harboring its own biological and clinical features [1]. The most common subtypes are gastrointestinal stromal tumors (GISTs), leiomyosarcomas and liposarcomas. Since STS can originate from all types of soft tissue, such as muscles, fat, tendons, blood vessels and nerve sheaths, they can arise at any site of the body, but the most common localizations are the extremity, the abdomen/retroperitoneum and the trunk. It is mainly a disease of the elderly, with a median age of 65 years at time of diagnosis, although some STS subtypes have a peak incidence during childhood (rhabdomyosarcoma) or adolescence (synovial sarcoma) [1].

Etiology

Most STS arise *de novo* and have an unknown etiology. Only in rare cases a genetic or environmental cause can be found. Examples include radiation-associated (angio)sarcoma, human herpes virus 8-induced or HIV/AIDS-associated Kaposi sarcoma, neurofibromatosis-type 1-associated malignant peripheral nerve sheath tumors and the Li-Fraumeni syndrome (*TP53* germline mutation) [1, 4-8].

Diagnostic work-up

Most patients present with a painless and slowly growing mass, and therefore undergo imaging, depending on the tumor localization an MRI and/or CT scan. Given the importance of a correct diagnosis regarding treatment and prognosis, usually an imaging-guided biopsy is taken and examined by an expert pathologist, who uses morphology, immunohistochemistry and/or additional molecular diagnostic tests. The STS subtype will be categorized according the classification of the World Health Organization [1] and graded according to the French Fédération Nationale des Centres de Lutte Contre le Cancer (FNCLCC) system, based on tumor differentiation, mitotic count and tumor necrosis [9]. Additionally, a staging CT scan will be performed to check for metastatic disease.

Treatment of soft tissue sarcoma

Currently, the treatment of STS is uniform for most of the different STS subtypes. Patients with localized disease are usually treated with surgery, optionally preceded or followed by

radiotherapy, isolated limb perfusion or systemic therapy [10]. Indications for neoadjuvant/ adjuvant treatment include a large tumor size, high tumor grade, inconvenient tumor localization and/or positive resection margins amongst others [10]. For certain STS subtypes, effective systemic therapy is available. For example imatinib for GIST patients and chemotherapy regimens for patients with small blue round cell sarcomas (e.g. embryonal rhabdomyosarcoma), but for most STS subtypes systemic treatment is not indicated in case of localized disease.

With respect to the non-GIST, non-small blue cell sarcomas, approximately 10-15% of the patients present with metastatic disease at time of diagnosis [11] and up to 40% of the patients with initially localized disease will develop metastases over time [12]. For these patients cure is generally not possible anymore, and treatment with palliative intent remains. Only in selected cases with oligometastatic disease, for example in patients with a solitary (lung) metastasis, long-term survival can be achieved [13-16]. Despite the heterogeneity amongst the different STS subtypes in terms of biology and sensitivity to chemotherapy, first-line treatment is similar for most STS subtypes, consisting of doxorubicin-based regimens [10]. Most patients receive doxorubicin monotherapy with a response rate of 10-15% and a median overall survival of 12-18 months [17-21]. For fit patients in need of a response, combination therapy with doxorubicin plus ifosfamide can be considered, prolonging the progression-free survival but not overall survival [17]. Recently, the combination of doxorubicin plus olaratumab was conditionally approved as first-line treatment, based on a phase II trial showing an improvement of 2.5 months in progression-free survival and almost a year in overall survival [18]. However, the phase III ANNOUNCE trial has failed to confirm the beneficial effect of the combination therapy compared to doxorubicin monotherapy [22]. As a consequence, the European Medicines Agency (EMA) has withdrawn its conditional marketing authorization.

In second-line treatment and beyond, a histology-driven/STS subtype-specific choice of treatment is becoming much more common. Examples include trabectedin in leiomyosarcoma and liposarcoma subtypes other than well-differentiated liposarcoma [23-25], eribulin in liposarcoma [26], pazopanib in non-adipocytic STS [27, 28], gemcitabine-based regimens in leiomyosarcoma [29, 30] or taxanes in angiosarcoma [31]. Additionally, a few promising agents in the pipeline are being explored in early phase clinical trials, such as regorafenib in non-adipocytic STS [32, 33] and therapies directed against the NY-ESO-1 antigen in synovial sarcoma and myxoid liposarcoma [34, 35]. Also immune checkpoint inhibitors are being investigated for their efficacy in various STS subtypes, including pembrolizumab [36], nivolumab and ipilimumab [37], but results obtained so far are disappointing.

Liposarcoma

Liposarcoma is one of the most common STS subtypes, representing approximately 20% of all STS. These tumors are derived from lipoblasts/adipocytes, and can be divided into four major subtypes based on distinct morphological and genetic features: well-differentiated liposarcoma (WDLPS), dedifferentiated liposarcoma (DDLPS), myxoid liposarcoma (MLPS) and pleomorphic liposarcoma (PLPS). A small part of liposarcomas cannot be further defined, resulting in a residual group of liposarcomas not otherwise specified (LPS NOS). The most common liposarcoma subtype is WDLPS, accounting for approximately 50% of all liposarcomas. It is a low-grade tumor with no metastatic potential, and – depending on tumor localization – is sometimes also called an atypical lipomatous tumor. It is molecularly characterized by amplification of 12q14-15, including the gene *MDM2*. In approximately 10% of the WDLPS, dedifferentiation into the more aggressive and high-grade DDLPS subtype occurs, thereby gaining the ability to metastasize. The remaining 90% of DDLPS arise *de novo*, are also characterized by amplification of 12q14-15 and are most frequently localized in the retroperitoneum. The third subtype, MLPS, accounts for approximately a third of all liposarcomas and is characterized by a translocation of t(12;16)(q13;p11), resulting in the FUS-CHOP (also called FUS-DDIT3) fusion protein. Approximately a third of the MLPS patients will develop metastatic disease, which is related to the presence of a round cell component and thereby the grade of the tumor. PLPS is the rarest but also the most aggressive liposarcoma subtype, harboring complex karyotypic aberrations. Up to 50% of the patients with PLPS will develop metastases, resulting in a poor prognosis [1].

Outline of this thesis

Because of the rarity, complexity and heterogeneity of the disease, not only diagnosing and treating these patients can be difficult, but also conducting research is challenging. Items that have been investigated for other more common cancers are still unexplored in STS and knowledge of these tumors is lagging behind, resulting in many ‘gaps’ in the STS biology, pathophysiology, diagnosis and treatment. This thesis contains research on a variety of subjects on multiple aspects of STS; from translational basic research to clinical research, from localized STS to advanced/metastatic STS, from diagnosis to evaluation of the current treatment, and from one specific STS subtype to all STS subtypes.

The **first part** of this thesis concentrates on the molecular biology of different STS subtypes. A better understanding of the tumor biology and pathophysiology is key in the identification and development of new treatment strategies. In **chapter 2**, the genomic landscape of metastatic STS, and more specifically GIST and leiomyosarcomas, was unraveled by using whole genome sequencing, along with the identification of targetable features for

systemic treatment. In addition to genomic alterations, also microRNAs can greatly impact the behavior of tumors. In **chapter 3**, the role of two specific microRNAs, miR-26a and miR-3913, and their effect on proliferation in liposarcoma (WDLPS andDDLPS) was explored. In the last chapter of this part, **chapter 4**, the biology of recurrent WDLPS was investigated on a microRNA and genome-wide DNA methylation level by comparing paired primary and recurrent WDLPS tumor samples.

The **second part** of this thesis focuses specifically on liposarcomas, one of the largest sarcoma subgroups, and the heterogeneity amongst these lipomatous tumors. Although clear differences in tumor size, depth and heterogeneity between benign lipomas and malignant WDLPS have been described in literature, in daily clinical practice there is a considerable overlap in these features. It can be difficult to distinguish between these two tumor types based on imaging, or even after biopsy based on morphology. In **chapter 5**, we developed a more objective and less invasive method to differentiate WDLPS from lipomas using a radiomics approach. Liposarcomas can arise at any site of the body, but are mainly localized in the extremity or retroperitoneum. In **chapter 6**, the impact of primary tumor location on recurrence and survival of patients with liposarcoma was assessed. The last chapter of part two, **chapter 7**, focuses on one specific liposarcoma location, the extremity, and investigated the differences in treatment, recurrence and survival between the different liposarcoma subtypes on this location.

In the **third part** of the thesis, the surgical treatment of localized STS is evaluated. **Chapter 8** assessed the treatment of WDLPS in the extremity, suggesting that there might be overtreatment of these patients and introducing the concept of active surveillance in this patient subgroup. Because of the rarity and complexity, more evidence is becoming available indicating that centralization has beneficial effects on the outcomes of STS patients in the last two decades. In **chapter 9**, the centralization of STS surgery in the Netherlands was evaluated on a nationwide level, together with its effect on surgical outcomes and the survival of Dutch STS patients. In **chapter 10**, one of these surgical outcomes, the unplanned resections or so called 'whoops' resections, was further examined for its effect on other surgical outcomes, such as the status of the resection margins, number of re-resections, use of adjuvant radiotherapy and plastic surgery.

In the last and **fourth part** of this thesis, the systemic treatment for advanced/metastatic STS is evaluated. **Chapter 11** gives a concise overview of the current systemic treatment and the promising developments in the pipeline for locally advanced or metastatic STS. In the last decade, two new agents have become available for patients with advanced STS who had failed on first-line doxorubicin-based treatment; pazopanib and trabectedin. In **chapter 12**, the impact of these changes in the treatment for STS patients with synchronous metastases has been assessed on a nationwide level. Finally, the association between

pazopanib-induced toxicity and survival in patients with advanced STS was investigated in **chapter 13**. This study was performed based on the hypothesis that the occurrence of toxicity is related to the anti-tumor activity of the drug, and that toxicity therefore can be used as a biomarker of efficacy.




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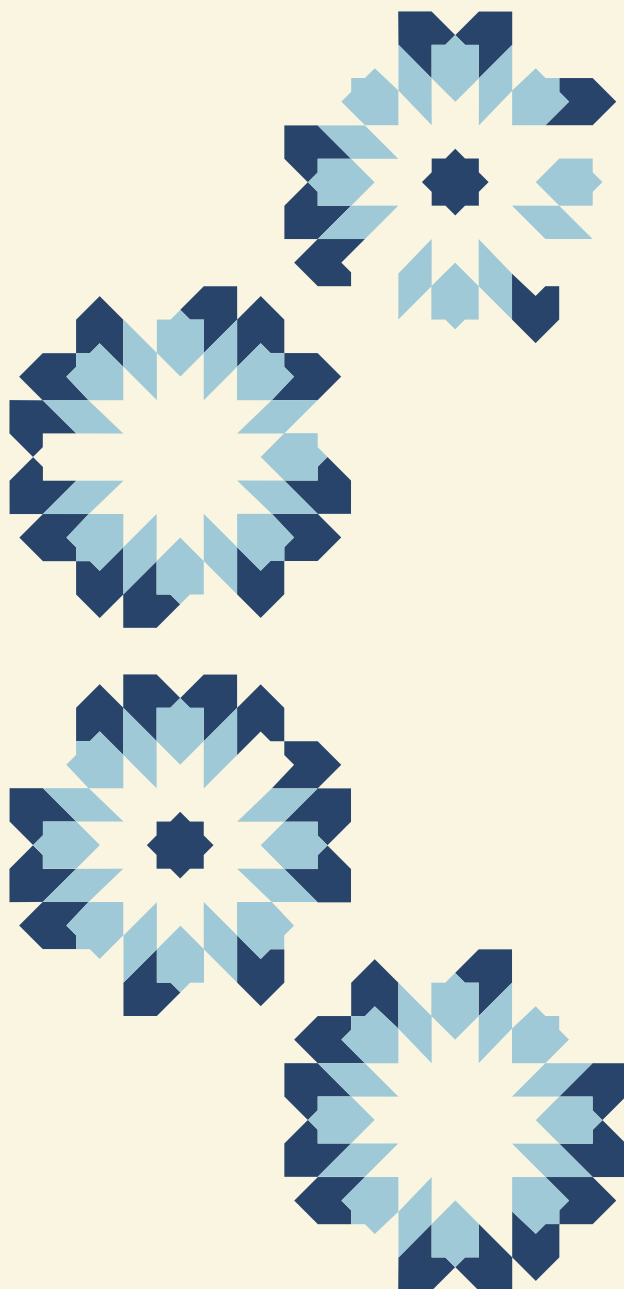
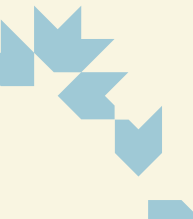
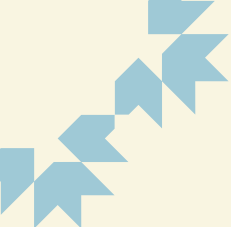
As outlined by this introduction, the mosaic theme reflects on multiple aspects of this thesis: the heterogeneity within the STS spectrum, the diversity of the subjects in this thesis and the variety of outcomes of the different chapters. Furthermore, the mosaic is still incomplete and the gaps have to be filled in further, which is — hopefully — partly done by this thesis.

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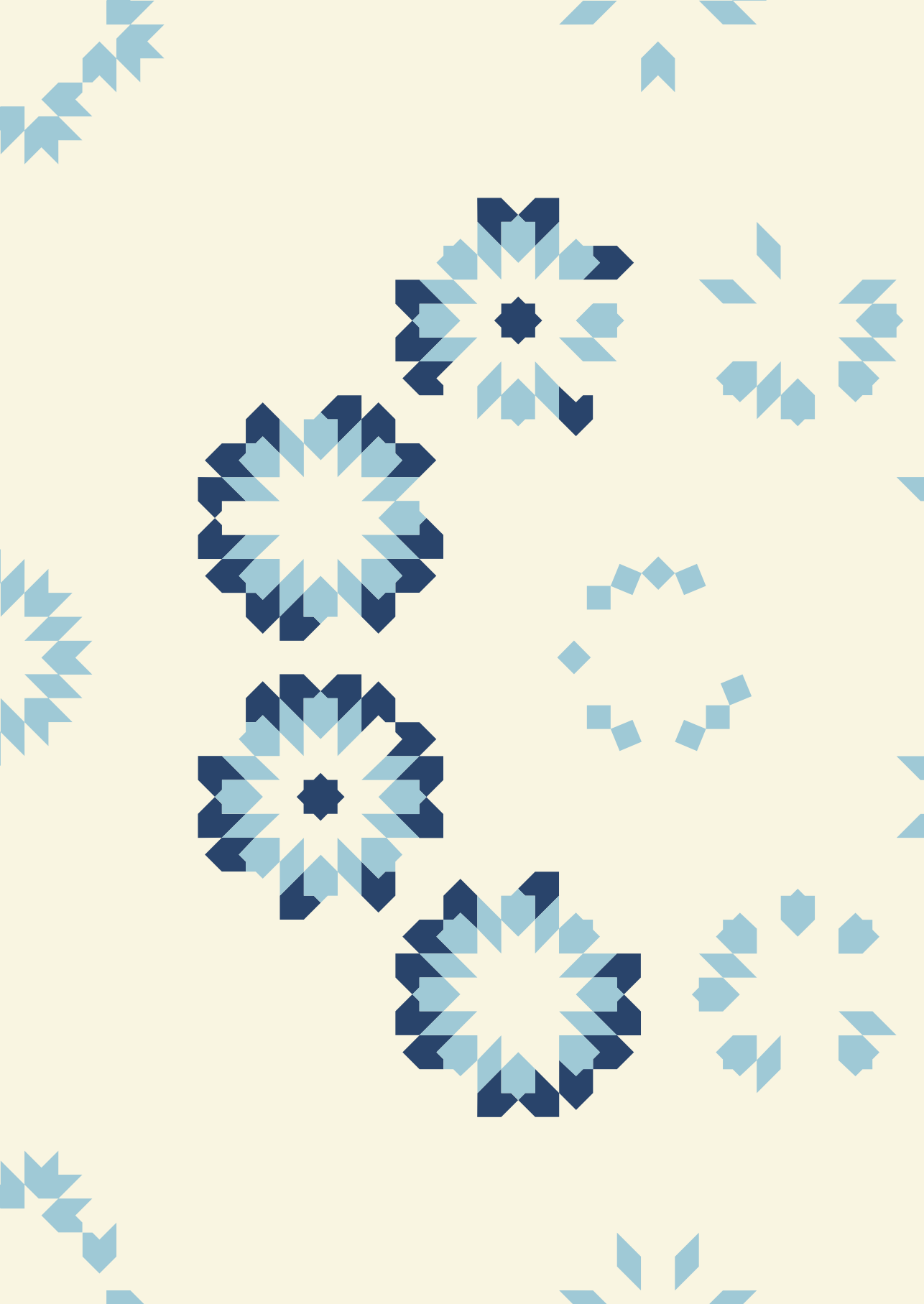
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PART I

EXPANDING THE INSIGHT
INTO THE BIOLOGY OF
SARCOMAS

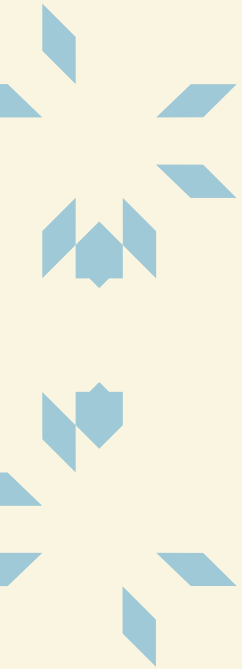


CHAPTER 2

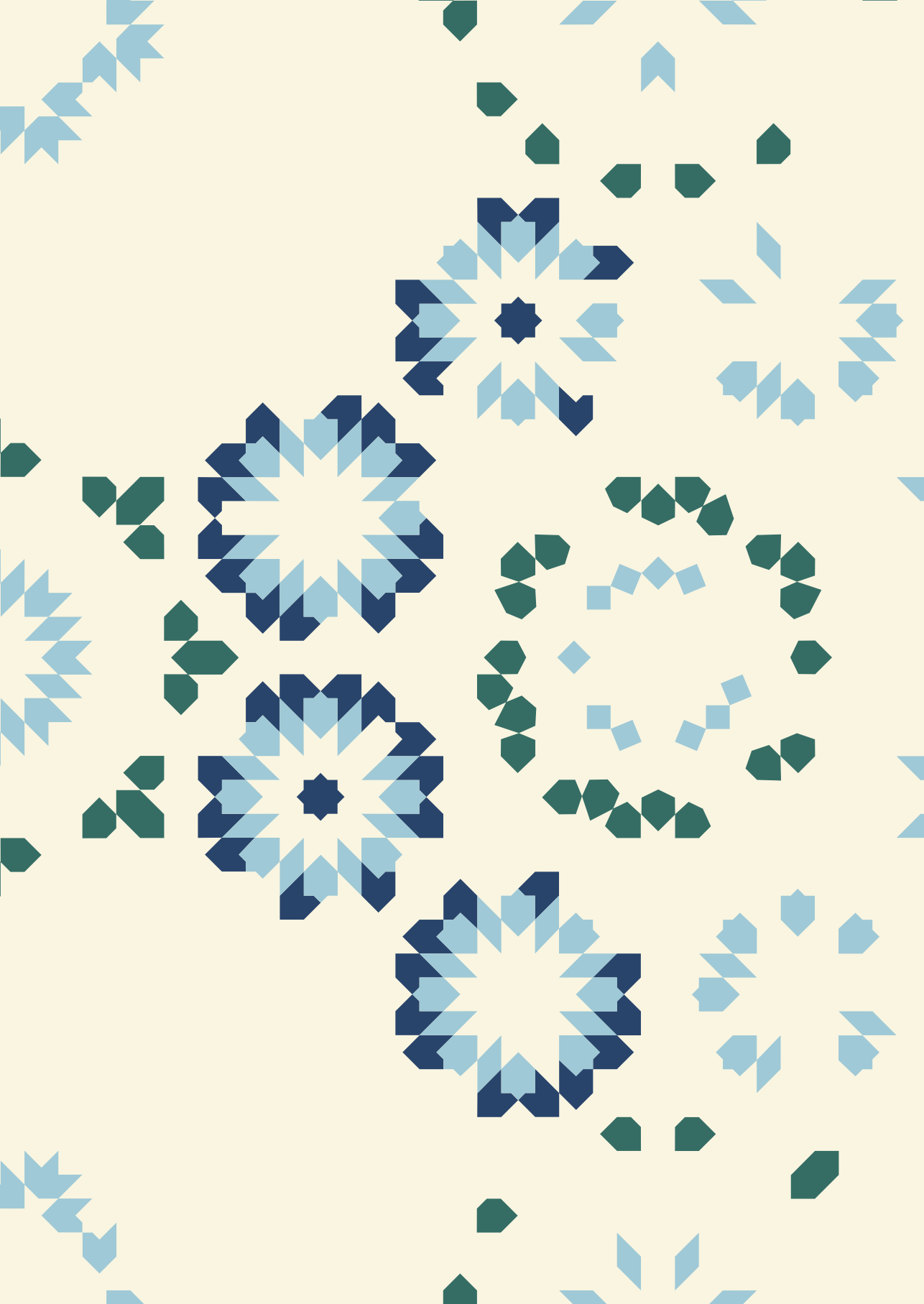
GENOMIC LANDSCAPE OF METASTATIC SOFT TISSUE SARCOMA REVEALS NEW POTENTIAL ACTIONABLE TARGETS

M. Vos, H.J.G. van de Werken, J. van Riet, N. Steeghs,
M.P.J.K. Lolkema, I.M.E. Desar, J.J. de Haan, H. Gelderblom,
J.W.M. Nin, E. Cuppen, S. Sleijfer, E.A.C. Wiemer

Manuscript in preparation.



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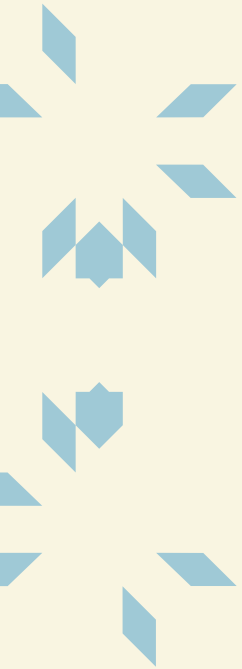


CHAPTER 3

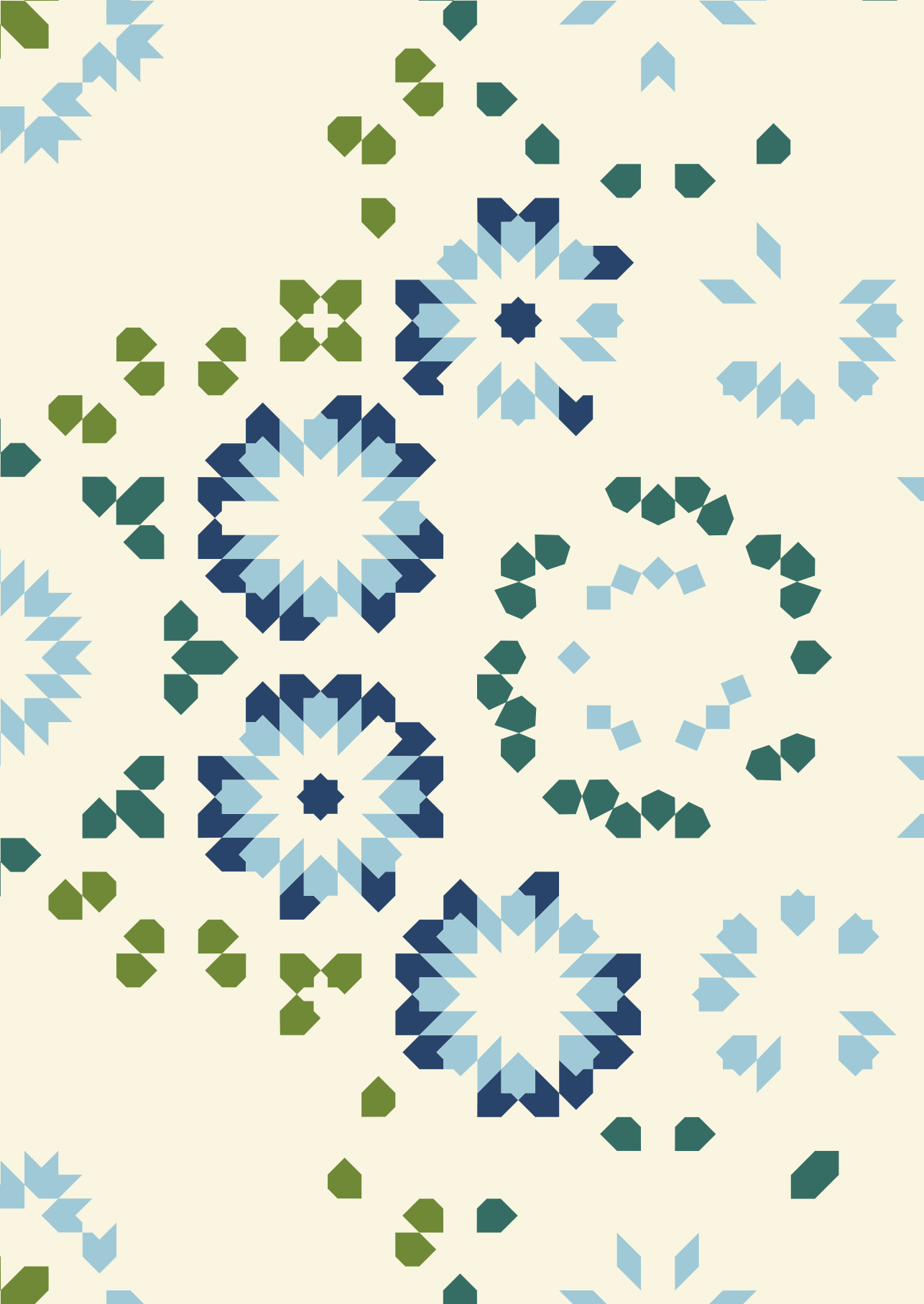
OVEREXPRESSION OF MIR-26A AND MIR-3913 IN WELL-DIFFERENTIATED AND DEDIFFERENTIATED LIPOSARCOMA AND ITS FUNCTIONAL CONSEQUENCES

M. Vos, A.L.M. Vriends, P.F. van Kuijk, A. Sacchetti,
D.J. Grünhagen, C. Verhoef, S. Sleijfer, E.A.C. Wiemer

Manuscript in preparation.



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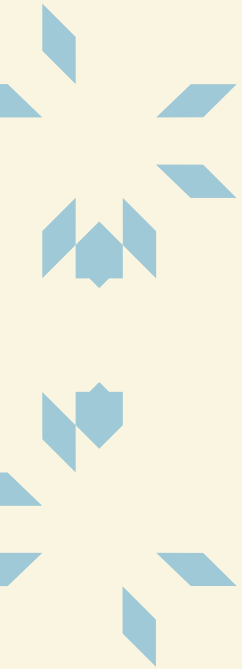


CHAPTER 4

MICRORNA EXPRESSION AND DNA METHYLATION PROFILES DO NOT DISTINGUISH BETWEEN PRIMARY AND RECURRENT WELL-DIFFERENTIATED LIPOSARCOMA

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Abstract

Approximately one-third of the patients with well-differentiated liposarcoma (WDLPS) will develop a local recurrence. Not much is known about the molecular relationship between the primary tumor and the recurrent tumor, which is important to reveal potential drivers of recurrence. Here we investigated the biology of recurrent WDLPS by comparing paired primary and recurrent WDLPS using microRNA profiling and genome-wide DNA methylation analyses. In total, 27 paired primary and recurrent WDLPS formalin-fixed and paraffin-embedded tumor samples were collected. MicroRNA expression profiles were determined using TaqMan® Low Density Array (TLDA) cards. Genome-wide DNA methylation and differentially methylated regions (DMRs) were assessed by methylated DNA sequencing (MeD-seq). A supervised cluster analysis based on differentially expressed microRNAs between paired primary and recurrent WDLPS did not reveal a clear cluster pattern separating the primary from the recurrent tumors. The clustering was also not based on tumor localization, time to recurrence, age or status of the resection margins. Changes in DNA methylation between primary and recurrent tumors were extremely variable, and no consistent DNA methylation changes were found. As a result, a supervised clustering analysis based on DMRs between primary and recurrent tumors did not show a distinct cluster pattern based on any of the features. Subgroup analysis for tumors localized in the extremity or the retroperitoneum also did not yield a clear distinction between primary and recurrent WDLPS samples. In conclusion, microRNA expression profiles and DNA methylation profiles do not distinguish between primary and recurrent WDLPS and no putative common drivers could be identified.

Introduction

Soft tissue sarcomas form a heterogeneous group of rare, mesenchymal tumors, of which liposarcomas comprise one of the largest subgroups [1]. Of all 100-120 patients diagnosed annually with liposarcoma in the Netherlands [2], the most common subtype is well-differentiated liposarcoma (WDLPS). WDLPS are mostly localized in the extremities and the retroperitoneum, and the prognosis of these patients is significantly better than those of patients with dedifferentiated liposarcoma [2]. However, WDLPS have a risk of dedifferentiation, potentially leading to metastatic disease with concurrent dismal prognosis. The rate of dedifferentiation in WDLPS in the extremities is extremely low, while in the retroperitoneum the risk of dedifferentiation is higher [1]. Molecularly, WDLPS are characterized by amplification – on a neochromosome – of the 12q14-15 region, which includes the genes *MDM2* and *CDK4* [1]. Treatment of WDLPS consists of complete surgical resection of the tumor, occasionally combined with neoadjuvant/adjuvant radiotherapy for tumors localized in the retroperitoneum. Unfortunately, approximately one-third of the patients will develop a local recurrence. Whereas the biology and behavior of primary WDLPS has been widely studied, there is a lack of insight in changes in microRNA expression and DNA methylation profiles between primary and recurrent WDLPS.

MicroRNAs have been proven to play a significant role in tumorigenesis [3-5], including in soft tissue sarcomas and more specifically liposarcomas [6-11]. So far, microRNA expression profiles have been used to differentiate between different liposarcoma subtypes [6-9, 12, 13] or to predict patient outcome [10, 11, 14, 15]. However, it is unclear whether primary WDLPS and their recurrent tumors can be distinguished by their microRNA profiles, which would suggest that microRNAs may be involved in the process of recurrence.

DNA methylation is an epigenetic process that fulfils an essential role in physiological and biological processes [16], and can be an important pathological driver in cancer [17, 18]. DNA methylation patterns can be utilized as biomarker [19, 20], to classify cancer (sub)types [21, 22] or to predict outcome [20, 23]. Genome-wide DNA methylation analysis used to be technically challenging and costly, but recently a new method was developed showing accurate genome-wide analysis of CpG-methylation by using the DNA methylation-dependent restriction enzyme *LpnPI* and subsequent DNA sequencing of the restriction fragments [24]. This methylated DNA sequencing (MeD-seq) technology is cost-effective, accurate and reproducible with high coverage, suitable for high-throughput epigenetic profiling, even on FFPE material. For liposarcoma in general and recurrent WDLPS specifically, the knowledge of epigenetics is limited. Only a few studies report on the role of DNA methylation in liposarcomas, but mostly focus on one specific DNA region in more aggressive liposarcomas subtypes [25, 26]. Some studies report a link between DNA methylation and microRNAs, for example methylation-

induced silencing of miR-193b in dedifferentiated liposarcoma but not in WDLPS [27] and low expression of miR-193b, due to downregulation by promoter methylation, resulting at least partly from an increased expression of DNA methyltransferase-1 [28].

In this study, we molecularly compared primary and recurrent WDLPS at microRNA and DNA methylation level aiming to discover differences and/or similarities that give insight in the biology of recurrent WDLPS.

Materials and methods

Patients and samples

Patients with available tumor samples of a primary and matching first recurrent WDLPS who were treated with surgery only were included. The formalin-fixed and paraffin-embedded (FFPE) tissue blocks were obtained through PALGA, the Dutch nationwide pathology registry, and the pathology department of the Maria Skłodowska-Curie Institute-Oncology Center together with anonymized clinicopathological information. The resection margins were defined as R0 (microscopically negative margins), R1 (microscopically positive margins), R2 (macroscopically positive margins) or Rx (unknown/not assessed). Although recurrence after R1/R2 resections can be considered as progressed WDLPS rather than truly recurrent WDLPS, these will be referred to as recurrent WDLPS as well. To calculate time to recurrence, the resection dates stated in the pathology reports were used. Each pair received an individual number with index numbers designating the primary tumor (.1) or recurrent tumor (.2).

The experimental protocol was reviewed and approved by the Medical Ethics Committee of the Erasmus MC (MEC-2016-213). All experimental procedures were performed in accordance with the relevant guidelines and regulations, including the Helsinki Declaration. The use of anonymous or coded left-over material for scientific purposes is part of the standard treatment agreement with patients and therefore additional informed consent was not asked.

RNA and DNA isolation

The archival tumor samples were examined by an expert pathologist to confirm the initial histopathological diagnosis and to determine the percentage of tumor cells. The diagnosis of WDLPS was based either on the presence of lipomatous cells with fibrous septa and spindle cells with hyperchromatic irregular nuclei, or on the amplification of the *MDM2* gene using FISH in case morphological atypia was less conspicuous. Only sections containing approximately 100% tumor cells were used for isolation. Total RNA was isolated using the RecoverAll™ Total Nucleic Acid Isolation Kit (Ambion/Life Technologies) and total DNA was isolated using the AllPrep® DNA/RNA FFPE kit (Qiagen), both according to manufacturer's instructions.

MicroRNA expression profiling

MicroRNA expression was determined using TaqMan® Low Density Array (TLDA) cards (A card v2.0, B card v3.0, Applied Biosystems/Thermo Fisher Scientific). Megaplex™ RT Primers (Human Pool, pool A v2.1, pool B v3.0, Applied Biosystems/Thermo Fisher Scientific) were used for cDNA synthesis, followed by a standard pre-amplification protocol using Megaplex™ PreAmp Primers (Human Pool, pool A v2.1, pool B v3.0, Applied Biosystems/Thermo Fisher Scientific). The TLDA cards were analyzed using a 7900HT Real-Time PCR system (Applied Biosystems). The paired samples were processed in three batches for logistical and technical reasons, with each primary and its matching recurrent tumor being placed within the same batch.

Statistical analysis of microRNA profiling data

The expression of each microRNA in a sample was normalized to the median Ct-value of all detectable microRNAs in that sample. The normalized relative expression was subsequently calculated for each microRNA and log-transformed. Since the samples were processed in multiple batches, potential batch-effects were investigated using PCA-plots in R (S1 Fig). To correct for the observed batch-effects, ComBat was used [29]. Only microRNAs detected in at least 50% of the samples were included in the statistical analyses. A paired t-test was performed to identify microRNAs that were differentially expressed between paired primary and recurrent WDLPS samples. A two-sided p-value <0.05 was considered statistically significant. To adjust for multiple testing, a false discovery rate (FDR) of 0.25 was used. For all microRNA clustering analyses, the software program Cluster 3.0 was used followed by Java TreeView for visualization of the clustering results. The microRNA expression datasets generated and analyzed during the current study have been deposited to the Gene Expression Omnibus (GEO) data repository under submission number GSE137722.

MeD-seq sample preparations

MeD-seq analyses were essentially carried out as previously described [24]. DNA samples were digested by *LpnPI* (New England Biolabs). Stem-loop adapters were blunt-end ligated to repaired input DNA and amplified to include dual indexed barcodes using a high fidelity polymerase to generate an indexed Illumina NGS library. The amplified end product was purified on a Pippin HT system with 3% agarose gel cassettes (Sage Science). Multiplexed samples were sequenced on Illumina HiSeq2500 systems for single reads of 50bp according to manufacturer's instructions. Dual indexed samples were demultiplexed using bcl2fastq software (Illumina).

MeD-seq data analysis

Data processing was carried out using specifically created scripts in Python. Raw fastq files were subjected to Illumina adaptor trimming and reads were filtered based on *LpnPI* restriction site occurrence between 13-17bp from either 5' or 3' end of the read and mapped to hg38 using bowtie2. Genome-wide individual *LpnPI* site scores were used to generate read count scores for the following annotated regions (www.ensembl.org): transcription start sites (TSS, 1 kb before and 1 kb after), CpG-islands and gene bodies (1kb after TSS till TES). Detection of differentially methylated regions (DMRs) was performed between two datasets using the χ^2 -test on read counts. Significance was called by either Bonferroni or FDR using the Benjamini-Hochberg procedure.

In addition, a genome-wide sliding window was used to detect sequentially differentially methylated *LpnPI* sites. Statistical significance was called between *LpnPI* sites in predetermined groups using the χ^2 -test. Neighboring significantly called *LpnPI* sites were binned and reported. Annotation of the overlap was reported for TSS, CpG-islands and gene body regions. DMR thresholds were based on *LpnPI* site count, DMR sizes (in bp) and fold changes of read counts as mentioned in the figure legends before performing hierarchical clustering. The differentially methylated datasets generated and analyzed during the current study have been deposited to the Sequence Read Archive (SRA) under submission number PRJNA574561.

Results

Patient samples

In total 27 pairs of patient samples were collected: 16 from the Erasmus MC Cancer Institute, 9 from the Netherlands Cancer Institute, and 2 from the Maria Skłodowska-Curie Institute-Oncology Center. The extremity was the most common localization (N = 15), followed by the retroperitoneum (N = 8). Fourteen patients were female, 13 patients were male. The median age at time of diagnosis of the primary tumor was 59 years (interquartile range [IQR] 50–64) and the median time to recurrence was 3.7 years (IQR 1.9–6.5). In a number of patients (N = 8, 29.6%), the status of the resection margins of the primary tumor was unknown, not assessed or not specified (Rx) in the pathology report. Of those patients of whom the status of the resection margins was reported, all primary resections were R0 or R1 resections, except for one patient (no. 17) with tumor localization in the esophagus, who underwent a R2 resection. Resections of the recurrent tumors resulted in 4 patients in R2 resections (Table 1).

Table 1. Patient and tumor characteristics.

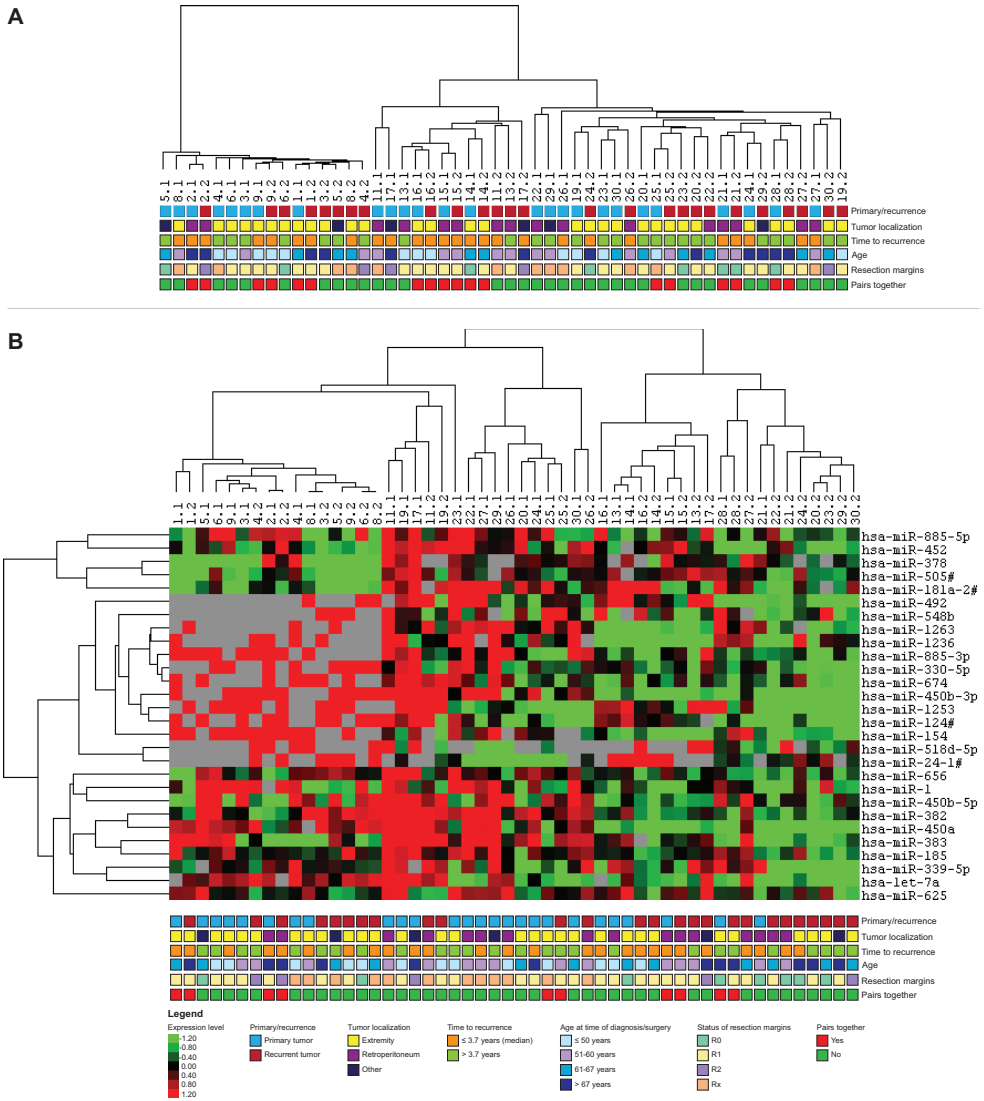
Sample	Age [†]	Sex	Localization	Resection margins	Time to recurrence [†]	No. of DMRs
1.1	64	Female	Upper leg	R1	3.7	32,854
1.2	68			R1		
2.1	78	Male	Retroperitoneal	R1	1.9	2,430
2.2	79			R2		
3.1	58	Female	Upper leg	R1	10.6	4,410
3.2	69			R1		
4.1	50	Male	Upper leg	Rx	8.3	1,061
4.2	59			R2		
5.1	62	Male	Axilla	R0	5.3	1,191
5.2	67			Rx		
6.1	31	Female	Upper leg	R1	8.5	2,732
6.2	39			R0		
8.1	60	Male	Lower leg	Rx	1.0	724
8.2	61			Rx		
9.1	38	Female	Upper leg	R1	2.1	675
9.2	40			R1		
10.1	68	Female	Mediastinum	R0	1.3	1,747
10.2	69			R1		
11.1	52	Female	Retroperitoneal	Rx	2.6	1,028
11.2	54			Rx		
13.1	50	Female	Retroperitoneal	R1	8.1	3,659
13.2	58			R1		
14.1	64	Male	Upper leg	R0	0.6	636
14.2	64			R1		
15.1	55	Female	Retroperitoneal	R1	2.0	1,920
15.2	57			R1		
16.1	48	Male	Lower leg	R1	0.4	473
16.2	48			R1		
17.1	70	Male	Esophagus	R2	0.1	586
17.2	70			R2		
19.1	43	Male	Upper leg	R1	4.7	7,644
19.2	48			R1		
20.1	64	Male	Upper leg	R1	6.5	21,585
20.2	70			R1		

Sample	Age [†]	Sex	Localization	Resection margins	Time to recurrence [‡]	No. of DMRs
21.1	52	Male	Retroperitoneal	R0	3.5	1,481
21.2	56			R0		
22.1	59	Female	Retroperitoneal	Rx	4.2	314
22.2	63			R1		
23.1	47	Male	Upper leg	R1	16.6	1,119
23.2	63			R0		
24.1	76	Female	Upper leg	R1	3.0	372
24.2	79			R0		
25.1	49	Female	Upper leg	Rx	3.9	482
25.2	53			R1		
26.1	50	Female	Retroperitoneal	Rx	2.1	2,513
26.2	53			Rx		
27.1	60	Male	Retroperitoneal	Rx	1.5	1,377
27.2	61			R1		
28.1	71	Female	Upper leg	R0	6.1	1,910
28.2	77			R1		
29.1	60	Male	Trunk	Rx	13.8	2,819
29.2	74			R1		
30.1	61	Female	Upper leg	R1	4.6	294
30.2	66			R2		

DMR, differentially methylated region. [†]Age at time of surgery. [‡]in years.

MicroRNA profiling of paired primary-recurrent WDLPS samples

After correction for batch effect, samples 10.1 and 10.2 were excluded from further microRNA analyses (S1 Fig). First, an unsupervised hierarchical clustering analysis was performed to group the samples based on their microRNA expression profiles without prior knowledge of the origin of the sample (primary or recurrent). This clustering did not show a clear distinction between primary and recurrent WDLPS samples, neither a discriminative pattern based on tumor localization, time to recurrence, age nor the status of the resection margins could be observed (Fig 1A). In 9 of the 26 pairs, the primary and recurrent tumor samples clustered together (indicated by the red squares in the bottom row of the figure). All of these pairs had a short time to recurrence (before the median time to recurrence of 3.7 years), except one pair with a time to recurrence of 3.9 years and one pair with a time to recurrence of 6.1 years.



▲ Fig 1. Hierarchical clustering based on the microRNA expression levels of 26 paired primary and recurrent WDLPS tumor samples.

(A) Results of an unsupervised clustering analysis, depicted with time to recurrence, tumor localization, age and the status of the resection margins. Tumor pairs that cluster together in the same branch of the cluster tree are indicated with red boxes in the bottom line of the figure. (B) Results of a supervised clustering analysis based on the expression of 28 significant differentially expressed microRNAs ($p < 0.05$, $FDR < 0.25$), together with time to recurrence, tumor localization, age, the status of the resection margins and an indication of primary-recurrent pairs that cluster together. Grey designates missing expression values.

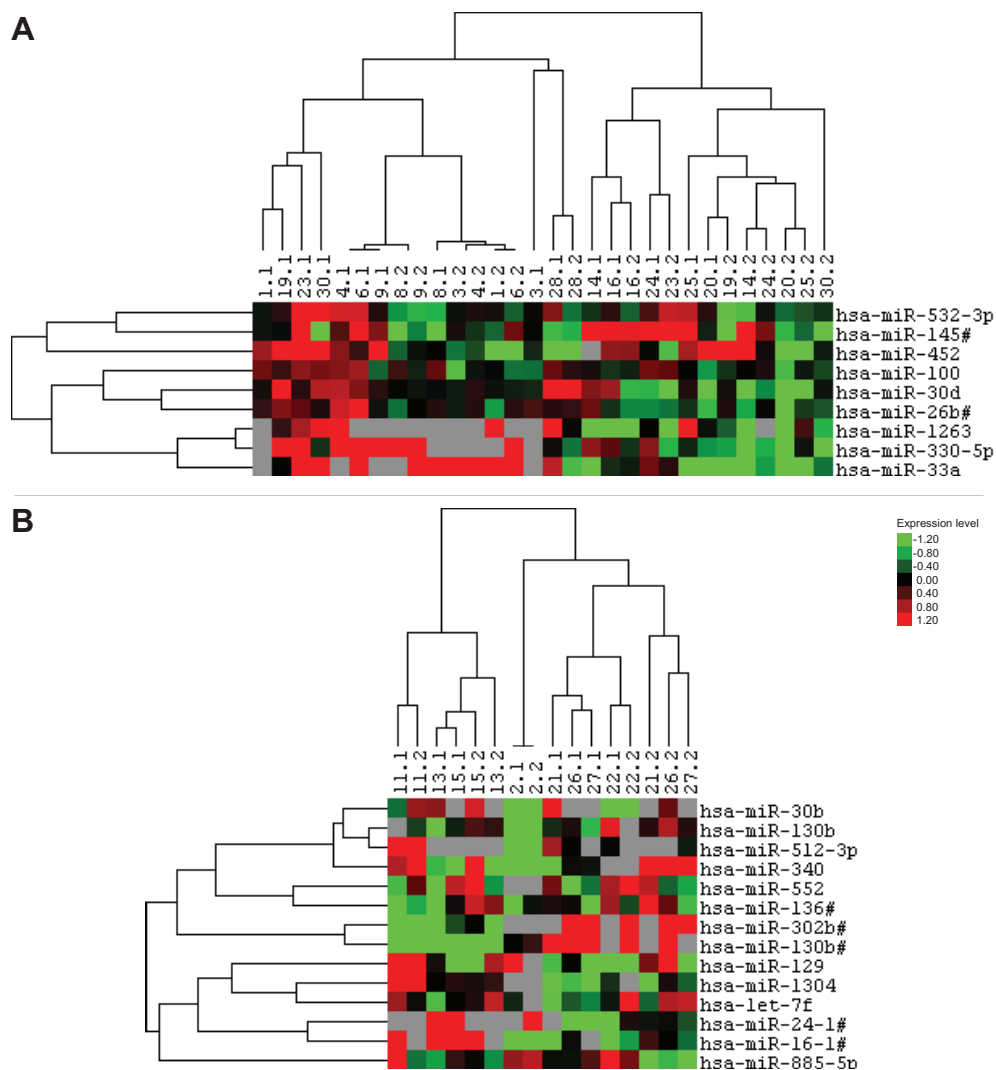
Next, a supervised analysis was performed based on the expression levels of the 28 significant differentially expressed microRNAs ($p < 0.05$, $FDR < 0.25$) (Fig 1B, S1 Table). The heat map indicated no clear discriminative pattern between primary and recurrent WDLPS, nor a distinction based on tumor localization, time to recurrence, age or the status of the resection margins. Five pairs clustered together, but clustering of these pairs also did not seem to be driven by one of the clinicopathological parameters.

Since microRNA expression is reported to be (partially) tissue specific [30], it may be influenced by the localization of the tumor. Therefore, additional sub-analyses for the two largest subgroups regarding tumor localization were performed: the extremity (N = 15 pairs, Fig 2A) and the retroperitoneum (N = 8 pairs, Fig 2B). For the tumor samples localized in the extremity, 68 microRNAs were significantly differentially expressed between primary and recurrent WDLPS of which 9 had an $FDR < 0.25$ (Fig 2A, S2 Table). A cluster analysis based on the expression of these microRNAs did not seem to depend on primary/recurrence, time to recurrence, age or status of the resection margins. For the retroperitoneal WDLPS, only 14 microRNAs were significantly differentially expressed, of which none had an $FDR < 0.25$ (S2 Table). Therefore, the microRNAs with $p < 0.05$ without FDR correction were used to generate a heat map for this subgroup (Fig 2B). Again, no distinction between primary and recurrent samples was observed.

DNA methylation patterns of paired primary and recurrent WDLPS samples

When comparing differentially methylated DNA regions (DMRs) between individual primary and recurrent WDLPS pairs, it was noted that the DNA methylation differences were extremely variable between pairs (Table 1), although most of the pairs with a short time to recurrence (before median time to recurrence of 3.7 years) tended to have a lower number of DMRs. However, samples with a longer time to recurrence, for example sample pairs 23 and 28, also displayed relative low numbers of DMRs, and sample pair 1, which had a short time to recurrence, exhibited the largest number of DMRs (Table 1). These DNA methylation differences seemed to be inconsistent among the individual pairs and could not be identified when comparing primary tumors versus recurrent tumors as a group. In the total group, only a relatively small number of 470 DMRs were identified, located on various chromosomes (S3 Table). When these DMRs were used for a supervised hierarchical clustering analysis, no clear clustering of the 27 primary and recurrent samples was observed (Fig 3). Likewise, no distinction was detected based on the clinicopathological parameters (Fig 3). Five of the pairs clustered together, but again across these samples no similarities in terms of time to recurrence, localization, or the status of the resection margins could be identified.

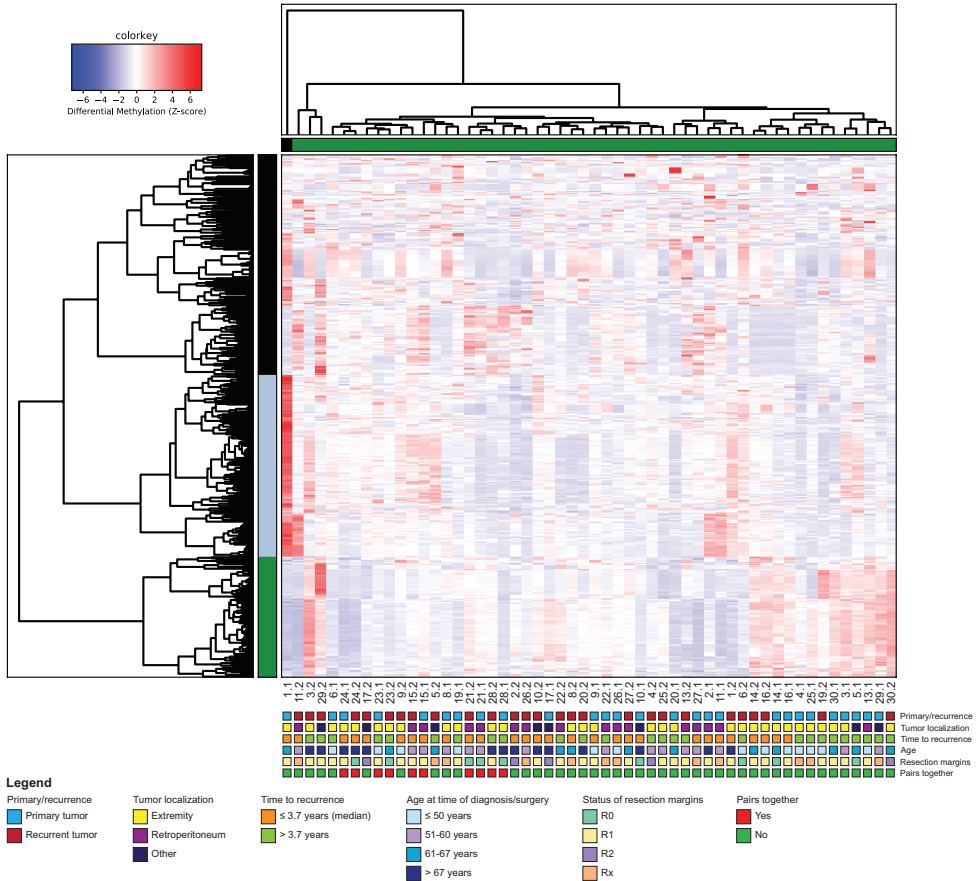
A relatively high number of the observed 470 DMRs was located at chromosome 12 (S3



▲ Fig 2. Hierarchical clustering based on the microRNA expression levels of paired primary and recurrent WDLPS tumor samples of the two main tumor localizations. Grey designates missing expression values. **(A)** Results of a supervised clustering analysis based on nine differentially expressed microRNAs ($p < 0.05$, $FDR < 0.25$; $N = 15$ pairs) between primary and recurrent WDLPS of the extremity. **(B)** Results of a supervised clustering analysis based on 14 differentially expressed microRNAs ($p < 0.05$; $N = 8$ pairs) between primary and recurrent WDLPS of the retroperitoneum.

Table), including DMRs linked to the genes *MDM2*, *CDK4* and *MIR26A* (S4 Table). These DMRs might indicate a possible difference in methylation of (regions of) chromosome 12 between primary and recurrent WDLPS, albeit the fold changes between the groups are relatively low (S4 Table). The highest fold change observed was 2.03 for the gene *RP11-611E13.2*, a

relatively unknown gene located on chr12q15, the same region as *MDM2*, encoding a non-coding RNA. For *MDM2*, which is amplified in WDLPS, eight DMRs were found, with a fold change of 1.29 for the highest DMR.



▲ Fig 3. Hierarchical clustering based on differentially methylated DNA regions (DMRs) between primary and recurrent WDLPS samples. The heat map depicts a supervised clustering of the 27 paired WDLPS samples based on 455 differentially methylated regions (DMRs), excluding sex chromosomal regions (N = 15 DMRs), together with the clinicopathological features time to recurrence, tumor localization, age and the status of the resection margins.

Since DNA methylation patterns are also tissue-specific [24, 31, 32] and may be affected by tumor localization, subgroup analyses for the two main localizations were performed: the extremity (N = 15 pairs) and the retroperitoneum (N = 8 pairs). For the tumor samples located in the extremity, 631 DMRs were identified between primary and recurrent samples. Also here, no clear clustering pattern could be identified based on primary/recurrent WDLPS, time to recurrence or the status of the resection margins (Fig 4A). For the tumor samples

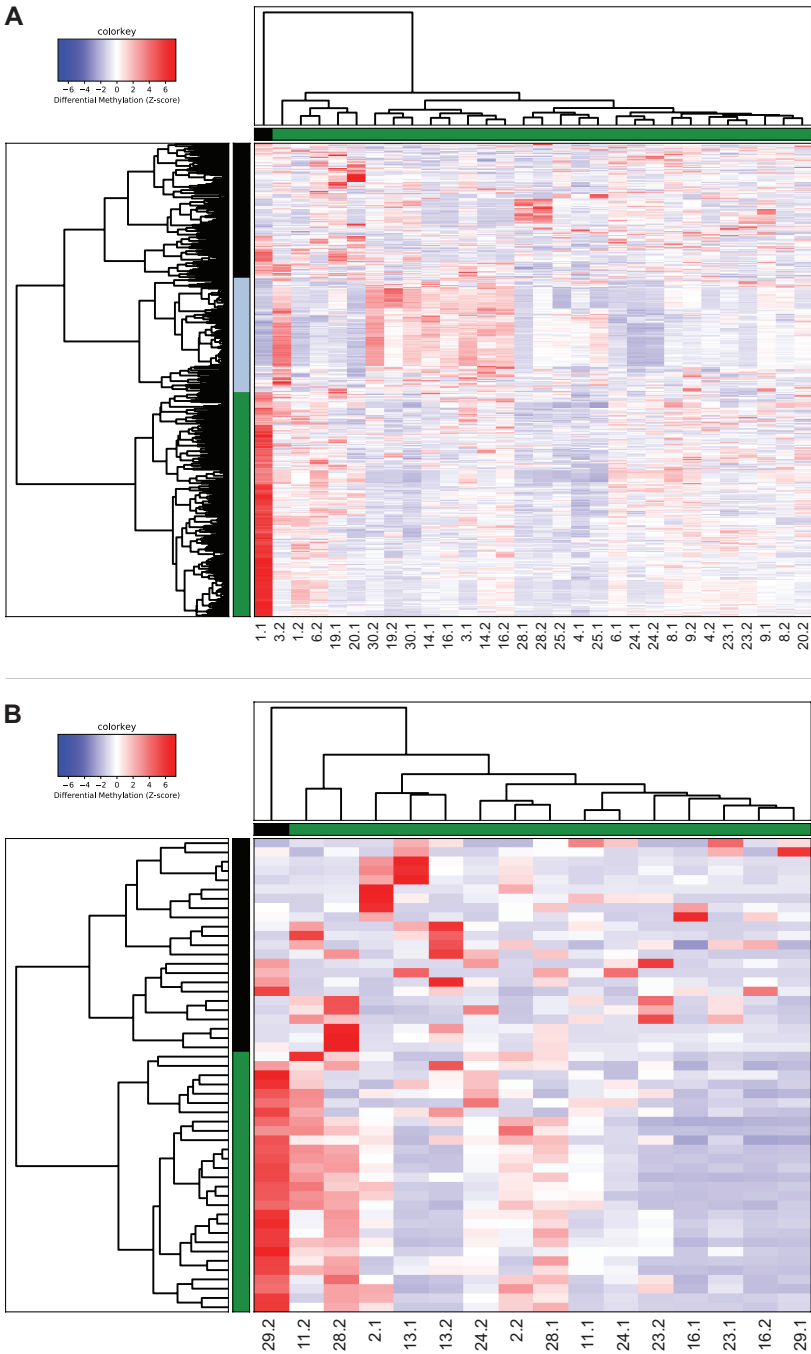
localized in the retroperitoneum, 1,071 DMRs were identified. To prevent the clustering from being blurred by background noise due to the higher number of DMRs, the clustering analysis for the retroperitoneal tumors was based on the DMRs with a fold change >2 ($N = 53$ DMRs). Again, this did not lead to a clear distinction between primary and recurrent WDLPS samples (Fig 4B).

Discussion

To the best of our knowledge, this is the first paper comparing paired primary WDLPS samples to recurrent WDLPS samples at a molecular level. We aimed to gain more insight into the biology of (recurrent) WDLPS and thereby the process of recurrence. The finding that no clear distinction could be made between primary and recurrent WDLPS based on differentially expressed microRNAs or differentially methylated DNA regions suggests that there are no common alterations or that the alterations in microRNA expression and DNA methylation are very heterogeneous and variable between individual patients.

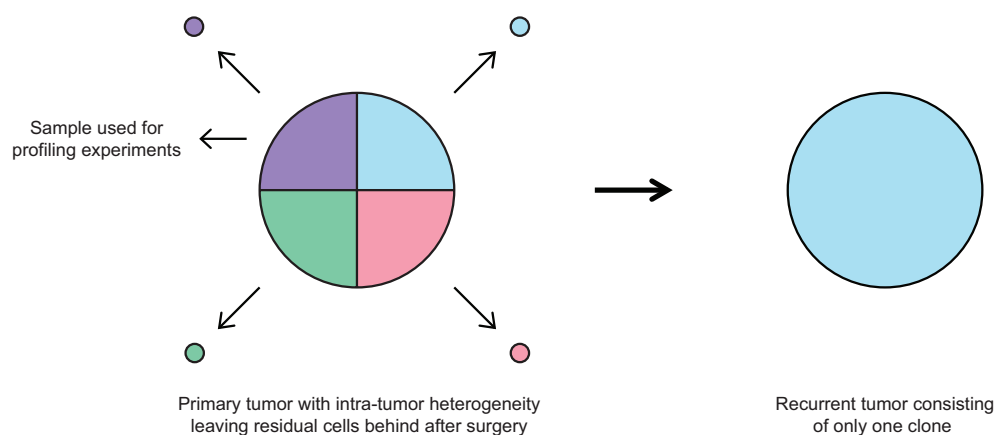
In the unsupervised microRNA clustering analysis, 7 of the 13 pairs (54%) with a short time to recurrence (before median time to recurrence) clustered together, compared to 2 of the 13 pairs (15%) with a longer time to recurrence. This might point towards a recurrence through the outgrowth of a residue in these patients, rather than a recurrence that originates from a single tumor cell. Alternatively, it might suggest that early recurrent tumors resemble each other more closely than late recurrent tumors, because they have had less time to change.

Of the 28 differentially expressed microRNAs, miR-1263 was the most significant differentially expressed microRNA, a relatively unknown microRNA whose role in cancer has not been established yet, followed by miR-885-5p. Upregulation of this microRNA has been linked to enhanced proliferation and migration [33], and the development of liver and lung metastases in colorectal cancer [34]. In contrast, miR-885-5p suppressed proliferation, migration and invasion *in vitro* in osteosarcoma cells, and was downregulated in osteosarcoma patients with low expression levels being associated with a poor prognosis [35]. In our study, miR-885-5p was downregulated in the recurrent tumors, possibly matching the findings in osteosarcoma with low levels of miR-885-5p being associated with more proliferation and a poorer prognosis. Lastly, in our comparison of primary and recurrent WDLPS we did not detect differential expression of the microRNAs that were previously found to be important for sarcomagenesis in WDLPS, such as miR-628 [6], miR-675 [6], miR-26a [8], miR-451 [8] or miR-193b [28]. However, these microRNAs were all discovered in comparisons with 'normal' fat tissue.



▲Fig 4. Hierarchical clustering based on differentially methylated DNA regions (DMRs) between paired WDLPS tumor samples for the two main localizations. (A) Results of the hierarchical supervised clustering, excluding sex chromosomal regions (N = 27), based on 604 DMRs of the 15 paired WDLPS samples localized in the extremity. **(B)** Results of the hierarchical supervised clustering analysis based on the 51 DMRs with a fold change ≥ 2 , excluding sex chromosomal regions (N = 2), of the 8 paired retroperitoneal WDLPS samples.

Remarkably, only 470 DMRs with relatively low fold changes were identified between primary and recurrent WDLPS, which is a relatively small number considering the thousands of potential DNA methylation sites in the genome. Possibly, this can be explained by the low-grade nature of this tumor type [1]. Furthermore, there was large variability in the number of DMRs between the pairs, ranging from 294 to 32,854 DMRs. Given our extensive efforts to compose a homogenous dataset by selecting only WDLPS without any neoadjuvant/adjuvant treatment and using only sections almost entirely consisting of tumor tissue, it seems that the inter-tumor heterogeneity is abundant. This heterogeneity – in DNA methylation as well as in microRNA expression – could also be due to intra-tumor heterogeneity, such as exists in other cancers. The concept of intra-tumor heterogeneity describes the observation that a tumor may exist of different tumor cells with distinct molecular and genomic profiles. If the used primary tumor sample was taken of one part of the tumor, but the recurrence mainly consists of cells from another part of the tumor or of cells that had a relatively small contribution to the primary tumor, this might explain the differences in microRNA expression profiles and DNA methylation patterns, even in case of a short time to recurrence (Fig 5). However, currently it is unknown whether such an intra-tumor heterogeneity is present in WDLPS.



▲Fig 5. Schematic overview of the concept of intra-tumor heterogeneity in the context of the current study. If the primary tumor sample that was used for the experiments mainly consists of one specific cancer cell subtype, but the recurrent tumor is a recurrence of mainly other cancer cell subtypes, this might explain the large variability in DNA methylation and microRNA expression, even in case of short time to recurrence.

A relatively high number of DMRs occurred in chromosome 12, including DMRs linked to *MDM2*, suggesting that hypermethylation of chromosome 12 plays a role in recurrence. However, with the MeD-seq method one cannot reliably discriminate copy-number variations from actual differences in DNA methylation. Since WDLPS is characterized by amplification of a specific region on chromosome 12 (12q14-15) [1, 36], including *MDM2* and *CDK4* amongst others, we cannot reliably distinguish between additional amplification or actual changes in DNA methylation.

A limitation of the study was that in approximately a third of the patients the status of the resection margins of the primary surgery was not specified in the pathology report. Unfortunately, due to the retrospective nature of the study, which is inevitable when studying extremely rare diseases like WDLPS, we were not able to retrieve these. However, this percentage (29.6%) of missing resection margins is not unusual and in line with the number (24.0%) of pathology reports lacking information on the resection margins in a nationwide study on sarcoma care in the Netherlands [37]. The strengths of this study were the relatively large sample size and the use of paired samples collected from multiple centers. Both microRNAs and DNA methylation are known to vary – to a certain extent – between individuals [38, 39], and by using paired samples, we aimed to eliminate or minimize this inter-individual variability, so that only microRNAs and DMRs involved in sarcomagenesis would remain in the analyses.

The results of this study suggest that there are no common alterations on microRNA or DNA methylation level that are possibly involved as drivers in the process of recurrence. The next question is whether recurrent WDLPS has different molecular abnormalities upfront, i.e. in the primary tumor, than those who do not recur. Therefore, for a future research project we would recommend to compare primary WDLPS samples of patients who did not develop a recurrence to primary WDLPS tumor samples of patients who did develop a recurrence.

Conclusion

Primary and recurrent WDLPS cannot be distinguished based on microRNA expression profiles and DNA methylation patterns. Although no common alterations for recurrence could be revealed, a role for microRNAs and DNA methylation in the process of recurrence cannot be ruled out completely, since the aberrations contributing to recurrence might be very heterogeneous and variable between individuals. Alternatively, other molecular events may underlie WDLPS recurrence.

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Competing interests

R. Boers, J. Boers, W.F.J. van IJcken and J. Gribnau declare a conflict of interest as shareholders of Methylomics B.V. This does not alter our adherence to PLOS ONE policies on sharing data and materials. The other authors declare no conflicts of interest.

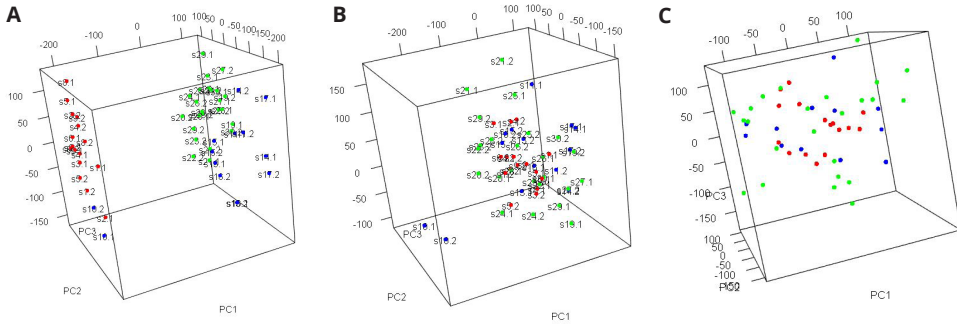


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Supporting information



▲S1 Fig. Visualization of principal component analyses (PCA) using the microRNA expression data as input. The panels depict the PCA before (A) and after (B) correction for batch effects. Based on the analyses shown in panel B, data from sample 10.1 and 10.2 were excluded from further microRNA analyses, resulting in the PCA analysis in the third panel (C).

S1 Table. Differentially expressed microRNAs. All differentially expressed microRNAs ($p < 0.05$, $FDR < 0.25$, $N = 28$ microRNAs) between primary and recurrent WDLPS of 26 paired tumor samples.

microRNA	Upregulated	in Fold change	% detection	p-value	FDR
hsa-miR-1263	Primary	1.209	73%	0.0001	0.041
hsa-miR-885-5p	Primary	1.987	100%	0.0006	0.132
hsa-miR-885-3p	Primary	4.699	87%	0.0010	0.132
hsa-miR-656	Primary	1.493	100%	0.0013	0.132
hsa-miR-450b-3p	Primary	2.205	88%	0.0014	0.132
hsa-miR-330-5p	Primary	2.167	85%	0.0016	0.132
hsa-miR-492	Primary	1.802	73%	0.0017	0.132
hsa-miR-452	Primary	2.541	98%	0.0018	0.132
hsa-miR-383	Primary	2.850	98%	0.0026	0.164
hsa-miR-548b	Recurrence	4.178	69%	0.0032	0.168
hsa-miR-382	Primary	1.842	100%	0.0033	0.168
hsa-miR-378	Primary	2.460	85%	0.0035	0.168
hsa-miR-450a	Primary	2.347	98%	0.0040	0.168
hsa-miR-1236	Primary	3.319	75%	0.0041	0.168
hsa-miR-505#	Primary	1.412	96%	0.0045	0.171
hsa-miR-181a-2#	Primary	2.224	96%	0.0060	0.216
hsa-miR-1	Primary	2.416	98%	0.0071	0.232
hsa-miR-625	Primary	1.724	100%	0.0073	0.232
hsa-miR-1253	Primary	1.135	85%	0.0079	0.240
hsa-miR-450b-5p	Primary	1.966	100%	0.0096	0.248
hsa-miR-674	Primary	4.721	83%	0.0096	0.248
hsa-miR-154	Primary	3.068	83%	0.0107	0.248
hsa-miR-185	Primary	1.659	100%	0.0108	0.248
hsa-miR-339-5p	Primary	3.076	96%	0.0109	0.248
hsa-miR-518d-5p	Recurrence	3.008	50%	0.0112	0.248
hsa-let-7a	Primary	1.695	94%	0.0114	0.248
hsa-miR-24-1#	Recurrence	79.356	62%	0.0116	0.248
hsa-miR-124#	Primary	1.521	85%	0.0121	0.249

S2 Table. Differentially expressed microRNAs in subgroup analyses of the extremity and retroperitoneum. All differentially expressed microRNAs between 15 paired primary and recurrent WDLPS tumor samples of the extremity ($p < 0.05$, FDR < 0.25 , N = 9 microRNAs) (A) and of the 8 paired primary and recurrent WDLPS tumor samples of the retroperitoneum ($p < 0.05$, no FDR, N = 14 microRNAs)(B).

(A) Extremity

microRNA	Upregulated in	Fold change	% detection	p-value	FDR
hsa-miR-532-3p	Primary	1.731	100%	0.0001	0.024
hsa-miR-1263	Primary	2.248	63%	0.0001	0.024
hsa-miR-145#	Primary	1.731	100%	0.0008	0.155
hsa-miR-452	Primary	2.609	97%	0.0015	0.221
hsa-miR-100	Primary	1.303	100%	0.0028	0.221
hsa-miR-30d	Primary	1.895	100%	0.0029	0.221
hsa-miR-26b#	Primary	1.422	100%	0.0029	0.221
hsa-miR-33a	Recurrence	1.128	83%	0.0031	0.221
hsa-miR-330-5p	Primary	2.029	80%	0.0034	0.221

(B) Retroperitoneum

microRNA	Upregulated in	Fold change	% detection	p-value	FDR
hsa-miR-30b	Recurrence	1.632	63%	0.0036	0.955
hsa-miR-130b	Recurrence	1.524	88%	0.0051	0.955
hsa-miR-512-3p	Primary	2.444	50%	0.0090	0.955
hsa-miR-340	Recurrence	5.300	88%	0.0090	0.955
hsa-miR-302b#	Recurrence	3.381	69%	0.0144	0.955
hsa-miR-552	Recurrence	1.612	88%	0.0162	0.955
hsa-miR-1304	Recurrence	1.585	81%	0.0201	0.955
hsa-miR-129	Recurrence	1.085	94%	0.0214	0.955
hsa-miR-24-1#	Recurrence	4.316	63%	0.0221	0.955
hsa-miR-130b#	Recurrence	1.476	81%	0.0235	0.955
hsa-miR-136#	Recurrence	1.808	100%	0.0378	0.955
hsa-let-7f	Recurrence	1.884	94%	0.0382	0.955
hsa-miR-885-5p	Primary	2.717	100%	0.0400	0.955
hsa-miR-16-1#	Primary	1.710	81%	0.0483	0.955

S3 Table. List of the numbers of DMRs per chromosome.

Chromosome	Count
chr1	40
chr2	11
chr3	3
chr4	50
chr5	10
chr6	11
chr7	4
chr8	3
chr9	3
chr10	40
chr11	10
chr12	68
chr13	3
chr14	1
chr15	2
chr16	12
chr17	13
chr18	5
chr19	70
chr20	4
chr21	19
chr22	3
chrX	5
chrY	10
No accurate location*	70
Total	470

*DMRs found in repetitive genomic locations that lack an accurate UCSC chromosomal reference

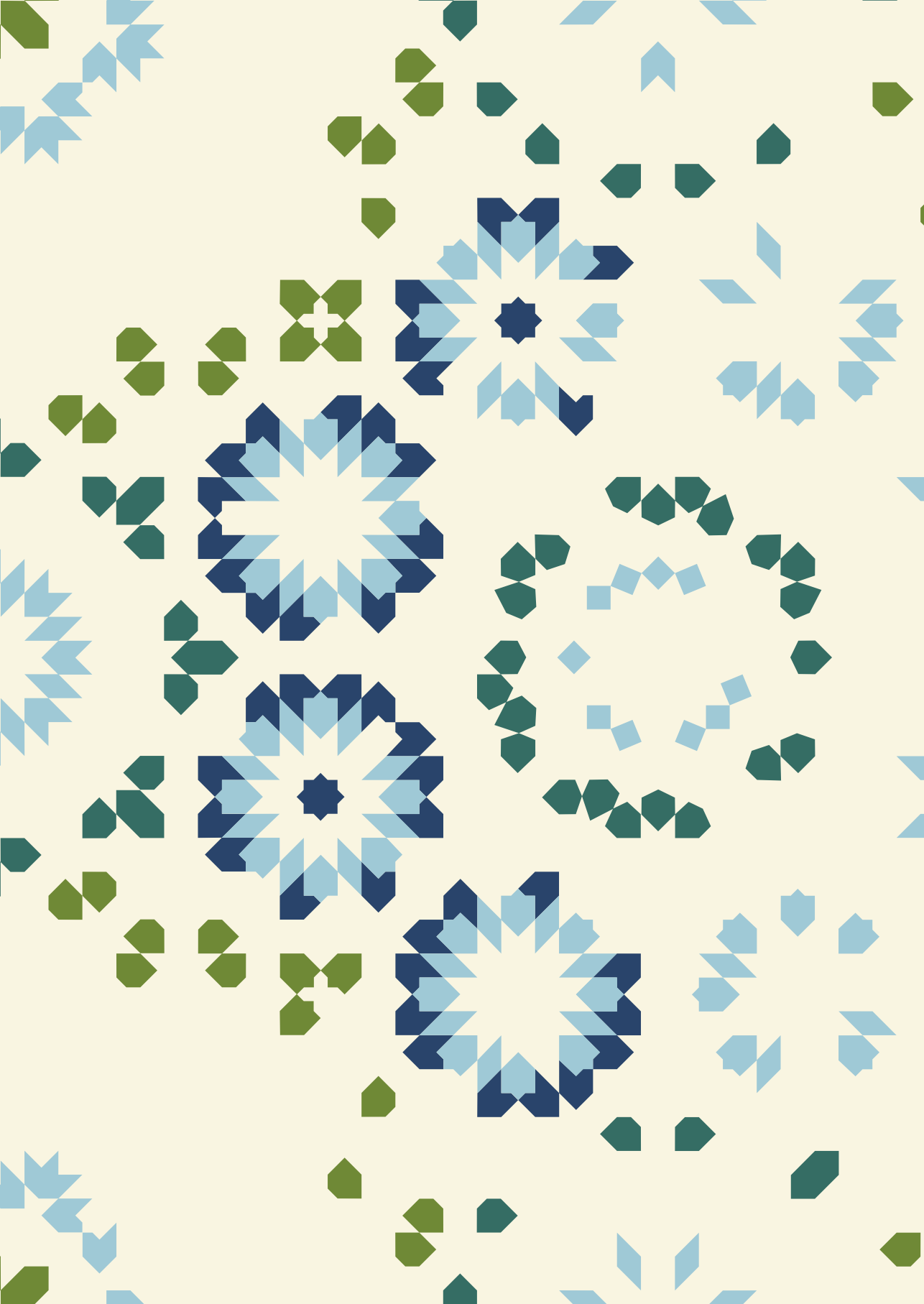
S4 Table. Top 100 genes with a DMR. Top 100 genes that contain at least one differentially methylated DNA region (DMR) after Bonferroni correction, excluding genes/DMRs located on the sex chromosomes, found by MeD-seq on 27 paired primary and recurrent WDLPS tumor samples.

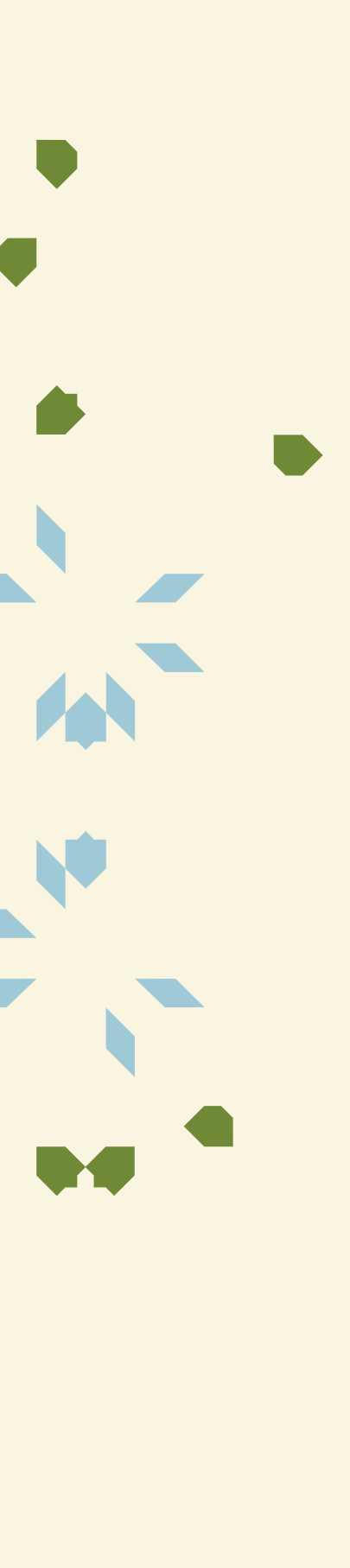
No.	Gene	No. of DMRs	Fold Change*	Hypermethylated in	Location
1	RP11-611E13.2	3	2.033	Recurrence	chr12
2	CENPIP1	1	1.661	Primary	chr13
3	FLG-AS1	3	1.590	Primary	chr1
4	HRNR	3	1.590	Primary	chr1
5	MYRFL	3	1.526	Recurrence	chr12
6	RP11-571M6.17	1	1.512	Recurrence	chr12
7	TSFM	2	1.512	Recurrence	chr12
8	AVIL	3	1.512	Recurrence	chr12
9	LYRM4	1	1.376	Recurrence	chr6
10	SLC35E3	5	1.350	Recurrence	chr12
11	OS9	2	1.298	Recurrence	chr12
12	RP11-571M6.7	3	1.298	Recurrence	chr12
13	NOC4L	1	1.293	Primary	chr12
14	MDM2	8	1.293	Recurrence	chr12
15	AC133749.1	1	1.289	Primary	chr12
16	CPM	5	1.289	Primary	chr12
17	CYP27B1	2	1.279	Recurrence	chr12
18	AL671532.6	1	1.278	Recurrence	chr14
19	AC025263.3	3	1.278	Recurrence	chr12
20	MIR26A2	2	1.276	Recurrence	chr12
21	CTDSP2	1	1.276	Recurrence	chr12
22	RP11-159A18.1	1	1.244	Recurrence	chr12
23	AC126281.1	7	1.239	Primary	chr4
24	DUX4L8	6	1.239	Primary	chr4
25	AL671532.5	1	1.239	Recurrence	chr14
26	SHC2	2	1.234	Recurrence	chr19
27	TBC1D22A	1	1.233	Recurrence	chr22
28	RP11-571M6.18	1	1.227	Recurrence	chr12
29	EXOC2	1	1.227	Primary	chr6
30	LRP8	1	1.225	Recurrence	chr1
31	LINC00854	6	1.225	Primary	chr17
32	RP3-470B24.5	1	1.215	Recurrence	chr6
33	AL671532.1	4	1.209	Recurrence	chr14
34	RNA5S9	3	1.203	Primary	chr1
35	AL713899.1	3	1.203	Primary	chr 1
36	GRTP1	1	1.192	Recurrence	chr13
37	SCNN1D	1	1.191	Primary	chr1
38	EXD3	1	1.190	Primary	chr9
39	DUX4L2	6	1.182	Primary	chr4
40	AC126281.4	3	1.182	Primary	chr4
41	AGAP2-AS1	2	1.182	Recurrence	chr12
42	AGAP2	3	1.182	Recurrence	chr12
43	AL845259.5	4	1.178	Primary	chr10

No.	Gene	No. of DMRs	Fold Change*	Hypermethylated in	Location
44	DUX4L20	2	1.178	Primary	chr10
45	TSPAN31	3	1.170	Recurrence	chr12
46	CFAP46	1	1.164	Primary	chr10
47	ABCC5	3	1.160	Recurrence	chr3
48	RAB3IP	1	1.157	Recurrence	chr12
49	TCEB3CL2	2	1.153	Recurrence	chr18
50	KATNAL2	3	1.153	Recurrence	chr18
51	AL732375.7	3	1.151	Primary	chr10
52	DIP2C	1	1.150	Primary	chr10
53	PCNT	1	1.146	Primary	chr21
54	DUX4L4	4	1.144	Primary	chr4
55	AC126281.5	3	1.144	Primary	chr4
56	CDK4	2	1.144	Recurrence	chr12
57	RNA5517	2	1.140	Primary	chr1
58	BEST3	2	1.138	Recurrence	chr12
59	NLRP4	2	1.135	Recurrence	chr19
60	TCEB3CL	3	1.133	Recurrence	chr18
61	TCEB3C	2	1.133	Recurrence	chr18
62	MIR8078	1	1.132	Recurrence	chr18
63	ROCK1P1	1	1.132	Recurrence	chr18
64	ANKRD33B	1	1.114	Recurrence	chr5
65	MARCH9	2	1.114	Primary	chr12
66	CTD-3220F14.1	7	1.113	Primary	chr19
67	METTL21B	2	1.109	Recurrence	chr12
68	RP11-571M6.15	2	1.109	Recurrence	chr12
69	RP11-49K24.9	2	1.109	Recurrence	chr18
70	HMGGA2	2	1.109	Recurrence	chr12
71	LMF1	1	1.105	Primary	chr16
72	RP11-611O2.1	1	1.102	Primary	chr12
73	SLC16A3	1	1.102	Recurrence	chr17
74	CSNK1D	1	1.102	Recurrence	chr17
75	RP13-638C3.3	2	1.101	Primary	chr17
76	FOXK2	1	1.101	Primary	chr17
77	YBEY	1	1.100	Primary	chr21
78	AL845259.7	3	1.100	Recurrence	chr10
79	LRRC10	2	1.097	Recurrence	chr12
80	EHMT1	1	1.097	Primary	chr9
81	TMTC2	1	1.092	Primary	chr12
82	TERT	1	1.092	Recurrence	chr5
83	PLEKHG4B	2	1.090	Primary	chr5
84	RP11-620J15.2	1	1.088	Primary	chr12
85	DBET	3	1.087	Primary	chr4
86	RNA5510	2	1.085	Primary	chr1
87	RNA5511	2	1.085	Primary	chr1
88	RNA5512	2	1.085	Primary	chr1
89	RNA55P19	1	1.082	Primary	chr1

No.	Gene	No. of DMRs	Fold Change*	Hypermethylated in	Location
90	RNA5SP162	1	1.082	Primary	chr1
91	DUX4L23	1	1.082	Primary	chr10
92	CTD-3162L10.1	5	1.080	Primary	chr19
93	TMEM242	2	1.078	Recurrence	chr6
94	AL671532.2	1	1.076	Recurrence	chr14
95	DLGAP2	1	1.076	Recurrence	chr8
96	CPSF6	1	1.074	Primary	chr12
97	RNA5S1	2	1.072	Primary	chr1
98	RNA5S2	2	1.072	Primary	chr1
99	RNA5S3	2	1.072	Primary	chr1
100	RNA5S4	3	1.072	Primary	chr1

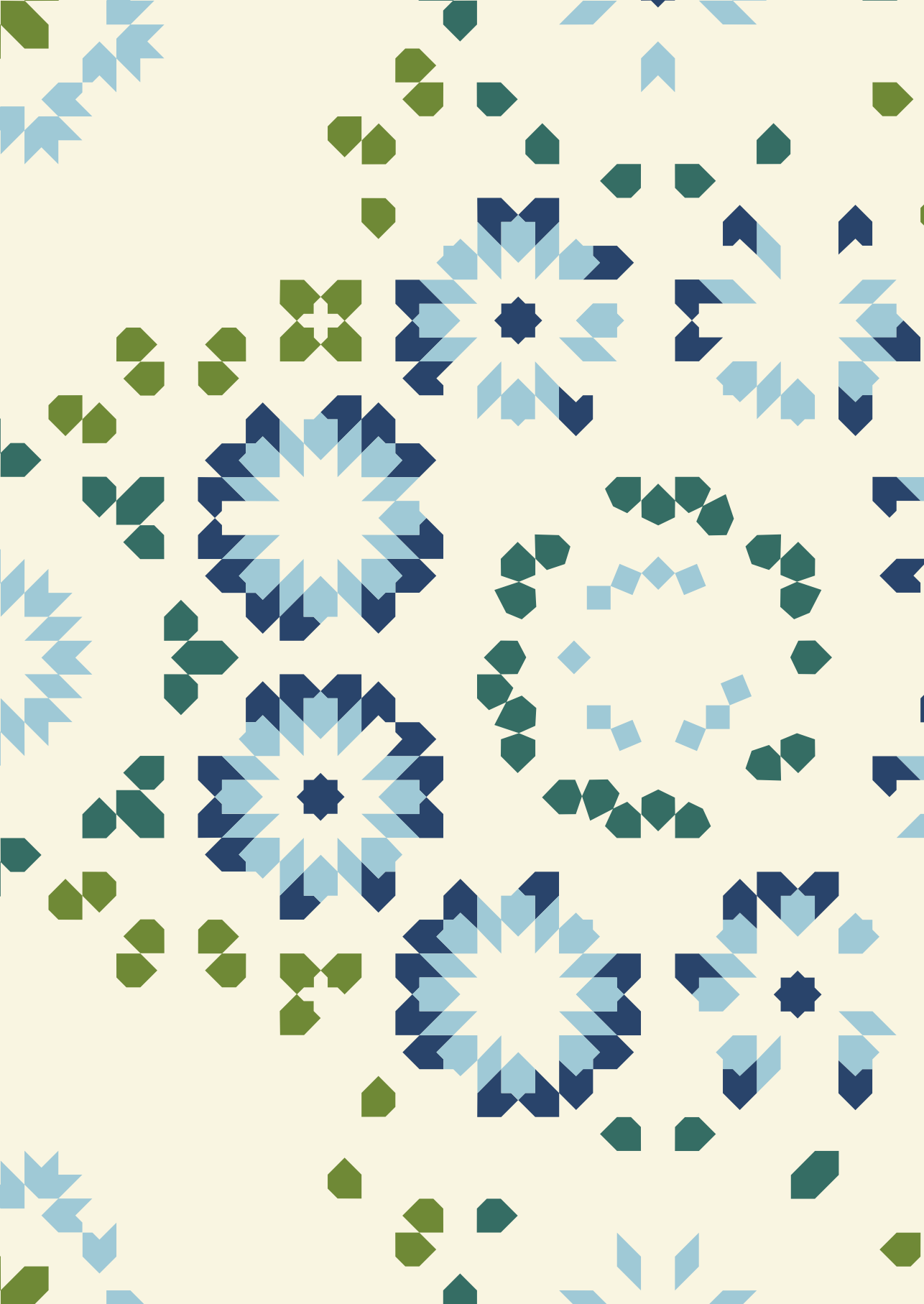
*Fold change of first/top DMR of the relevant gene





PART II

HETEROGENEITY WITHIN THE LIPOSARCOMA SPECTRUM





CHAPTER 5

RADIOMICS APPROACH TO DISTINGUISH BETWEEN WELL DIFFERENTIATED LIPOSARCOMAS AND LIPOMAS ON MRI

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Br J Surg. 2019 Dec;106(13):1800-1809.

Abstract

Background: Well differentiated liposarcoma (WDLPS) can be difficult to distinguish from lipoma. Currently, this distinction is made by testing for *MDM2* amplification, which requires a biopsy. The aim of this study was to develop a non-invasive method to predict the *MDM2* amplification status using radiomics features derived from MRI.

Methods: Patients with an *MDM2*-negative lipoma or *MDM2*-positive WDLPS and a pre-treatment T1-weighted MRI scan who were referred to Erasmus MC between 2009 and 2018 were included. When available, other MRI sequences were included in the radiomics analysis. Features describing intensity, shape and texture were extracted from the tumour region. Classification was performed using various machine learning approaches. Evaluation was performed through a 100 times random-split cross-validation. The performance of the models was compared with the performance of three expert radiologists.

Results: The data set included 116 tumours (58 patients with lipoma, 58 with WDLPS) and originated from 41 different MRI scanners, resulting in wide heterogeneity in imaging hardware and acquisition protocols. The radiomics model based on T1 imaging features alone resulted in a mean area under the curve (AUC) of 0.83, sensitivity of 0.68 and specificity of 0.84. Adding the T2-weighted imaging features in an explorative analysis improved the model to a mean AUC of 0.89, sensitivity of 0.74 and specificity of 0.88. The three radiologists scored an AUC of 0.74 and 0.72 and 0.61 respectively; a sensitivity of 0.74, 0.91 and 0.64; and a specificity of 0.55, 0.36 and 0.59.

Conclusion: Radiomics is a promising, non-invasive method for differentiating between WDLPS and lipoma, outperforming the scores of the radiologists. Further optimization and validation is needed before introduction into clinical practice.

Introduction

Lipomatous tumours are the most commonly observed soft tissue tumours, mostly owing to the high incidence of benign lipomas. Also within the malignant spectrum of soft tissue tumours (soft tissue sarcomas), liposarcoma is among the most frequently observed subtypes [1]. Well differentiated liposarcoma (WDLPS) represents the largest subgroup of liposarcomas; these low-grade, locally aggressive tumours are characterized by amplification of the *MDM2* gene [1]. In rare cases, WDLPS can progress into a more aggressive subtype: dedifferentiated liposarcoma (DDLPS), which has a poorer prognosis [1].

Several differences between lipoma and WDLPS on MRI have been described in the literature: size, location, tumour depth and intra-tumour heterogeneity. However, as there can be considerable overlap between these features, distinguishing between the two tumour types remains difficult, even for trained radiologists [2-6]. As the differences between lipoma/WDLPS and DDLPS are more obvious, this distinction can accurately be made solely by eye [5, 7-10].

An accurate diagnosis is needed to provide patients with the correct treatment and follow-up. Whereas lipomas do not necessarily need to be excised, patients with WDLPS are generally considered candidates for surgery [11]. Currently, the standard way to differentiate lipoma from WDLPS is through a biopsy, which is tested for *MDM2* amplification using fluorescence *in situ* hybridization (FISH). Amplification of the *MDM2* gene is present in WDLPS, but absent in lipoma [1, 12, 13]. Taking a biopsy is an invasive and painful procedure for the patient, and is associated with risks, depending on tumour location, and potential sampling error.

The field of radiomics is based on the hypothesis that there is a relationship between medical imaging features and the underlying biological information, such as genetic aberrations [14]. Radiomics approaches have already been used in soft tissue sarcomas to predict other outcomes, such as differentiating between benign and malignant soft tissue tumours in general (not specifically lipomatous tumours) [15], between intermediate- and high-grade soft tissue sarcomas [16], and predicting the risk of lung metastases from soft tissue sarcoma of the extremities [17]. Based on these results, it was hypothesized that radiomics might also be able to differentiate WDLPS from lipoma.

The aim of this study was to develop a model that predicts the *MDM2* amplification status using a radiomics approach, thereby differentiating WDLPS from lipoma. MRI scans obtained during routine diagnostic evaluation were used. Additionally, the performance of this model was compared with that of three trained radiologists reading the images. Finally, patients with DDLPS were included and classified by the radiologists to confirm that these tumours have distinct imaging features and can be identified without the help of additional models or tests.

Methods

Patients with a pathologically confirmed diagnosis of lipoma, WDLPS or DDLPS, a known *MDM2* amplification status tested by FISH, and with at least a T1-weighted MRI sequence available before treatment (if applicable) were included. All patients were either referred to/discussed at, or diagnosed/treated at the Erasmus MC Cancer Institute, Rotterdam, the Netherlands, between December 2009 and August 2018. As a result, some of the MRI scans were made in the referring hospitals. The study was reviewed and approved by the local medical ethics review committee (MEC-2016-339), and performed in accordance with national and international legislation. Need for informed consent was waived owing to the retrospective and anonymized nature of the study.

5

To explore the potential predictive value of different MRI sequences, several additional sequences were included, when available. Based on their use in clinical practice, the sequences were grouped into: plain T1 (T1); T1 with fat saturation (T1-FS) including T1 inversion recovery (IR) approaches (T1-IR; a combination of Spectral Presaturation with Inversion Recovery (SPIR), Short-T1 Inversion Recovery (STIR), Spectral Attenuated Inversion Recovery (SPAIR) and Turbo Inversion Recovery Magnitude (TIRM)); T1 with gadolinium contrast (T1-GD); T1 with fat saturation and gadolinium contrast (T1-FS-GD) including T1-IR with GD; T2 imaging (T2) including T2-Fast Field Echo (T2-FFE) and T2*; and T2-FS including T2-IR.

Segmentation

The lipoma and WDLPS lesions were segmented semi-automatically on the T1 images to indicate the regions of interest (ROIs) [18]. All images were segmented independently by either a medical masters student or a PhD candidate with an MD degree. Both were blinded to the type of lipomatous tumour. To validate segmentation accuracy, a sample set was verified by a musculoskeletal radiologist, specialized in soft tissue sarcomas. Median tumour size, defined as the maximum diameter in centimetres, and tumour volume, with corresponding i.q.r. values, were extracted from the segmentations. The DDLPS images were used only for visual classification by the radiologists, and therefore not segmented.

To transfer the segmentations to the other sequences, all sequences were spatially aligned to the T1 sequence using automated image registration (elastix software [19]), thereby compensating for patient movement between scans. Quality assurance was done by visual inspection.

Radiomics feature extraction

Quantitative imaging features related to intensity, shape and texture were extracted from the ROIs using PyRadiomics software [20, 21]. More details can be found in *Appendix S1*

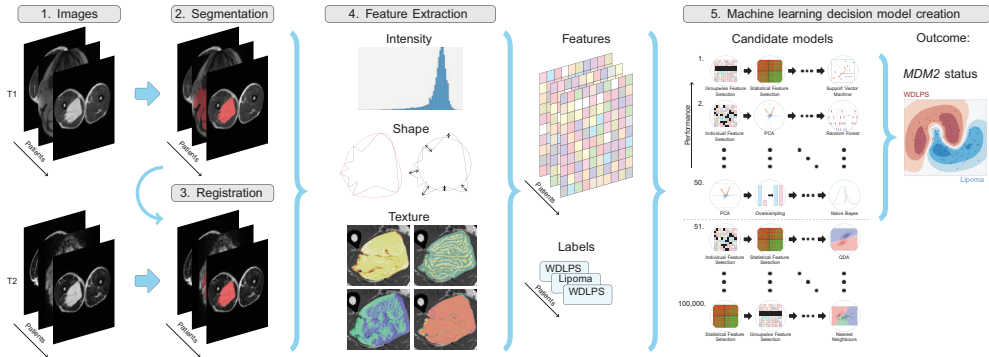
(supporting information). The shape features quantified were morphological properties such as volume and similarity to a circle. Intensity features were quantified using first-order statistics such as the mean and standard deviation. Texture features quantified more complex properties, such as the presence of heterogeneity and speckle patterns. When a scan type was missing for a patient, the feature values for the missing image type were imputed.

Additional features

Several additional features were selected based on the available literature and clinical relevance, including patient characteristics (age, sex and tumour location (extremity, trunk, head and neck or pelvis)) and manually scored features (tumour depth (superficial or deep), unilobular or multilobular tumour, atypical appearance on T1 image (yes or no)). These are referred to as patient and manually scored features respectively. Tumours were considered superficial when entirely located above the fascia, or as deep-seated when located beneath the fascia, or with invasion of the fascia.

Decision model creation

To create a decision model from the features, the Workflow for Optimal Radiomics Classification (WORC) toolbox [22] was used. A schematic overview of the radiomics methodology is shown in *Fig. 1*. In WORC, decision model creation is divided into several steps. These steps include, for example, selection of features that offer the highest predictive value and machine learning to discover the patterns in these features that distinguish between WDLPS and lipoma. For each of these steps, numerous algorithms have been proposed in the literature. WORC performs an exhaustive search amongst these algorithms, in a fully automated way, and establishes the combination of algorithms that maximizes the prediction accuracy. As the single best solution may be a coincidental finding, the 50 best performing solutions were combined into a single model, with the purpose of creating a more robust model and boosting performance. More details can be found in *Appendix S2* (supporting information).



▲ Fig. 1 Schematic overview of the radiomics approach. Inputs to the algorithm are T1- and T2-weighted magnetic resonance images of well differentiated liposarcoma (WDLPS) and lipoma. (1). Processing steps include segmentation of the tumour on the T1 image (2), registration of the T1 to the T2 image to transform this segmentation to the T2 image (3), feature extraction from both the T1 and T2 images (4) and the creation of a decision model from the features (5), using an ensemble of the best 50 workflows from 100 000 candidate workflows; workflows are different combinations of the different processing and analysis steps (for example the classifier used).

5

Experimental set-up

To assess the predictive value of the T1 imaging features, and the additional patient and manually scored features, five models were trained and tested based on: imaging features only (model 1); patient features only (model 2); manually scored features only (model 3); a combination of imaging features and manually scored features (model 4); and volume only (model 5). The fifth model was included because WDLPS is generally larger than lipoma [3]. Additionally, to investigate the potential of the features independent of volume, these five models were evaluated on a volume-matched cohort, that is a subset of the data in which the distribution of tumour volume was similar among WDLPS and lipoma. These models were trained on the full data set, but tested only on patients from the volume-matched cohort.

Next, the potential value of other MRI sequences was explored by training and testing multiple imaging-based radiomics models using combinations of the various MRI sequences. When a model showed more potential than the T1 imaging-only model, it was evaluated on the volume-matched cohort as well.

Evaluation

Model evaluation was performed through cross-validation. The data were randomly split for 100 iterations, using 80 per cent for training and 20 per cent for testing. In each iteration, automatic workflow optimization was performed on the training set in an internal ten times random split cross-validation (Fig. S1, supporting information). Thus, the models were optimized solely on the training set; the test set was used only for evaluation of the final model. All splitting was done in a stratified manner to keep the balance between WDLPS and lipoma similar in all data sets.

Performance was evaluated using the area under the curve (AUC) of the receiver operating characteristic (ROC) curve, accuracy, sensitivity, specificity, negative predictive value and positive predictive value, averaged over the 100 cross-validation iterations. Positive *MDM2* amplification status (WDLPS) was defined as the positive class. Ninety-five per cent confidence intervals for the mean performance measures were constructed using the corrected resampled *t* test based on all 100 cross-validation iterations, thereby taking into account that the samples in the cross-validation splits were not statistically independent [23].

Model insights

Insight into the model was gained by ranking the patients from typical to atypical for both lipoma and WDLPS, based on the consistency of the model predictions. This was determined by the number of times (percentage) that a patient was classified correctly when included in the test set. Typical examples were patients who were always classified correctly; and atypical vice versa. In addition, to identify the individual imaging features included in the radiomics model and to assess their respective contribution to the model, univariable statistical testing of the imaging features was undertaken using the Mann–Whitney *U* test. *P* values were corrected for multiple testing using the Bonferroni correction.

Classification by radiologists

Three radiologists with expertise in soft tissue tumours classified the lipomatous tumours; radiologists 1, 2 and 3 had 3, 10 and 5 years of experience respectively. First, the radiologists had to classify the tumours as either DDLPS or WDLPS/lipoma (non-DDLPS), to confirm that DDLPS can be recognized visually. Regardless of whether a tumour was classified as DDLPS or not, the tumours subsequently had to be classified as *MDM2*-negative (lipoma) or *MDM2*-positive (WDLPS/DDLPS). The classification was done using a ten-point scale to indicate the certainty of the radiologists. The radiologists had access to all sequences that were available for each patient, as well as the age and sex.

Results

In total, 138 tumours were included: 58 patients had an *MDM2*-negative lipoma, 58 had an *MDM2*-positive WDLPS and 22 had an *MDM2*-positive DDLPS. Most patients were men (60.1 per cent) and had a deep-seated tumour located in a leg. Median WDLPS size was 20.4 cm and median volume was 36.3 cl, compared with 12.3 cm and 12.9 cl for lipoma (Table 1). Most of the patients underwent surgery: 32 with a lipoma, 50 with a WDLPS and 19 of those with a DDLPS. The eight patients with a WDLPS who did not have surgery were treated

conservatively with an active surveillance approach, whereas the three with aDDLPS who did not have surgery had an inoperable tumour.

The 116 lipoma and WDLPS scans came from 41 different MRI scanners; there was wide heterogeneity in imaging hardware and acquisition protocols used, reflected in differences in magnetic field strength (1.5T, 98 scans; 1T, 10 scans; 3T, 8 scans), manufacturer (Siemens, Munich, Germany, 45 scans; Philips, Amsterdam, the Netherlands, 45 scans; GE, Chicago, Illinois, USA, 26 scans), scanner model (19 different ones), slice thickness, repetition time and echo time. Additional sequences besides T1 were available in subsets of patients: T1-FS in 55 patients (47.4 per cent), T1-GD in 42 patients (36.2 per cent), T1-FS-GD in 80 patients (69.0 per cent), T2 in 76 patients (65.5 per cent) and T2-FS in 92 patients (79.3 per cent) (*Table S1*, supporting information).

5

Table 1. Characteristics of the patients with lipomatous tumours

	No. of patients*(n =138)
Age (years)†	64 (54–71)
Sex ratio (M: F)	83 : 55
Diagnosis	
Lipoma	58 (42.0)
WDLPS	58 (42.0)
DDLPS	22 (15.9)
Tumour location	
Upper extremity	14 (10.1)
Lower extremity	71 (51.4)
Trunk	37 (26.8)
Head and neck	6 (4.3)
Retroperitoneum and pelvis	6 (4.3)
Paratesticular	4 (2.9)
Tumour depth	
Superficial	20 (14.5)
Deep	118 (85.5)
Tumour size (cm)†	
Lipoma	12.3 (9.3–15.5)
WDLPS	20.4 (15.9–26.3)
Tumour volume (cl)†	
Lipoma	12.9 (4.6–25.0)
WDLPS	36.3 (22.9–85.5)

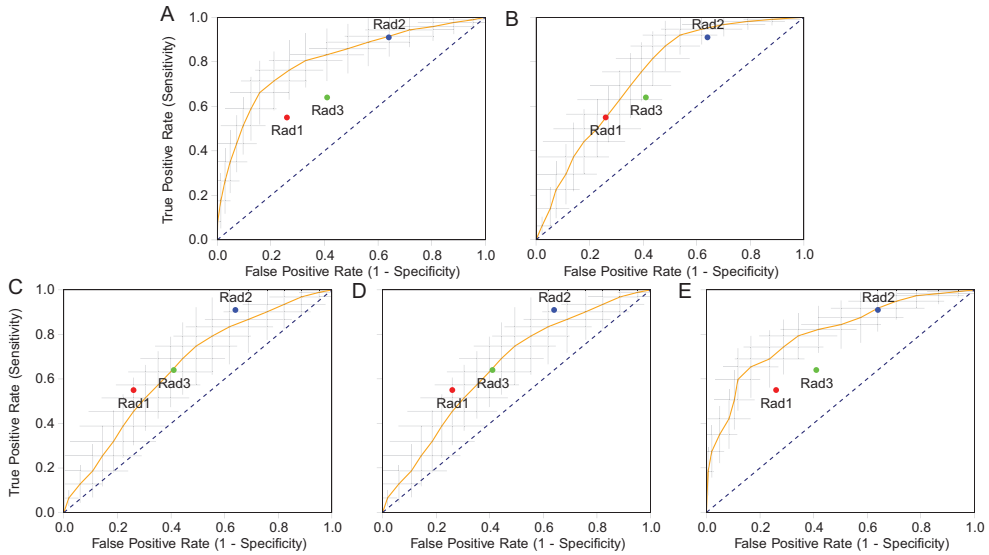
*With percentages in parentheses unless indicated otherwise; †values are median (i.q.r.). WDLPS, well differentiated liposarcoma; DDLPS, dedifferentiated liposarcoma.

Evaluation of radiomics models based on T1 imaging and additional features

The performances of models 1–5 are shown in *Fig. 2* and *Table S2* (supporting information). Model 1, based on the T1 imaging features, resulted in an AUC of 0.83, sensitivity of 0.68 and specificity of 0.84. Model 2, based on patient features, had a lower AUC (0.75), higher sensitivity (0.77), but lower specificity (0.59). Similarly, model 3, based on manually scored features, also had a lower AUC (0.72), higher sensitivity (0.76) and lower specificity (0.57). Model 4, combining the imaging and manually scored features, performed worse than model 1, implying that imaging features are sufficient as input. Finally, model 5, based on volume alone, performed similarly to model 1 with an AUC of 0.83, sensitivity of 0.67 and specificity of 0.84. Although the performance metrics were similar for models 1 and 5, the ROC curves in *Fig. 2* show some differences. The ROC curve for the volume model (*Fig. 2e*) has some sharp bends, while that for the T1 imaging model is smoother (*Fig. 2a*).

Evaluation of the radiomics models with additional MRI sequences

Most models with an additional MRI sequence had a similar performance to the T1 imaging model (*Table S3*, supporting information). However, the model combining the T1 and T2 imaging features showed a clear improvement in performance, with an AUC of 0.89, sensitivity of 0.74 and specificity of 0.88. The distribution of patient characteristics and the distribution of WDLPS and lipoma were similar across patients who had a T2 scan, indicating that the added value is within the T2 imaging features and not a result of incidental correlation with these characteristics, for example owing to selection bias.



▲ Fig. 2 Receiver operating characteristic (ROC) curves for the radiomics models based on the T1-weighted MRI sequence.

a Using imaging features only, **b** using patient features only, **c** using manually scored features only, **d** using T1 imaging features combined with manually scored features, and **e** using volume only. The shaded area indicates the 95 per cent confidence intervals of the 100 times random-split cross-validation; the curve is fit through their means. The performance of the three radiologists is shown.

Evaluation of models on volume-matched cohort

Model 5, based on volume alone, illustrated that volume is indeed a strong predictive factor. The 17 tumours with a volume above 70 cl were all WDLPS, whereas the 21 tumours with a volume below 7 cl were all lipoma. In the volume-matched cohort, consisting of the other 78 tumours with a volume between 7 and 70 cl, the volume distributions for WDLPS and lipoma were more similar. As only the T2 scans provided additional value over the T1 imaging features, the T1+T2 imaging model was evaluated for the volume-matched cohort as well.

The performance of both imaging-based models (T1 and T1+T2) was worse on the volume-matched cohort (T1: AUC 0.69; T1+T2: AUC 0.81) (*Table 2*) than on the entire cohort (AUC 0.83 and 0.89 respectively) (*Table S3*, supporting information). The models based on the patient and manually scored features performed similarly to the models tested on the full cohort. The model based on volume alone still performed above chance (mean AUC 0.64), but considerably worse than on the entire data set. In this volume-matched data set, both the T1 imaging model (AUC 0.69, sensitivity 0.60, specificity 0.74) and the T1+T2 imaging model (AUC 0.81, sensitivity 0.66, specificity 0.84) performed considerably better than volume alone (*Table 2*). This showed that these models were not based solely on volume, and that other features provided additional predictive value over volume.

Table 2. Performance of radiomics models trained on the full cohort, but evaluated in the volume-matched cohort.

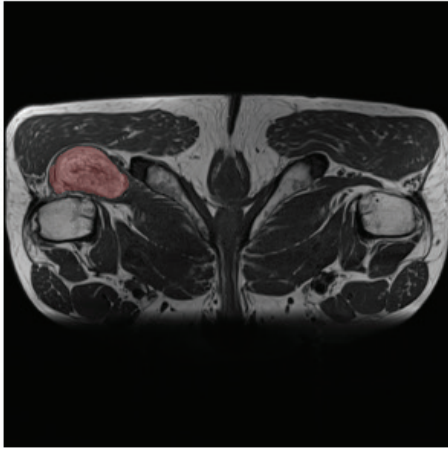
	T1 imaging features only	T1 + T2 imaging features	Patient features only	Manually scored features only	Volume only
AUC	0.69 [0.58, 0.80]	0.81 [0.72, 0.90]	0.74 [0.64, 0.84]	0.67 [0.56, 0.77]	0.64 [0.53, 0.74]
Accuracy	0.67 [0.57, 0.76]	0.75 [0.66, 0.83]	0.66 [0.56, 0.75]	0.60 [0.51, 0.69]	0.66 [0.57, 0.74]
Sensitivity	0.60 [0.45, 0.75]	0.66 [0.52, 0.79]	0.69 [0.55, 0.83]	0.70 [0.53, 0.87]	0.50 [0.36, 0.64]
Specificity	0.74 [0.60, 0.87]	0.84 [0.71, 0.96]	0.62 [0.48, 0.76]	0.51 [0.36, 0.65]	0.82 [0.71, 0.92]
NPV	0.66 [0.54, 0.77]	0.72 [0.60, 0.83]	0.68 [0.56, 0.79]	0.65 [0.49, 0.80]	0.62 [0.53, 0.71]
PPV	0.72 [0.58, 0.85]	0.81 [0.69, 0.93]	0.65 [0.54, 0.76]	0.59 [0.49, 0.69]	0.74 [0.61, 0.87]

Values are mean [95 per cent c.i.] over the cross-validation iterations. AUC, area under the curve; NPV, negative predictive value; PPV, positive predictive value.

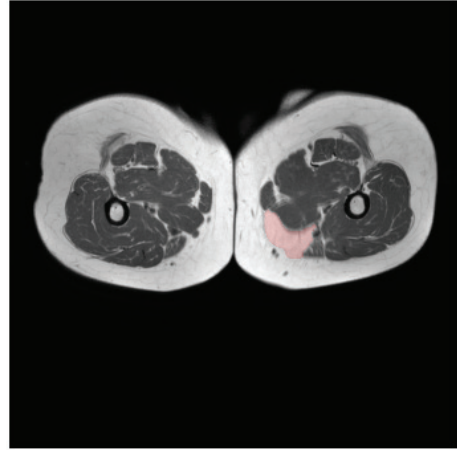
Model insights

Of the 116 lipomatous tumours, 69 (26 WDLPS, 43 lipoma) were always classified correctly by model 1 in all 100 cross-validation iterations. In contrast, 13 tumours (9 WDLPS, 4 lipoma) were always classified incorrectly. *Fig. 3* shows four MRI slices of such typical and atypical examples of lipoma and WDLPS. The lesions that were always classified incorrectly were checked for possible sampling error of the biopsy. The *MDM2* amplification status of eight of the 13 tumours always classified incorrectly was already determined on the resection specimen (6 WDLPS, 2 lipoma). For the other five patients, in whom it was tested on the biopsy (3 WDLPS, 2 lipoma), pathological examination of the resection specimen confirmed the diagnosis, except for one patient with a lipoma who did not undergo surgery. In the other patient with a lipoma, the resection specimen again tested negative for *MDM2* amplification. The three WDLPS resection specimens were not retested.

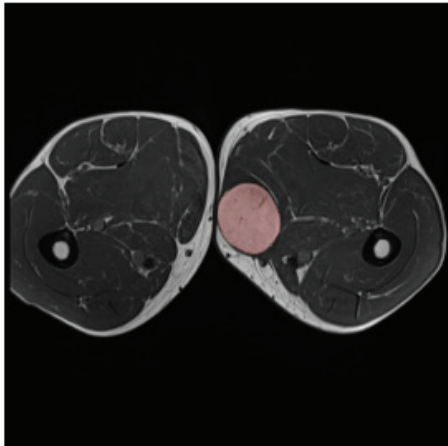
Analysis of feature importance was done for the volume-matched cohort, as the results on the full data set were dominated by volume-related measures. In total, 16 individual features were found to be significant after Bonferroni correction on the volume-matched cohort (*Fig. S2*, supporting information). These included 11 shape features (including several volume-related statistics), four texture features and one intensity feature.



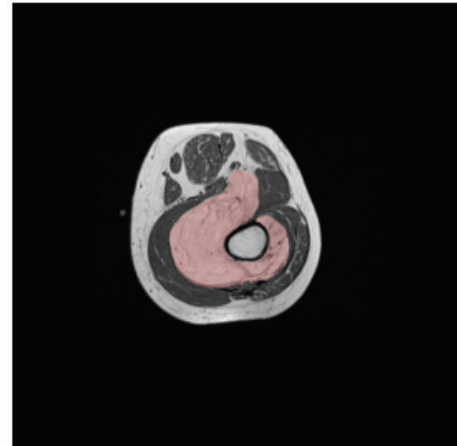
(A) Typical Lipoma



(B) Atypical Lipoma



(C) Atypical WDLPS



(D) Typical WDLPS

▲Fig. 3 Examples of typical and atypical lipomas and well differentiated liposarcomas.

a Typical lipoma, **b** atypical lipoma, **c** atypical well differentiated liposarcoma (WDLPS) and **d** typical WDLPS. The typical examples are from two patients always classified correctly by the T1 imaging model; the atypical examples are from two patients always classified incorrectly by the T1 imaging model.

Radiomics models compared with radiologists

On the entire cohort, the AUCs of all three radiologists (0.74, 0.72 and 0.61 for radiologist 1, 2 and 3 respectively) (*Table S4*, supporting information) were below the lower limit of the 95 per cent c.i. of the T1 imaging model (0.75 to 0.90) (*Fig. 2* and *Table S2*, supporting information), as well as of the 95 per cent c.i. of the T1+T2 imaging model (0.83 to 0.95) (*Table S3*, supporting information). The radiologists achieved sensitivity values similar to (0.64 and 0.74) or higher (0.91) than those of the radiomics models (T1: 0.68; T1+T2: 0.74), but their specificity was

much lower (radiomics: 0.84 and 0.88 respectively; radiologists 1–3: 0.55, 0.36 and 0.59 respectively). The Cohen's κ value was 0.24, 0.04 and 0.40 for all pairs of radiologists, with a mean of 0.23, indicating poor inter-observer agreement.

On the volume-matched cohort, the radiologists had a performance (AUC 0.68, 0.74 and 0.55) (*Table S4*, supporting information) more similar to that of the T1 imaging model (AUC 0.69) (*Table 2*). On average, the T1 imaging model still performed better in terms of specificity (radiomics: 0.74; radiologists 1–3: 0.58, 0.37 and 0.50), whereas the radiologists again performed better on sensitivity (radiomics: 0.60; radiologists 1–3: 0.65, 0.88 and 0.60). However, the T1+T2 imaging model performed much better (AUC 0.81, sensitivity 0.66, specificity 0.84) than both the T1 imaging model and the radiologists. On this cohort, the Cohen's κ values were 0.18, -0.04 and 0.34 for all pairs of radiologists, with a mean of 0.16, again indicating poor inter-observer agreement.

Distinction between dedifferentiated liposarcoma and well differentiated liposarcoma/lipoma

Besides classifying lipoma and WDLPS, the radiologists also classified the scans from 22 patients withDDLPS to evaluate whetherDDLPS can indeed be identified by imaging only, without the help of additional models. Radiologists 1–3 had an AUC of 0.97, 0.91 and 0.90 respectively; a sensitivity of 0.95, 0.95 and 0.91; and a specificity of 0.95, 0.56 and 0.89 in distinguishingDDLPS from non-DDLPS (WDLPS/lipoma) (*Table S4*, supporting information).

Discussion

This study shows that there is a relationship between quantitative MRI features and the *MDM2* amplification status, and that radiomics is a promising non-invasive method for differentiating lipoma from WDLPS. Although the radiologists were able to distinguish betweenDDLPS and non-DDLPS, they were outperformed by the T1 and T1+T2 imaging models in differentiatingWDLPS from lipoma. Moreover, the agreement between radiologists was very poor, whereas the radiomics-based predictions were objective and reproducible (given a tumour segmentation).

Remarkably, the model trained on volume alone had a similar performance to the T1 imaging model, which included many additional features. However, in the volume-matched data set, the T1 imaging model performed considerably better than the volume-only model, indicating that other features do provide additional predictive value. It is already known that WDLPS is on average larger than lipoma [3], and the relationship with volume (or size) in our data set was also strong; the database did not contain lipoma larger than 70 cl or WDLPS smaller than 7 cl although these do exist [24, 25]. However, all WDLPS lesions start as small

tumours and grow over time, so the measured tumour volume depends on the moment of presentation, and a small or intermediate tumour volume is therefore not a reliable biomarker. Future research should include expansion of the data set to make the volume distributions more representative (including lipoma larger than 70 cl and WDLPS smaller than 7 cl), thereby making the radiomics model less volume-dependent.

The models trained solely on either the patient or manually scored features performed slightly worse than the model trained on the T1 imaging features only. As the combined model did not outperform the T1 imaging model, the manually scored features did not add much in the search for the best radiomics model. Additionally, the manually scored features may be observer-dependent, and thus prone to subjectivity. Although patient features (age, sex and tumour location) are objective, the distribution in the present data set may not be representative of clinical practice. For example, none of the patients with WDLPS were younger than 35 years, there were no lipomas among patients older than 82 years, no lipomas in the head and neck region, and no WDLPS in the pelvis or shoulder/trunk; all these might occur in daily clinical practice. Therefore, the imaging-only models have more potential as an objective tool in clinical practice.

The results of present study are similar to those of Thornhill and colleagues [26], who used a comparable approach and showed that lipomas can be distinguished from liposarcomas by texture and shape analysis. Strong points of the present study include the larger sample size (116 *versus* 44 in Thornhill *et al.*). Thornhill and co-workers also included other liposarcoma subtypes in their model, such asDDLPS and myxoid liposarcoma (8 of 20 included liposarcomas). These other liposarcoma subtypes have distinct radiological features [5, 10], which in general can be easily discriminated from lipomas by experienced radiologists. By solely including the two tumour types that are the most difficult to distinguish (WDLPS and lipoma) in the radiomics model, the present data set is more challenging and more clinically relevant. In contrast to the cases described by Thornhill *et al.*, the diagnosis of all patients in the present data set was confirmed by verifying the *MDM2* amplification status using FISH, the current standard for diagnosing and differentiating between lipoma and WDLPS [1, 12, 13]. The present radiomics model only requires routine MRI scans (T1, and optionally T2) without contrast injection; the other sequences did not add any predictive value to the model. As almost all standard MRI protocols include a T1 and T2 sequence, the present radiomics method is generalizable, feasible and applicable for use in daily practice. Finally, these radiomics models were developed and evaluated on a heterogeneous data set, thereby increasing the chance that the reported performance can be reproduced in a routine clinical setting when using other MRI scanners.

Advantages of using a radiomics approach over pathological assessment to differentiate between lipoma and WDLPS include sparing patients an invasive and painful biopsy, and saving the substantial costs of a radiologist performing the imaging-guided biopsy and of the

pathologist assessing it, including the costs of molecular testing by FISH. Radiomics makes use of MRI images obtained during routine diagnostic evaluation and patients do not need to undergo any additional procedure. When radiomics becomes a widely available tool, patients with WDLPS can be identified and referred to a soft tissue sarcoma expert centre at an earlier stage, with potential beneficial effects on further diagnostics, treatment and follow-up.

Several limitations of this study should be noted, besides the volume bias already mentioned. First, segmentation of ROIs of the tumours was done manually, which inherently leads to both inter-observer and intra-observer variability, as has been quantified for other cancer types [27-29]. Variability in segmenting the ROIs might lead to variability in the extracted imaging features and subsequently influence the classification of tumours. Additionally, manual segmentation is rather time-consuming. This could be addressed by use of automated segmentation tools that might be available in the future. Second, variation in imaging protocols might have influenced the imaging statistics. No restrictions were put on the T1 MRI sequences regarding field strength, slice thickness, or other MRI acquisition settings, as selecting a single protocol is an unrealistic reflection of daily clinical practice and would have made the results non-generalizable. Instead, this study shows that the present radiomics approach is robust to these variations by both training and testing the model on heterogeneous data. Third, the model is based on retrospectively collected data, which might have led to selection and information bias. This potential selection bias might have occurred particularly in the lipoma subgroup, as usually only large and atypical lipomas are referred to a sarcoma centre. However, this probably made the data set even more challenging and relevant, as these can be seen as the complex cases. Addition of the 'small and typical' lipomas would have made the classification easier, and radiomics is not needed to make the distinction for such lipomas.

The present radiomics model could serve as a non-invasive, quick and low-cost alternative to a biopsy. Although the model needs optimization to match the accuracy of a biopsy, there could be a certain patient group for whom the model may already be useful. For example, patients at high risk of complications of biopsy, or those in whom the radiomics model can predict the *MDM2* amplification status with a high degree of certainty, could already be treated according to the prediction of the radiomics model. Although further research is required to identify which patients could benefit most from the present model, initial misclassification of a WDLPS as a lipoma would not harm the patient, considering that active surveillance seems a safe option in patients without (invalidating) symptoms and/or tumour growth, at least in the short term [30]. In addition, the performance of the radiomics model improved substantially when T2 images were added. However, only 65.5 per cent of the patients had a T2 scan available, so for a follow-up study it is proposed to use MRI with at least both T1 and T2 sequences.

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Disclosure

The authors declare no other conflicts of interest.

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Supporting information

Appendix S1: Radiomics feature extraction

In this study, radiomics features quantifying intensity, shape and texture were extracted. Intensity features were extracted using the histogram of all intensity values within the Regions of Interest (ROIs) and included several first order statistics such as the mean, standard deviation and kurtosis. Shape features were extracted by solely using the ROI and included shape descriptions such as the compactness, roundness and circular variance. Additionally, the volume and orientation of the ROI were used. Texture features were extracted using the Gray Level Co-occurrence Matrix, Gray Level Size Zone Matrix Gray Level Run Length Matrix and Neighborhood Grey Tone Difference Matrix. All features were extracted using the defaults for MR images from PyRadiomics.

The used dataset is highly heterogeneous in terms of acquisition protocols. Especially the variations in slice thickness and contrast may cause feature values to be highly dependent on the acquisition protocol. The slice thickness varies between 2.5mm and 10mm. Hence, extracting robust 3D features may be hampered by these variations, especially for the low resolutions. To overcome this issue, all features are extracted per 2D axial slice and aggregated over all slices. Due to the slice thickness and pixel spacing heterogeneity, the images were not resampled. Due to variations in especially the magnetic field strength, echo time, and repetition time, the image contrast highly varies, which will affect the feature values. To overcome this, each 3D MRI is normalized using z-scoring before feature extraction. The code to extract the features has been published open-source (<https://github.com/MStarmans91/LipoRadiomicsFeatures>)

Appendix S2: Technical details on decision model creation

The Workflow for Optimal Radiomics Classification (WORC) toolbox¹ makes use of adaptive algorithm optimization to create the optimal performing workflow from a variety of methods. We define a workflow as a sequential combination of algorithms and their respective parameters.

WORC includes algorithms to perform feature imputation, feature selection, feature scaling, oversampling, and machine learning. Feature selection was performed to eliminate features which are not useful to distinguish between WDLPS and lipoma. These included; 1) a group-wise search, in which specific groups of features (i.e. intensity, shape, and the several subgroups of texture features as defined in Supplementary Materials 1) are selected or deleted; 2) a variance threshold, in which features with a low variance are removed; and 3) principal component analysis (PCA), in which only those linear combinations of features were kept which explained a large part of the variance in the features.

Feature scaling was performed to make all features have the same scale, as otherwise

the machine learning methods may focus only on those features with large values. This was done through z-scoring, i.e. subtracting the mean value followed by division by the standard deviation. In this way, all features had a mean of zero and a variance of one.

Oversampling was used to make sure the classes (i.e. WDLPS and lipoma) were balanced in the training dataset. These include 1) random oversampling, which randomly repeats patients of the minority class; and 2) SMOTE², which creates new synthetic patients using a combination of the patients in the minority class.

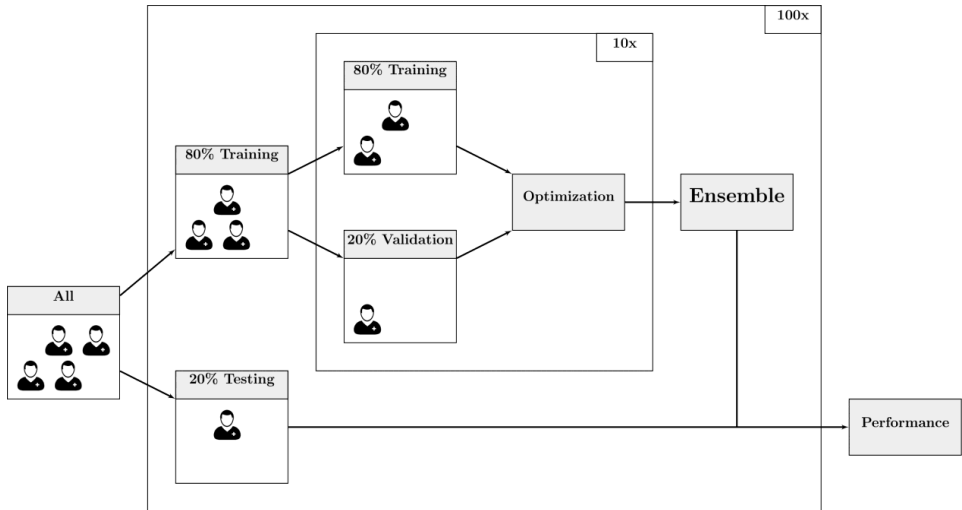
Lastly, machine learning methods were used to determine a decision rule to distinguish between WDLPS and lipoma. These included 1) logistic regression; 2) support vector machines; 3) random forests; 4) naive Bayes; and 5) linear and quadratic discriminant analysis.

Most of the included methods require specific settings or parameters to be set, which may have a large impact on the performance. As these parameters have to be determined before executing the workflow, these are so-called "hyperparameters". In WORC, we treat all parameters of all methods as hyperparameters, since they may all influence the decision model creation. Hence, we simultaneously determine which combination of algorithms and hyperparameters performs best.

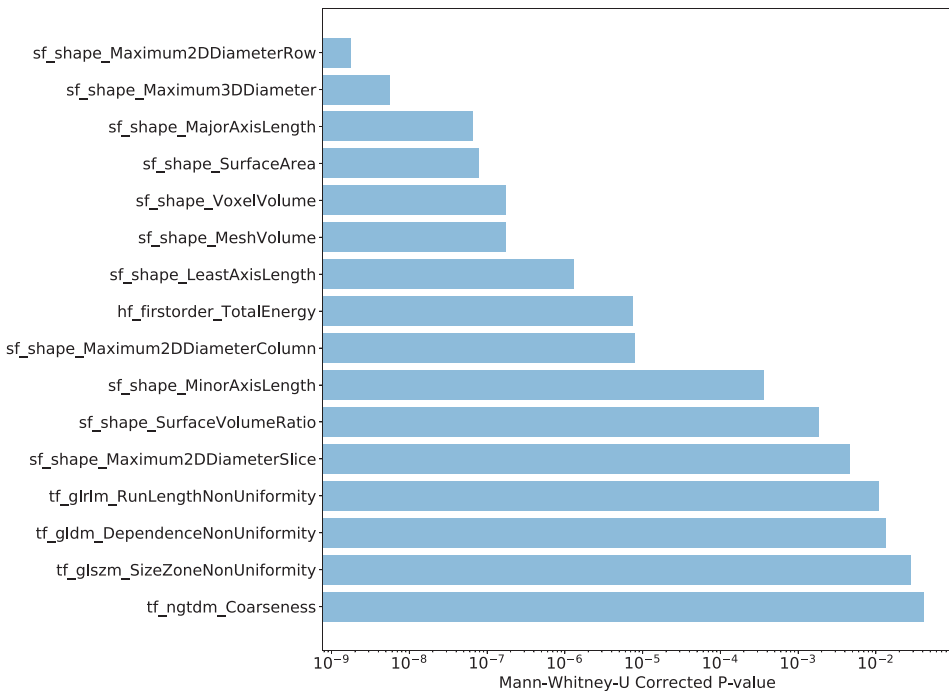
In the training phase, a total of 100,000 pseudo-randomly generated workflows is created and executed. The workflows are ranked from best to worst based on the F1-score, which is the harmonic average of precision and recall. Due to the large number of workflows executed, there is a chance that the best performing workflow is overfitting, i.e. looking at too much detail or even noise in the training dataset. Hence, to create a more robust model and boost performance, WORC combines the 50 best performing methods into a single decision model, which is known as ensembling. The ensemble is created through averaging of the probabilities, i.e. the chance of a patient being WDLPS or lipoma, of these 50 workflows.

1. Workflow for Optimal Radiomics Classification (WORC). <https://github.com/MStarmans91/WORC>.
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Supporting figures



▲**Figure S1.** Visualization of the 100x stratified random-split cross-validation, including a second cross-validation within the training set to perform the automatic workflows optimization. Optimization was done solely on the training set in order to prevent overfitting on the test set. The ensemble averages the predictions of the best 50 performing workflows to create a more robust model.



▲**Figure S2.** P-values of Mann-Whitney U tests of feature values for WDLPS and lipomas. Only the features that had a corrected P-value <0.05 were included in the graph. The labels on the y-axis correspond to the feature names: see Appendix S1 for more details.

Supporting tables

Table S1. Several properties of the acquisition protocols of the 116 T1-weighted MRI sequences of patients with lipoma or well-differentiated liposarcoma (WDLPS) that were used to build the radiomics model.

Property	N	%		
Magnetic field strength				
1T	10	9.6		
1.5T	98	84.5		
3T	8	6.9		
Manufacturer				
Siemens	45	38.8		
Philips	45	38.8		
GE	26	22.4		
Setting (Unit)	Mean	Std.	Min.	Max.
Slice Thickness (mm)	4.77	1.14	2.5	10.0
Repetition time (ms)	555	108	280	831
Echo time (ms)	13.2	4.3	5.7	37
Available MRI sequences		N	%	
T1	116	100		
T1-FS	55	47.4		
T1-GD	42	36.2		
T1-FS-GD	80	69.0		
T2	76	65.5		
T2-FS	92	79.3		

Std.: standard deviation, min.: minimum value, max.: maximum value, mm: millimeters, ms: milliseconds, FS: Fat Saturation, GD: gadolinium contrast.

Table S2. Performance of the radiomics models based on T1 imaging features only; patient features only; manually scored features only; the combination of T1 imaging and manually scored features; and of volume only on the full dataset. Performance for the radiomics models is reported for each experiment as mean [95% confidence interval] over the cross-validation iterations.

	Model 1 T1 imaging features	Model 2 Patient features	Model 3 Manually scored features	Model 4 T1 imaging + manually scored features	Model 5 Volume
AUC	0.83 [0.75, 0.90]	0.75 [0.64, 0.85]	0.72 [0.62, 0.81]	0.69 [0.58, 0.79]	0.83 [0.75, 0.91]
Accuracy	0.68 [0.67, 0.84]	0.68 [0.59, 0.76]	0.67 [0.57, 0.76]	0.61 [0.51, 0.70]	0.76 [0.67, 0.84]
Sensitivity	0.68 [0.53, 0.82]	0.77 [0.63, 0.90]	0.76 [0.58, 0.94]	0.53 [0.37, 0.68]	0.67 [0.52, 0.81]
Specificity	0.84 [0.72, 0.95]	0.59 [0.45, 0.72]	0.57 [0.43, 0.71]	0.69 [0.54, 0.84]	0.84 [0.71, 0.97]
NPV	0.73 [0.63, 0.82]	0.73 [0.61, 0.85]	0.73 [0.59, 0.86]	0.60 [0.50, 0.69]	0.75 [0.66, 0.83]
PPV	0.82 [0.70, 0.93]	0.66 [0.58, 0.73]	0.64 [0.54, 0.74]	0.64 [0.51, 0.76]	0.81 [0.69, 0.93]

AUC: area under the curve, NPV: negative predictive value, PPV: positive predictive value

Table S3. Performance of radiomics models trained on features extracted from various MRI sequences on the full dataset. Performance is reported as mean [95% confidence interval] over the cross-validation iterations.

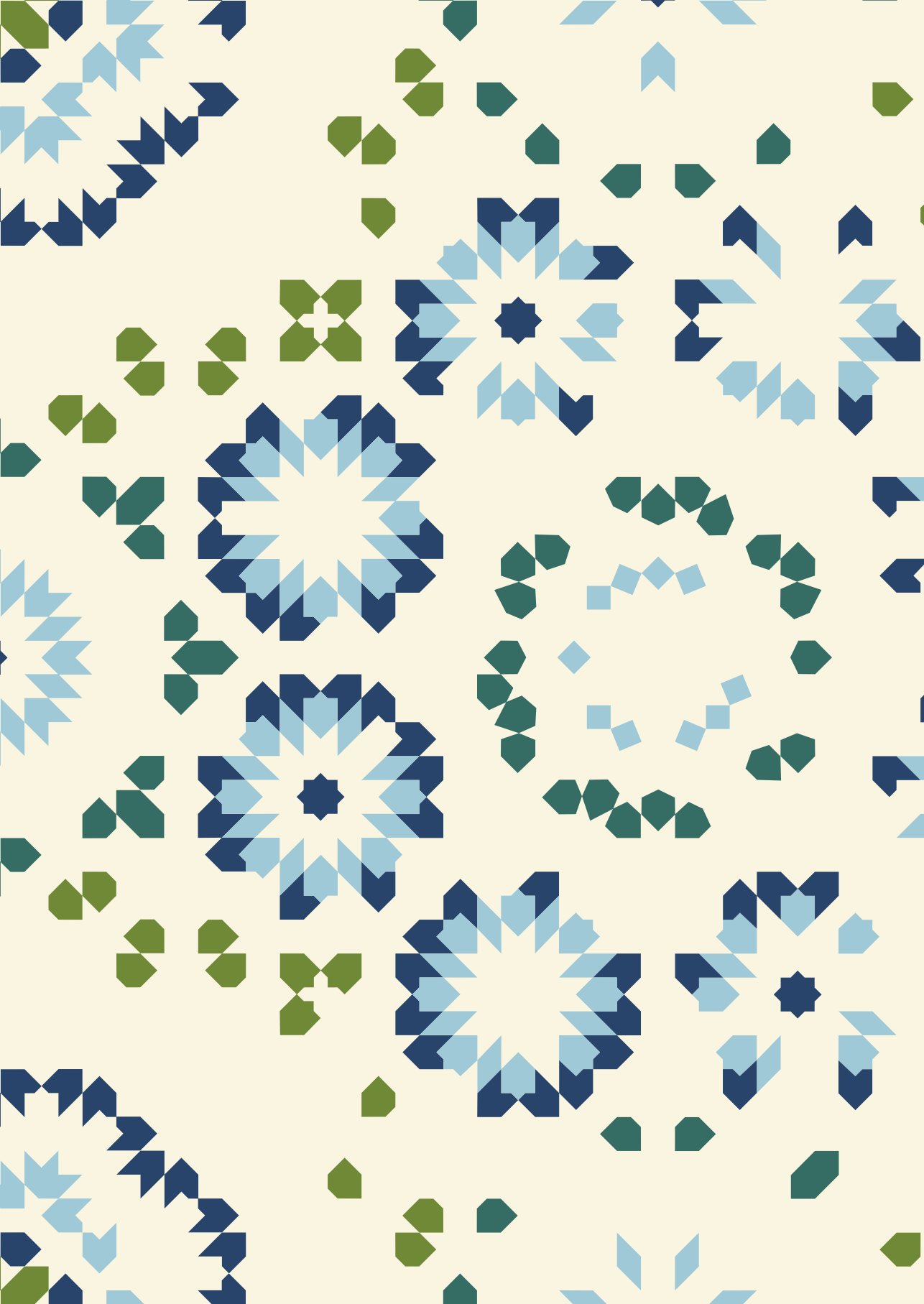
	T1	T1 + T1-FS	T1 + T1-GD	T1 + T1-FS-GD	T1 + T2	T1 + T2-FS
AUC	0.83 [0.75, 0.90]	0.84 [0.75, 0.92]	0.81 [0.72, 0.90]	0.81 [0.73, 0.89]	0.89 [0.83, 0.95]	0.81 [0.73, 0.88]
Accuracy	0.68 [0.67, 0.84]	0.77 [0.69, 0.85]	0.76 [0.67, 0.84]	0.75 [0.66, 0.83]	0.81 [0.74, 0.87]	0.74 [0.66, 0.81]
Sensitivity	0.68 [0.53, 0.82]	0.69 [0.56, 0.82]	0.69 [0.56, 0.82]	0.66 [0.51, 0.81]	0.74 [0.61, 0.86]	0.66 [0.53, 0.79]
Specificity	0.84 [0.72, 0.95]	0.84 [0.73, 0.95]	0.77 [0.71, 0.83]	0.84 [0.72, 0.95]	0.88 [0.78, 0.98]	0.82 [0.70, 0.93]
NPV	0.73 [0.63, 0.82]	0.74 [0.65, 0.82]	0.73 [0.64, 0.82]	0.72 [0.63, 0.81]	0.78 [0.69, 0.86]	0.72 [0.63, 0.80]
PPV	0.82 [0.70, 0.93]	0.83 [0.72, 0.93]	0.80 [0.69, 0.91]	0.81 [0.69, 0.93]	0.88 [0.78, 0.97]	0.79 [0.68, 0.90]

AUC: area under the curve, NPV: negative predictive value, PPV: positive predictive value, FS: Fat Saturation, GD: gadolinium contrast

Table S4. Performance of the three radiologists in differentiating between well-differentiated liposarcomas and lipomas on both the full and volume-matched cohort, and in differentiating dedifferentiated liposarcoma (DDLPS) and non-DDLPS (well-differentiated liposarcoma (WDLPS)/lipomas).

	Full cohort			Volume-matched cohort			DDLPS vs. non-DDLPS		
	Rad. 1	Rad. 2	Rad.3	Rad. 1	Rad. 2	Rad. 3	Rad. 1	Rad. 2	Rad. 3
AUC	0.74	0.72	0.61	0.68	0.74	0.55	0.97	0.91	0.90
Accuracy	0.64	0.64	0.61	0.62	0.63	0.55	0.95	0.62	0.89
Sensitivity	0.74	0.91	0.64	0.65	0.88	0.60	0.95	0.95	0.91
Specificity	0.55	0.36	0.59	0.58	0.37	0.50	0.95	0.56	0.89
NPV	0.68	0.81	0.62	0.61	0.74	0.54	0.99	0.98	0.98
PPV	0.62	0.59	0.61	0.62	0.59	0.56	0.78	0.29	0.61

AUC: area under the curve, NPV: negative predictive value, PPV: positive predictive value, rad.: radiologist





CHAPTER 6

IMPACT OF PRIMARY TUMOR LOCATION ON OUTCOME OF LIPOSARCOMA PATIENTS, A RETROSPECTIVE COHORT STUDY

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Eur J Surg Oncol. 2019 Dec;45(12):2437-2442.

Abstract

Background: Tumor location as a prognostic factor for patients with liposarcoma (LPS) has been studied modestly with varying outcomes. The aim was to establish the impact of tumor location on recurrence and survival of LPS patients.

Methods: A retrospective database of patients treated for LPS until December 2017 was used to assess 5-year local recurrence-free survival (LRFS), distant metastasis-free survival (DMFS) and disease-specific survival (DSS) per tumor location using the Kaplan-Meier method and log-rank test. A multivariable Cox regression analysis was performed to adjust for other prognostic factors.

Results: In total, 518 patients were identified with a median follow-up of 68 months (interquartile range 31–138). Patients with retroperitoneal/intrathoracic WDLPS orDDLPS ($p = 0.014$), or testicular WDLPS ($p = 0.026$) developed a local recurrence more often than patients with other tumor locations. No differences between LPS subtypes and tumor location in the development of metastases ($p = 0.600$) was observed. Five-year LRFS differed significantly between tumor locations ($p < 0.001$) as well as 5y-DSS ($p < 0.001$), but 5y-DMFS did not ($p = 0.241$), with retroperitoneal/intrathoracic LPS having a worse prognosis. Patients with WDLPS in the extremity, trunk or testicular region did not die of disease, except for the rare occasion of dedifferentiation upon recurrence. After adjustment for other prognostic factors, tumor location was only of prognostic value for DSS (retroperitoneal/intrathoracic vs. extremity: HR 5.08, 95% CI 2.41–10.71, $p < 0.001$).

Conclusion: For all tumor locations, DSS mimicked DMFS except for retroperitoneal/intrathoracic LPS, where DSS mimicked LRFS and where DSS was worse than DMFS. This implies that these patients die of local disease instead of metastatic disease.

Keywords: Liposarcoma; Survival; Tumor location; Prognostic factor

Introduction

Liposarcoma (LPS) is one of the most common subtypes of soft tissue sarcoma (STS), accounting for approximately 20% of all STS [1]. They arise from lipoblasts and adipocytes, and can therefore occur at any site of the body, but the most frequently observed locations are the extremity, the retroperitoneum and trunk [2]. Based on morphology and genetic aberrations, four subtypes can be distinguished: well-differentiated liposarcoma (WDLPS), dedifferentiated liposarcoma (DDLPS), myxoid liposarcoma (MLPS) and pleomorphic liposarcoma (PLPS) [2]. Some LPS cannot be further classified and form a residual group of LPS not otherwise specified (LPS NOS). For patients presenting with non-metastatic disease treatment usually consists of surgical removal of the tumor, optionally preceded or followed by radiotherapy, chemotherapy or an isolated limb perfusion (ILP). The choice for neoadjuvant/adjuvant treatment partially depends on the LPS subtype.

Unfortunately, a number of patients will develop a local recurrence and/or distant metastasis, or will die due to the disease. Previously identified prognostic factors for recurrence and survival include age, LPS subtype, tumor grade, tumor size and status of the resection margins [3-11], but the impact of primary tumor location as a prognostic factor has been studied modestly. Most of the studies compared multiple STS subtypes on one location [3-8], or just one of the LPS subtypes on multiple locations [12-15]. Until now, we identified only two articles studying primary LPS on multiple locations, but these two studies presented conflicting outcomes: one in which tumor location was of prognostic importance [16], and one in which location was not of significant importance [17]. The aim of this study was to establish the impact of tumor location in recurrence and survival in LPS patients.

6

Methods

Patient characteristics

Data of all patients diagnosed with and treated for LPS in the Erasmus MC Cancer Institute in Rotterdam, the Netherlands from June 1983 up to and including December 2017 were collected retrospectively. Patients with LPS NOS, distant metastases at time of diagnosis or with insufficient clinical data available were excluded.

Histological LPS subtypes were categorized according to the WHO classification and grading according to the FNCLCC [2]. Because of low numbers, tumors localized on the trunk and tumors localized in the head and neck region were combined, as well as retroperitoneal LPS with intrathoracic LPS. The resection margins were classified as R0 (microscopically negative margins), R1 (microscopically positive margins), R2 (macroscopically positive margins) or Rx (margins unknown/not assessed). During follow-up, information on vital status

(alive, death of disease, death of other/unknown cause) and recurrence (local and/or distant) were obtained. In case of retroperitoneal LPS, a local recurrence was defined as recurrence of disease within the abdomen, including multifocal recurrences. Due to the retrospective nature of our data source, no distinction between a multifocal peritoneal recurrence (e.g. two peritoneal tumor depositions) and peritoneal sarcomatosis, which perhaps represents a more advanced stage of disease, could be made. Distant metastasis of retroperitoneal LPS was defined as disease outside of the abdomen. Follow-up was performed according to national and international guidelines [18].

Statistical analysis

Categorical data were presented as numbers with percentages, and continuous data were presented as medians with corresponding interquartile ranges (IQR). Chi-square and Fisher's Exact tests were used when appropriate. The median follow-up time was calculated using the reversed Kaplan-Meier method [19].

Local recurrence-free survival (LRFS), distant metastasis-free survival (DMFS) and disease-specific survival (DSS) were defined as time (in months) between date of diagnosis and date of local recurrence, distant metastasis or death of disease, respectively. Time was censored at 5 years of follow-up for patients remaining free of local recurrences and distant metastasis or who were alive after 5 years of follow-up. The 5-year LRFS, DMFS and DSS were estimated using the Kaplan-Meier method and differences between subgroups were tested for their significance using the log-rank test.

To adjust for other prognostic factors, multivariable Cox regression analyses for LRFS, DMFS and DSS were performed. Firstly, the factors were tested univariably, and were added to the multivariable model in case the p-value was <0.05 , together with the factor coding for tumor location. The results were reported as hazard ratios (HR) with their corresponding 95% confidence intervals (95% CI). The main results of the Cox regression analyses are summarized in an overview, the complete results of both the univariable and multivariable analyses are presented in the supplemental tables. All statistical analyses were performed using SPSS (IBM SPSS Statistics, version 24).

Results

Patient characteristics

In total, 518 patients were identified who were diagnosed with and treated for LPS. There were slightly more males (56%) than females (44%), and the median age at time of diagnosis was 59 years (IQR 46–68). Most of the patients had a WDLPS (48%), followed by DDLPS (24%), MLPS (21%) and PLPS (8%). Most of the tumors were localized in one of the extremities

(49%), followed by the retroperitoneum/intrathoracic cavity (29%), trunk/head and neck region (15%) and testicular/inguinal region (7%). Most tumors were low-grade, due to the large proportion of WDLPS, and the median tumor size was 16 cm (IQR 10–23). A quarter of the patients received radiotherapy, mostly adjuvant, while a small fraction received chemotherapy (4%) or an ILP (6%) as part of their primary treatment. The median follow-up time was 68 months (IQR 31–138)(Table 1).

Tumor location versus liposarcoma subtype

More than half of the tumors localized in one of the extremities were WDLPS (51%), a third of the tumors MLPS (34%) and only a small proportion wereDDLPS (7%) or PLPS (8%). Retroperitoneal/intrathoracic LPS were mostly of theDDLPS (55%) and WDLPS (38%) subtype, while tumors localized on the trunk/head and neck region were mostly WDLPS (58%) and less often MLPS (17%) or PLPS (17%). At last, testicular/inguinal tumors were mainlyDDLPS (54%) and WDLPS (37%), and rarely PLPS (6%) or MLPS (3%)(Table 2).

6

Recurrence versus tumor location

In total, 36.7% of the patients developed a local recurrence (median time to local recurrence 23 months, IQR 11–58) and 17.4% developed distant metastasis (median time to metastasis 24 months, IQR 9–59)(Table 1). Since local recurrence and distant metastasis rates differ between LPS subtypes, the impact of tumor location was analyzed per subtype (Table 3). Patients with WDLPS developed significantly more often a local recurrence when the tumor was localized retroperitoneal/intrathoracic (53%) or in the testicular region (46%) than with tumors localized in the extremity (29%) or trunk/head and neck (30%, $p = 0.014$). Also patients with retroperitoneal/intrathoracicDDLPS experienced significantly more often a local recurrence (62%) than patients with other locations ofDDLPS (extremity 33%, trunk/head and neck 29%, testicular 37%, $p = 0.026$). In patients with MLPS ($p = 0.274$) and PLPS ($p = 0.703$) no differences in local recurrence rates between the different tumor locations were observed (Table 3).

Using Kaplan-Meier analysis, 5y-LRFS differed significantly between the different tumor locations ($p < 0.001$, Fig. 1A), with 5y-LRFS rates of 73.9% for patients with extremity LPS, 70.3% for patients with trunk/head and neck LPS, 64.5% for patients with testicular LPS and 35.8% for patients with retroperitoneal/intrathoracic LPS. After adjustment for other prognostic factors (LPS subtype, age, tumor size, status of the resection margins, neoadjuvant/adjuvant radiotherapy, chemotherapy and ILP) in a multivariable Cox regression analysis, tumor location was no longer of prognostic value (Table 4, Supplemental Table S1).

Table 1. Patient characteristics (N=518).

		N	%
Gender	Male	290	56.0
	Female	228	44.0
Age (years), median (IQR)		59 (46-68)	
Subtype	WDLPS	246	47.5
	DDLPS	126	24.3
	MLPS	107	20.7
	PLPS	39	7.5
Location	Extremity	254	49.0
	RPS + intrathoracic	150	29.0
	Trunk + head&neck	79	15.3
	Testis/inguinal	35	6.8
Grade	I	297	57.3
	II	36	6.9
	III	76	14.7
	Unknown	109	21.0
Resection margins	R0	149	28.8
	R1	197	38.0
	R2	45	8.7
	Rx	106	20.5
	No resection	21	4.1
Tumor size (cm), median (IQR)		16 (10-23)	
RTx	No	387	74.7
	Neoadjuvant	31	6.0
	Adjuvant	100	19.3
CTx	No	503	97.1
	Neoadjuvant	8	1.5
	Adjuvant	7	1.4
ILP	No	491	94.6
	Neoadjuvant	28	5.4
Local recurrence	No	328	63.3
	Yes	190	36.7
Distant metastases	TLR (months), median (IQR)	23 (10.8-58)	
	No	428	82.6
	Yes	90	17.4
Survival	TSD (months), median (IQR)	23.5 (8.8-58.5)	
	Alive	352	68.0
	Death of disease	122	23.6
	Death of other/unknown cause	44	8.5
	Follow-up (months), median (IQR)	68 (31-138)	

WDLPS, well-differentiated liposarcoma; DDLPS, dedifferentiated liposarcoma; MLPS, myxoid liposarcoma; PLPS, pleomorphic liposarcoma; RPS, retroperitoneal sarcoma; IQR, interquartile range; RTx, radiotherapy; ILP, isolated limb perfusion; CTx, chemotherapy; TLR, time to local recurrence; TSD, time to systemic disease

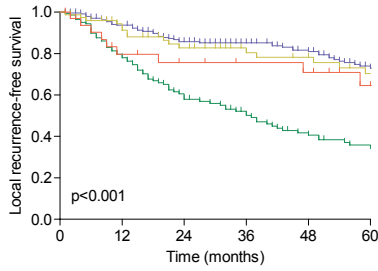
With regard to the distant metastasis rate, no significant differences between the different tumor locations in any of the LPS subtypes were observed (WDLPS: $p = 0.773$, DDLPS: $p = 0.321$, MLPS: $p = 0.556$, PLPS: $p = 0.512$, overall: $p = 0.600$)(Table 3). Additionally, there were no differences in the 5y-DMFS between the different tumor locations, with 5y-DMFS rates of 89.0% for patients with trunk/head and neck LPS, 84.8% for patients with extremity LPS, 80.1% for patients with testicular LPS and 77.3% for patients with retroperitoneal/intrathoracic LPS ($p = 0.241$, Fig. 1B). Also in the multivariable analysis no significant impact for tumor location was observed (Table 4, Supplemental Table S2).

Table 2. LPS subtype per primary tumor location

	Extremity	RPS + intrathoracic	Trunk + head&neck	Testis	Total
WDLPS	130 (51)	57 (38)	46 (58)	13 (37)	246 (48)
DDLPS	18 (7)	82 (55)	7 (9)	19 (54)	126 (24)
MLPS	86 (34)	7 (5)	13 (17)	1 (3)	107 (21)
PLPS	20 (8)	4 (3)	13 (17)	2 (6)	39 (8)
Total	254 (100)	150 (100)	79 (100)	35 (100)	518 (100)

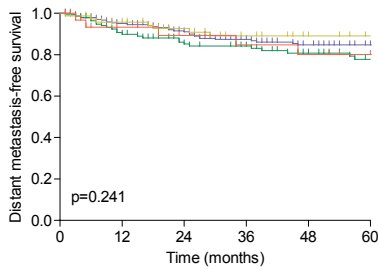
WDLPS, well-differentiated liposarcoma; DDLPS, dedifferentiated liposarcoma; MLPS, myxoid liposarcoma; PLPS, pleomorphic liposarcoma; RPS, retroperitoneal sarcoma

A - Local recurrence-free survival



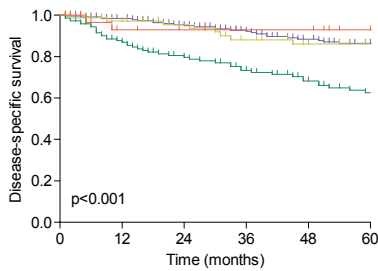
No. at risk		0	12	24	36	48	60
Extremity	254	205	163	131	111	87	
RPS/intrathoracic	150	97	66	52	37	27	
Trunk/H&N	79	61	45	36	32	24	
Testis	35	22	18	16	15	10	

B - Distant metastasis-free survival



No. at risk		0	12	24	36	48	60
Extremity	254	210	177	140	124	110	
RPS/intrathoracic	150	107	89	78	63	51	
Trunk/H&N	79	63	53	44	39	34	
Testis	35	25	21	18	17	14	

C - Disease-specific survival



No. at risk		0	12	24	36	48	60
Extremity	254	219	188	153	134	116	
RPS/intrathoracic	150	113	94	79	64	53	
Trunk/H&N	79	64	55	45	39	34	
Testis	35	25	22	20	20	15	

▲ Fig. 1. Five-year local recurrence-free survival (A), 5-year distant metastasis-free survival (B) and 5-year disease-specific survival (C) per tumor location of all patients diagnosed with and treated for liposarcoma. P-values were calculated using the log-rank test. RPS: retroperitoneal liposarcoma, H&N: head & neck.

Table 3. Number of patients with a local recurrence (LR) and/or distant metastasis (DM).

		LR, n (%)			DM, n (%)		
		No	Yes	p	No	Yes	p
WDLPS	Extremity	92 (71)	38 (29)	0.014	127 (98)	3 (2)	0.773
	RPS + intrathoracic	27 (47)	30 (53)		55 (96)	2 (4)	
	Trunk + head&neck	32 (70)	14 (30)		44 (96)	2 (4)	
	Testis	7 (54)	6 (46)		13 (100)	0 (0)	
	Total	158 (64)	88 (36)		239 (97)	7 (3)	
DDLPS	Extremity	12 (67)	6 (33)	0.026	16 (89)	2 (11)	0.321
	RPS + intrathoracic	31 (38)	51 (62)		58 (71)	24 (29)	
	Trunk + head&neck	5 (71)	2 (29)		5 (71)	2 (29)	
	Testis	12 (63)	7 (37)		12 (63)	7 (37)	
	Total	60 (48)	66 (52)		91 (72)	35 (28)	
MLPS	Extremity	65 (76)	21 (24)	0.274	59 (69)	27 (31)	0.556
	RPS + intrathoracic	4 (57)	3 (43)		5 (71)	2 (29)	
	Trunk + head&neck	10 (77)	3 (23)		10 (77)	3 (23)	
	Testis	0 (0)	1 (100)		0 (0)	1 (100)	
	Total	79 (74)	28 (26)		74 (69)	33 (31)	
PLPS	Extremity	17 (85)	3 (15)	0.703	10 (50)	10 (50)	0.512
	RPS + intrathoracic	3 (75)	1 (25)		3 (75)	1 (25)	
	Trunk + head&neck	9 (69)	4 (31)		9 (69)	4 (31)	
	Testis	2 (100)	0 (0)		2 (100)	0 (0)	
	Total	31 (79)	8 (21)		24 (62)	15 (38)	
Total	328 (63)	190 (37)	<0.001*	428 (83)	90 (17)	0.600*	

* χ^2 -test, all other tests were Fisher's Exact tests. WDLPS, well-differentiated liposarcoma; DDLPS, dedifferentiated liposarcoma; MLPS, myxoid liposarcoma; PLPS, pleomorphic liposarcoma; RPS, retroperitoneal sarcoma; LR, local recurrence; DM, distant metastasis

Remarkably, 7 of the patients with WDLPS developed metastatic disease, while this subtype is known for its lacking metastatic potential, unless the tumor undergoes dedifferentiation at recurrence. This was indeed the case for five out of the seven patients with metastatic 'WDLPS'. The sixth patient had multiple local recurrences which were all WDLPS, but at time of the last (multifocal) local recurrence also multiple lung lesions suspected for metastases were discovered. However, no biopsy or resection was performed on either the local recurrence or one of the lung lesions. A few months after the diagnosis of lung metastases the patient died. So, in our opinion it was likely that dedifferentiation also had occurred in this patient. The last patient developed a local recurrence and a paravertebral lesion simultaneously. The local recurrence was biopsied and showed WDLPS without any signs of dedifferentiation, but no biopsy of the paravertebral lesion was obtained. The patient is still alive, after 'palliative' radiotherapy of 24Gy, with a follow-up period of 60 months (42 months after discovery of the paravertebral lesion), so we doubt if this atypical paravertebral lesion indeed was a metastasis.

Table 4. Impact of tumor location on local recurrence-free survival (LRFS), distant metastasis-free survival (DMFS) and disease-specific survival (DSS) after adjustment for other prognostic factors. Complete results of the Cox regression analyses are shown in supplemental tables S1, S2 and S3.

		N	HR	95% CI	p
LRFS	Extremity	223	Ref		
	RPS + intrathoracic	135	1.46	0.92-2.32	0.110
	Trunk + head&neck	68	1.16	0.66-2.05	0.604
	Testis/inguinal	30	1.29	0.62-2.70	0.503
DMFS	Extremity	254	Ref		
	RPS + intrathoracic	150	1.78	0.86-3.70	0.123
	Trunk + head&neck	79	1.08	0.53-2.21	0.838
	Testis/inguinal	35	1.64	0.66-4.03	0.285
DSS	Extremity	223	Ref		
	RPS + intrathoracic	135	5.08	2.41-10.71	<0.001
	Trunk + head&neck	68	1.87	0.81-4.30	0.142
	Testis/inguinal	30	1.15	0.32-4.14	0.826

LRFS, local recurrence-free survival; DMFS, distant metastasis-free survival; DSS, disease-specific survival; RPS, retroperitoneum; RTx, radiotherapy; CTx, chemotherapy; ILP, isolated limb perfusion; Tx, treatment; HR, hazard ratio; 95% CI, 95% confidence interval.

Survival versus tumor location

The 5y-DSS differed significantly between the different tumor locations ($p < 0.001$, Fig. 1C), with the best prognosis for patients with testicular LPS (5y-DSS 93.0%), patients with extremity LPS (86.4%) and trunk/head and neck LPS (86.1%). Patients with retroperitoneal/intrathoracic LPS had a worse prognosis with a 5y-DSS rate of 62.2%. Also after adjustment for other prognostic factors, a retroperitoneal/intrathoracic tumor location had a worse prognosis compared to tumor location in the extremity (HR 5.08, 95% CI 2.41–10.71, $p < 0.001$, Table 4, Supplemental Table S3).

Since the group of retroperitoneal/intrathoracic LPS mainly consisted of patients with WDLPS or DDLPS (total 93%, Table 2), an additional DSS analysis for these two LPS subtypes was performed to explore whether the worse prognosis was due to large proportion of DDLPS in this subgroup. As expected, DDLPS patients with a retroperitoneal/intrathoracic location had the worst prognosis (5y-DSS 50.3%), together with patients with a DDLPS on the trunk/head and neck (44.4%), followed by DDLPS patients with a location in the extremity (84.0%) and testis (88.2%, $p = 0.023$, Supplemental Fig. S1A). Also when analyzing patients with WDLPS, patients with retroperitoneal/intrathoracic WDLPS had a worse prognosis (5y-DSS 80.5%), while WDLPS patients with tumor locations in the extremity (99.2%), trunk/head and neck (100%) or testis (100%) had an excellent prognosis ($p < 0.001$, Supplemental Fig. S1B). Only one patient with non-retroperitoneal WDLPS died of disease within 5 years of follow-up, which turned out to be treatment-related (5 days after ILP). In the total follow-up period, 4 patients with non-retroperitoneal WDLPS died of disease (after 75, 179, 210 and

226 months), all after dedifferentiation upon recurrence. Only of one patient with multiple local recurrences and lung metastases, dedifferentiation was not pathologically confirmed.

Neoadjuvant/adjuvant treatment in LPS

Neoadjuvant/adjuvant treatment of the primary tumor was also included in the Cox regression analyses for LRFS, DMFS and DSS. For LRFS, radiotherapy as well as chemotherapy and ILP tested significantly in univariable analysis, but only radiotherapy remained of significant influence in the multivariable analysis, reducing the risk of a local recurrence (HR 0.19, 95% CI 0.11–0.35, $p < 0.001$, Supplemental Table S1). Also for DMFS all three treatment modalities tested significantly in univariable analysis, but none of them remained significant in multivariable analysis (Supplemental Table S2). For DSS, only CTx and ILP tested significantly in univariable analysis, but again both lost their prognostic value in multivariable analysis (Supplemental Table S3).

6

Discussion

The results of this study show that primary tumor location has an impact on local recurrence-free survival and disease-specific survival, while no differences in distant metastasis-free survival were observed.

Despite that there was no difference in DMFS, patients with retroperitoneal/intrathoracic LPS have a worse prognosis than patients with a LPS localized elsewhere. Generally, patients with cancer die because of metastatic disease, but these data confirmed that retroperitoneal/intrathoracic LPS is one of the few entities where patients also can die because of local disease, as indicated by the worse LRFS. This is further underlined by Fig. 1, showing that the DSS is worse than the DMFS and that the DSS curve of patients with retroperitoneal/intrathoracic LPS mimics the LRFS curve. For the other tumor locations, the DSS curves resemble the DMFS curves more. The worse prognosis of these patients might be explained by the large proportion of patients with DDLPS or a higher percentage of irradical resections (R1/R2) in this subgroup. However, after adjusting for the status of resection margins and for LPS subtype in a multivariable analysis, still a worse DSS for patients with retroperitoneal/intrathoracic tumors was observed. Additionally, we separately analyzed the patients with WDLPS, and patients with a retroperitoneal/intrathoracic tumor location again had a worse prognosis than patients with WDLPS on other locations (Supplemental Fig. S1). Multiple explanations for the worse prognosis of retroperitoneal/intrathoracic LPS can be thought of, including delayed detection because of a lack of symptoms, allowing the tumor to grow silently and resulting in more complex and extensive surgery, but it is still unclear if it is indeed a matter of time or whether there is a biological reason for an unfavorable clinical

outcome.

Since patients with retroperitoneal/intrathoracic LPS die because of local disease and local control proved to be essential, we might need to reconsider the local treatment options, consisting of 1) surgery and 2) radiotherapy. Evidently, the goal of surgery is complete resection of the tumor, but especially for retroperitoneal sarcomas there is an ongoing discussion regarding the appropriate extent of resection. Usually, a 'simple' complete resection is performed, enucleating the tumor, sometimes in combination with en-bloc resection of an involved adjacent organ. However, there are clues that a compartmental resection, during which also uninvolved adjacent organs are resected to ensure wide margins, is associated with lower recurrences rates and improved overall survival [20-22]. However, these studies were based on retrospective data, which inherently leads to selection and information bias amongst others, and compartmental resections might lead to higher complications rates. Secondly, the use of neoadjuvant/adjuvant radiotherapy in this patient group might be reconsidered. Neoadjuvant/adjuvant radiotherapy as part of the primary treatment had no significant effect on DMFS or DSS, but did have a protective effect on LRFS in multivariable analysis in this study. Additionally, a previous study on extremity LPS also showed that an aggressive treatment approach (resection with wide margins and radiotherapy) resulted in excellent local control in extremity WDLPS, but also that this did not result in better disease-specific survival [23]. Given its toxicity, varying effectivity and missing effect on survival, we are currently reluctant in giving radiotherapy in our center, despite the better local control. Only a quarter of the patients received radiotherapy in this cohort, whereas this percentage might be higher in other centers/cohorts [23]. However, since local control appears to be crucial in retroperitoneal/intrathoracic LPS, the use of radiotherapy for the sake of local control needs to be reevaluated, which is currently being done in the STRASS trial. Although the first results of the STRASS trial – randomizing between neoadjuvant radiotherapy plus surgery versus surgery alone for patients with retroperitoneal sarcoma – overall showed no benefit of neoadjuvant radiotherapy in terms of abdominal recurrence-free survival, a subgroup analysis demonstrated that neoadjuvant radiotherapy might benefit the LPS subgroup [24]. However, the final results, including data on overall survival, are pending and needed to see whether the improved abdominal recurrence-free survival will result in improved overall survival.

To the best of our knowledge, there were only two studies comparing the outcomes of the different LPS subtypes taking all tumor locations into account: one study in which tumor location was not of prognostic value [17] and one study in which it was of prognostic value [16]. In the latter study, patients with retroperitoneal disease also had a worse prognosis than patients with tumors in the lower extremity, upper extremity or trunk. This study confirms these results, but contradicts the results of the other study. Possible explanations for the

different outcomes could be the distribution of LPS subtypes, the distribution of the different tumor locations or the number of included patients. The distribution of LPS subtypes was comparable between the two studies and our study, but in the study of Knebel et al. [17] only 130 patients were included, of whom almost 85% had a tumor localized in the extremity, approximately 10% in the retroperitoneum/pelvis, and only 4.5% in the trunk/head and neck region and 1% in the spermatic cord. On the contrary, Dalal et al. [16] included 801 patients with a distribution of the tumor locations comparable to ours, with 56.5% of the tumors localized in the extremity, 28% in the retroperitoneum and 11% in the trunk.

The survival rates observed in this study are comparable to the survival rates reported in literature. For retroperitoneal LPS, 5-year overall survival (5y-OS) rates of 60% (all LPS) [11], 57% (only 50% LPS included) [20] and 54% (58% LPS) [8] have been reported, compared to 5y-DSS of 62% in this study. For extremity LPS, a 5y-DSS rate of 80% [9], 12y-DSS rate of 87% for the upper extremity and 82% [16] for lower extremity LPS have been reported, compared to 5y-DSS of 86% in our study.

Evidently, our study has some limitations. Because of the retrospective nature, which is inevitable when studying rare diseases, selection bias and information bias may have been introduced. We tried to minimize the selection bias by including all LPS patients without any exclusion criteria except for insufficient available data and metastatic disease at diagnosis. Strengths of this study are the large number of included patients and that the results are based on daily clinical practice.

Currently, treatment is more or less similar for the different LPS subtypes or tumor locations, but more and more evidence is becoming available showing STS and even LPS is not a single entity. For each STS/LPS subtype, and maybe even for each tumor location, a different treatment approach might be needed and preferable.

Conclusion

A retroperitoneal/intrathoracic tumor location had a negative effect on disease-specific survival of LPS patients. These patients also developed local recurrent disease more often than patients with other tumor locations, but no differences in distant metastases were observed. This implies that these patients die of local disease instead of metastatic disease and that the local treatment options, including the extent of surgery and radiotherapy, should be reevaluated. Radiotherapy improved local control, but had no effect on distant metastasis-free survival or disease-specific survival in this cohort. Therefore, pending the final results on overall survival of the STRASS trial, the use of radiotherapy in retroperitoneal/intrathoracic LPS should be reconsidered, since in this patient group local control proved to be of essential importance. Lastly, patients with WDLPS in the extremity, trunk or testicular

region did not die of disease, except for rare cases in whom the tumor had dedifferentiated upon recurrence.

Conflicts of interest

None of the authors declare any conflicts of interest.

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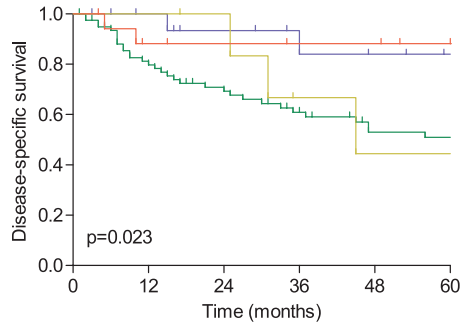
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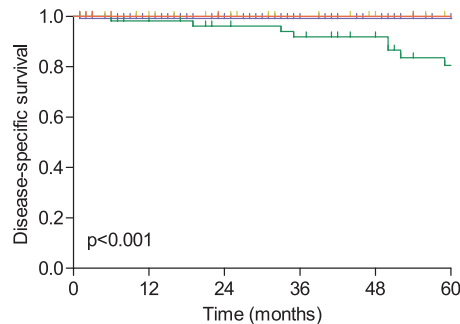
Appendix A. Supplementary data

A - Dedifferentiated liposarcoma



No. at risk	0	12	24	36	48	60
Extremity	18	15	12	10	8	6
RPS/intrathoracic	82	57	45	36	26	25
Trunk/H&N	7	7	6	3	2	2
Testis	19	14	12	11	11	8

B - Well-differentiated liposarcoma



No. at risk	0	12	24	36	48	60
Extremity	130	107	92	77	68	59
RPS/intrathoracic	57	52	46	42	36	26
Trunk/H&N	46	34	30	26	23	19
Testis	13	8	7	7	7	6

▲ **Supplemental Figure S1.** Five-year disease-specific survival per tumor location specified for patients with (A) dedifferentiated liposarcoma (DDLPS) and (B) well-differentiated liposarcoma (WDLPS). RPS: retroperitoneal liposarcoma, H&N: head & neck.

Supplemental Table S1. Complete results of the univariable and multivariable Cox regression analyses for local recurrence-free survival.

	Univariable				Multivariable			
	N	HR	95% CI	p	N	HR	95% CI	p
Age at time of diagnosis	518	1.02	1.00-1.03	0.006	456	1.02	1.00-1.03	0.009
Gender								
Male	290	Ref						
Female	228	1.14	0.86-1.52	0.363				
Subtype								
WDLPS	246	Ref			218	Ref		
DDLPS	126	2.07	1.50-2.85	<0.001	113	2.39	1.59-3.60	<0.001
MLPS	107	0.59	0.38-0.90	0.014	89	1.70	0.94-3.05	0.078
PLPS	39	0.49	0.24-1.00	0.051	36	1.66	0.70-3.94	0.249
Grade								
I	297	Ref						
II	36	1.14	0.66-1.95	0.646				
III	76	1.42	0.95-2.13	0.091				
Unknown	109	1.24	0.86-1.79	0.243				
Location								
Extremity	254	Ref			223	Ref		
RPS + intrathoracic	150	3.40	2.46-4.70	<0.001	135	1.46	0.92-2.32	0.110
Trunk + head&neck	79	1.23	0.77-1.98	0.388	68	1.16	0.66-2.05	0.604
Testis/inguinal	35	1.70	0.95-3.03	0.072	30	1.29	0.62-2.70	0.503
Tumor size	469	1.05	1.03-1.06	<0.001	456	1.02	1.01-1.04	0.012
Resection margins								
R0	149	Ref			136	Ref		
R1	197	1.49	1.02-2.20	0.042	191	1.17	0.74-1.86	0.503
R2	45	4.07	2.39-6.91	<0.001	44	3.66	1.96-6.81	<0.001
Rx	106	2.24	1.50-3.32	<0.001	85	1.39	0.84-2.32	0.204
RTx								
No	387	Ref			335	Ref		
Yes	131	0.21	0.13-0.35	<0.001	121	0.19	0.11-0.35	<0.001
CTx								
No	503	Ref			446	Ref		
Yes	15	2.48	1.15-5.32	0.020	10	1.69	0.75-3.81	0.210
ILP								
No	490	Ref			429	Ref		
Yes	28	0.36	0.15-0.88	0.026	27	0.71	0.28-1.84	0.479

WDLPS, well-differentiated liposarcoma; DDLPS, dedifferentiated liposarcoma; MLPS, myxoid liposarcoma; PLPS, pleomorphic liposarcoma; RPS, retroperitoneum; RTx, radiotherapy; CTx, chemotherapy; ILP, isolated limb perfusion; Tx, treatment; HR, hazard ratio; 95% CI, 95% confidence interval.

Supplemental Table S2. Complete results of the univariable and multivariable Cox regression analyses for distant metastasis-free survival.

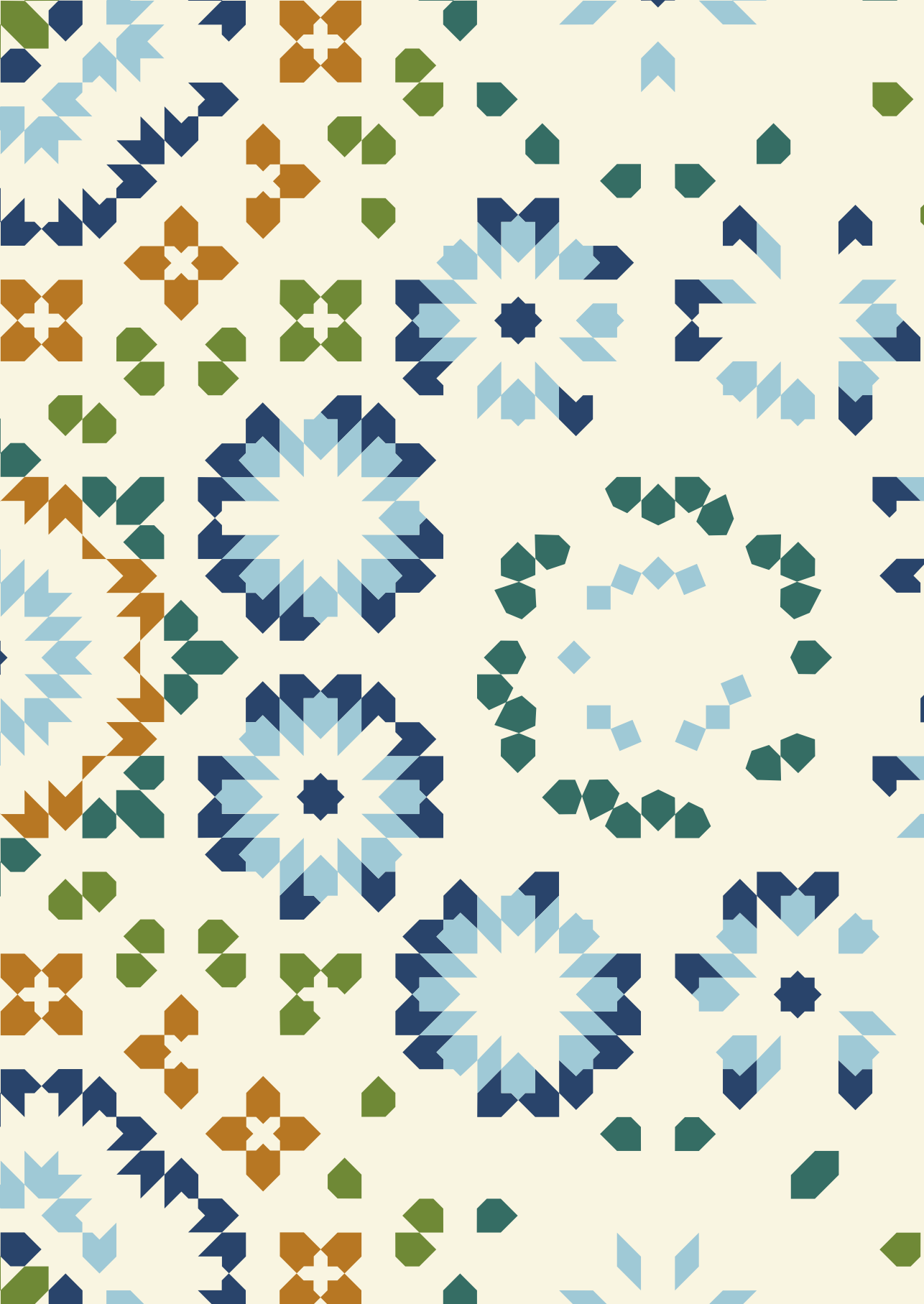
	Univariable				Multivariable			
	N	HR	95% CI	p	N	HR	95% CI	p
Age at time of diagnosis	518	0.99	0.98-1.01	0.180				
Gender								
Male	290	Ref						
Female	228	0.71	0.46-1.08	0.108				
Subtype								
WDLPS	246	Ref			246	Ref		
DDLPS	126	13.08	5.80-29.52	<0.001	126	6.74	2.46-18.44	<0.001
MLPS	107	10.34	4.57-23.37	<0.001	107	8.43	3.26-21.84	<0.001
PLPS	39	15.53	6.33-38.10	<0.001	39	9.02	2.96-27.46	<0.001
Grade								
I	297	Ref			297	Ref		
II	36	4.42	2.13-9.16	<0.001	36	1.20	0.53-2.72	0.670
III	76	8.47	4.85-14.80	<0.001	76	2.18	1.07-4.41	0.031
Unknown	109	4.60	2.59-8.15	<0.001	109	1.31	0.67-2.56	0.437
Location								
Extremity	254	Ref			254	Ref		
RPS + intrathoracic	150	1.38	0.86-2.21	0.187	150	1.78	0.86-3.70	0.123
Trunk + head&neck	79	0.82	0.42-1.60	0.569	79	1.08	0.53-2.21	0.838
Testis/inguinal	35	1.45	0.68-3.09	0.336	35	1.64	0.66-4.03	0.285
Tumor size	473	1.01	0.99-1.04	0.219				
Resection margins								
R0	149	Ref						
R1	197	0.73	0.45-1.19	0.204				
R2	45	1.71	0.84-3.46	0.137				
Rx	106	0.46	0.24-0.86	0.016				
RTx								
No	387	Ref			387	Ref		
Yes	131	2.11	1.39-3.21	0.001	131	1.11	0.65-1.88	0.702
CTx								
No	503	Ref			503	Ref		
Yes	15	2.95	1.19-7.28	0.019	15	1.87	0.71-4.96	0.208
ILP								
No	490	Ref			490	Ref		
Yes	28	2.97	1.65-5.35	<0.001	28	1.66	0.84-3.28	0.143

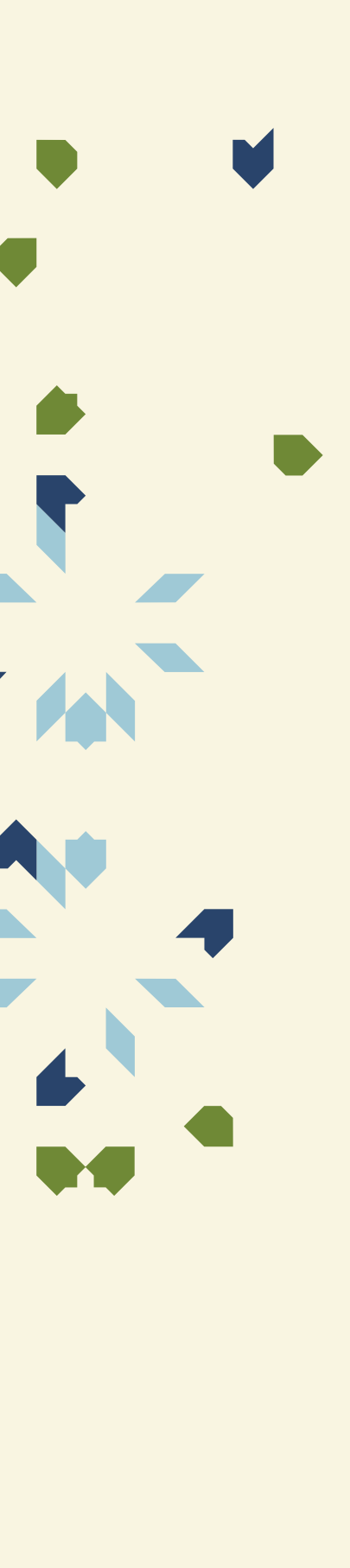
WDLPS, well-differentiated liposarcoma; DDLPS, dedifferentiated liposarcoma; MLPS, myxoid liposarcoma; PLPS, pleomorphic liposarcoma; RPS, retroperitoneum; RTx, radiotherapy; CTx, chemotherapy; ILP, isolated limb perfusion; Tx, treatment; HR, hazard ration; 95% CI, 95% confidence interval.

Supplemental Table S3. Complete results of the univariable and multivariable Cox regression analyses for disease-specific survival.

		Univariable			p	Multivariable			
		N	HR	95% CI		N	HR	95% CI	p
Age at time of diagnosis		518	1.02	1.00-1.03	0.029	456	1.01	1.00-1.03	0.126
Gender	Male	290	Ref						
	Female	228	0.90	0.63-1.29	0.569				
Subtype	WDLPS	246	Ref			218	Ref		
	DDLPS	126	7.28	4.44-11.94	<0.001	113	3.74	1.70-8.22	0.001
	MLPS	107	2.53	1.44-4.42	0.001	89	3.99	1.56-10.18	0.004
	PLPS	39	4.39	2.24-8.59	<0.001	36	7.17	2.45-20.99	<0.001
Grade	I	297	Ref			260	Ref		
	II	36	2.40	1.22-4.71	0.011	33	1.06	0.44-2.54	0.894
	III	76	5.64	3.52-9.02	<0.001	71	2.01	0.99-4.07	0.053
	Unknown	109	4.14	2.62-6.52	<0.001	92	1.90	0.95-3.81	0.071
Location	Extremity	254	Ref			223	Ref		
	RPS + intrathoracic	150	4.00	2.68-5.96	<0.001	135	5.08	2.41-10.71	<0.001
	Trunk + head&neck	79	0.88	0.45-1.74	0.718	68	1.87	0.81-4.30	0.142
	Testis/inguinal	35	0.74	0.26-2.08	0.568	30	1.15	0.32-4.14	0.826
Tumor size		473	1.05	1.03-1.07	<0.001	456	1.04	1.02-1.07	<0.001
Resection margins	R0	149	Ref			136	Ref		
	R1	197	1.11	0.70-1.76	0.671	191	0.63	0.34-1.14	0.127
	R2	45	4.08	2.28-7.29	<0.001	44	2.17	1.07-4.41	0.032
	Rx	106	0.68	0.40-1.18	0.172	85	0.61	0.30-1.23	0.167
RTx	No	387	Ref						
	Yes	131	0.87	0.57-1.33	0.523				
CTx	No	503	Ref			446	Ref		
	Yes	15	3.92	1.98-7.77	<0.001	10	1.10	0.44-2.71	0.845
ILP	No	490	Ref			429	Ref		
	Yes	28	1.93	1.08-3.43	0.026	27	1.69	0.76-3.75	0.198

WDLPS, well-differentiated liposarcoma; DDLPS, dedifferentiated liposarcoma; MLPS, myxoid liposarcoma; PLPS, pleomorphic liposarcoma; RPS, retroperitoneum; RTx, radiotherapy; CTx, chemotherapy; ILP, isolated limb perfusion; Tx, treatment; HR, hazard ration; 95% CI, 95% confidence interval.





CHAPTER 7

DIFFERENCES IN RECURRENCE AND SURVIVAL OF EXTREMITY LIPOSARCOMA SUBTYPES

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Abstract

Background: Liposarcomas can be divided into four subtypes and are most frequently located in the extremities. There are currently no studies comparing the clinical outcomes, such as local recurrence and distant metastasis, between the distinct subtypes of primary LPS of the extremity specifically.

Methods: Retrospective databases of two expertise centres (Rotterdam-R, Warsaw-W) of patients with liposarcoma located in the extremities from 1985 to 2015 were used to analyse 5-year local recurrence-free survival (5y-LRFS), 5-year distant metastasis-free survival (5y-DMFS) and 5-year overall survival (5y-OS).

Results: We identified 456 patients: 192 well-differentiated liposarcomas (WDLPS), 172 myxoid liposarcomas (MLPS), 54 pleomorphic liposarcomas (PLPS), 23 dedifferentiated liposarcomas (DDLPS) and 15 other subtypes. The frequency of (neo)adjuvant radiotherapy (R: 34.5% vs. W: 78.4%) and R0-resections (R: 41.0% vs. W: 84.1%) differed between the datasets. Local recurrences (LR) were observed most frequently in DDLPS (5y-LRFS 62.4%), followed by PLPS (71.4%), WDLPS (77.0%) and MLPS (84.5%, $p = 0.054$). Distant metastases (DM) were most commonly observed in PLPS (5y-DMFS 46.9%), followed by MLPS (74.0%), DDLPS (86.3%) and WDLPS (97.3%, $p < 0.001$). 5y-OS was poorest in patients with PLPS (47.6%) and DDLPS (54.4%), followed by MLPS (79.7%) and WDLPS (92.4%, $p < 0.001$). Male gender significantly increased the risk of LR and DM. The subtypes MLPS and PLPS were significant prognostic factors for DM and OS. Additionally, DDLPS and age had significant impact on OS.

Conclusion: In the largest cohort of extremity LPS patients reported to date, LPS subtypes show distinct patterns of LR, DM and OS, stressing that 'extremity LPS' is not a single entity.

Keywords: Liposarcoma; Extremity; Survival; Recurrence; Treatment

Introduction

Liposarcoma (LPS) is one of the most common subtypes of soft tissue sarcoma (STS), representing approximately 20% of all STS [1, 2]. They arise from lipoblasts and can be located throughout the body, but are most frequently localised in the extremities. Based on their morphological and genetic features, four major subtypes can be distinguished: well-differentiated liposarcoma (WDLPS), dedifferentiated liposarcoma (DDLPS), myxoid liposarcoma (MLPS) and pleomorphic liposarcoma (PLPS). Additionally, there is a small residual group with liposarcomas not otherwise specified (LPS NOS) [3]. These four major subtypes harbour distinct molecular aberrations. Where WDLPS (also known as atypical lipomatous tumours) and DDLPS – which are thought to arise from WDLPS – are characterized by amplification of the *MDM2* gene, MLPS harbours in >90% of the patients a translocation of chromosome t(12;16)(q13;p11) resulting in the expression of the fusion protein FUS-CHOP. The last subtype, PLPS, has a complex karyotype with multiple defects.

Treatment of extremity LPS without distant metastases usually consists of surgical resection, optionally preceded by neoadjuvant radiotherapy, chemotherapy or isolated limb perfusion, and/or followed by adjuvant radiotherapy or chemotherapy. Indications for neoadjuvant/adjuvant treatment include large tumour size, involvement of the neurovascular bundle (neoadjuvant treatment) and positive resection margins (adjuvant therapy) amongst others. Despite optimal (multimodality) treatment, the different LPS subtypes often relapse locally and/or at distant sites, a situation in which patients generally only have very little chance of cure. Few studies have investigated the factors impacting recurrences of primary LPS of the extremities, but these studies were conducted mostly on small patient groups, using all primary tumour sites, focussing on all STS types or just one of the LPS subtypes [4-14], rather than comparing the four major LPS subtypes in a larger cohort on one primary tumour site. This is in contrast with retroperitoneal STS or LPS, in which several studies already have been conducted and there is more clarity on different factors predicting recurrence and survival [15-17].

The Erasmus MC Cancer Institute in Rotterdam and Maria Skłodowska-Curie Institute-Oncology Center in Warsaw are two tertiary referral centres and expertise centres for STS, treating a substantial part of STS patients in the Netherlands and Poland, respectively. The aim of this study is to investigate the differences in recurrence and survival of the different subtypes of primary LPS of the extremity, including the influence of diverse clinicopathological factors, in the largest cohort of extremity LPS patients reported to date.

Methods

Patient characteristics

All patients treated for primary extremity LPS between 1986 and 2015 at the Erasmus MC Cancer Institute (Rotterdam-cohort), and between 1990 and 2015 at the Maria Skłodowska-Curie Institute-Oncology Center (Warsaw-cohort) were identified based on pathology reports, and data was collected retrospectively. Patients with metastatic disease or local recurrence at presentation, or with insufficient clinical information available were excluded. This study was performed in accordance with local ethics committee guidelines and national legislation.

Histologic subtypes were classified following the WHO classification of soft tissue tumours, and round-cell LPS was considered high-grade MLPS [3]. Cases in which tumour characteristics (particularly subtype) were not clear based on the pathology reports were reviewed by a pathologist with expertise in soft tissue sarcomas. Resection margins were classified as R0 (microscopic negative margins), R1 (microscopic positive margins), R2 (macroscopic positive margins) or Rx (unknown/not assessed). In the Rotterdam-cohort, patients who received radiotherapy were treated with standard schedules of 50 Gy (preoperatively) or 60-70 Gy (postoperatively). In the Warsaw-cohort, hypofractionated radiotherapy schedules of 5 x 4-5 Gy (preoperatively) and standard schedules of 60-70 Gy (post-operatively) were used. In patients who received both preoperative and postoperative radiotherapy, postoperative radiotherapy was used as a boost because of unsure resection margins. Follow-up schedules slightly differed between the two centres, but both included one-time imaging of the local site 3-6 months after surgery, more frequent follow-up visits in the first 3-5 year after treatment and regular imaging of the chest. Complete follow-up schedules are shown in Appendix table A. During follow-up, information about status of disease (recurrence of disease, local and/or distant) and vital status (alive/death) was obtained. For the Rotterdam-cohort, also data on cause of death (death of disease, death of other/unknown cause) was available. Of patients who were classified as dying of unknown cause, there was no information on cause of death available, as well as no signs of recurrent or metastatic disease at last follow-up. These deaths were not attributed or unlikely related to LPS or LPS treatment.

Statistical methods

Patients' characteristics were described using descriptive statistics. Fisher's Exact tests, χ^2 -tests (for categorical data) and Mann-Whitney U tests (for continuous data) were used when appropriate. Median follow-up time with corresponding interquartile range (IQR) was calculated by using the reversed Kaplan-Meier method and statistics by the log-rank test [18]. Patients with LPS subtypes other than the four major subtypes (WDLPS, DDLPS, MLPS, and PLPS) were excluded from the survival and Cox regression analyses, because this subgroup

is too small. Local recurrence-free survival (LRFS), distant metastasis-free survival (DMFS) and overall survival (OS) were defined as time (in months) between diagnosis and the occurrence of local recurrence (LR), distant metastasis (DM) and death from any cause respectively. Additionally, disease-specific survival (DSS) was analysed for the Rotterdam-cohort, defined as time (in months) to death of disease. Time was censored at 5 years follow-up for patients remaining LR-free, DM-free or alive. LRFS, DMFS, DSS and OS curves were estimated by using the Kaplan-Meier method and reported as 5-year survival rates with corresponding standard errors (SE). Differences between subgroups were tested for their significance by the log-rank test. Because of the long time period during which patients were included, additional analyses were performed to determine whether there were differences in outcome over the years. To this end, incidence years were clustered into groups of five years and Kaplan-Meier estimates with log-rank test statistics were calculated.

Diverse clinicopathological variables (age at diagnosis, gender, LPS subtype, grade, site, size, resection margins, neoadjuvant and adjuvant treatment) were assessed for their impact on LR, DM and OS by Cox regression models. A variable coding for either one of the centres was added to the multivariable analyses to correct for differences in baseline characteristics. Due to the relatively small number of events, it was not possible to set up a reliable Cox regression analysis for DSS in the Rotterdam-cohort. Variables with a p-value <0.05 in univariable analysis were included in the multivariable Cox proportional hazard regression models. The definitive models were obtained with an enter method. Hazard ratios (HRs) with corresponding 95% confidence intervals (CIs) are described. P-values <0.05 were considered statistically significant. SPSS was used for statistical analyses (IBM SPSS Statistics for Windows, Version 21.0).

Results

Patient characteristics

In total, 456 patients were identified: 229 in the Rotterdam-cohort and 227 in the Warsaw-cohort, with a median follow-up time of 61 months. In the Rotterdam-cohort, we observed 113 patients with WDLPS (49.3%), 77 patients with MLPS (33.6%), 13 patients withDDLPS (5.7%), 20 patients with PLPS and 6 patients with other LPS subtypes (2.6%). In the Warsaw-cohort, more aggressive subtypes were observed: MLPS was the most common subtype (95 patients, 41.9%), followed by WDLPS with 79 patients (34.8%), PLPS (34 patients, 15.0%), DDLPS (10 patients, 4.4%) and other LPS subtypes (9 patients, 4.0%). Subsequently, significantly more high grade tumours were observed in the Warsaw-cohort. In the Rotterdam-cohort, in total 34.5% received neoadjuvant/adjuvant radiotherapy, where in the Warsaw-cohort in total 78.9% of the patients received neoadjuvant/adjuvant radiotherapy ($p < 0.001$, Table 1). Furthermore, there were significantly more R0 (radical) resections in the Warsaw-cohort

(84.1%) compared to the Rotterdam-cohort (41.0%, $p < 0.001$) and patients in the Rotterdam-cohort experienced local recurrence more often (Rotterdam: 26.% vs. Warsaw: 11.9%, $p < 0.001$). The patients with other LPS subtypes were excluded from further analyses.

Table 1. Patient characteristics.

		Total (N=456)		Rotterdam (N=229)		Warsaw (N=227)		p-value ^a
		N	% of total	N	% of total	N	% of total	
Gender	Female	224	49.1	115	50.2	109	48.0	0.638
	Male	232	50.9	114	49.8	118	52.0	
Age (yrs.), median (IQR)		55 (43-66)		56 (43-67)		54 (44-64)		0.138
Subtype	WDLPS	192	42.1	113	49.3	79	34.8	0.014 ^b
	DDLPS	23	5.0	13	5.7	10	4.4	
	MLPS	172	37.7	77	33.6	95	41.9	
	PLPS	54	11.8	20	8.7	34	15.0	
	Other subtypes	15	3.3	6	2.6	9	4.0	
Site	Upper extremity	49	10.7	23	10.0	26	11.5	0.627
	Lower extremity	407	89.3	206	90.0	201	88.5	
Grade	I	230	50.4	145	63.3	85	37.4	<0.001 ^b
	II	56	12.3	18	7.9	38	16.7	
	III	89	19.5	31	13.5	58	25.6	
Resection margins	Unknown	81	17.8	35	15.3	46	20.3	<0.001 ^b
	Radical (R0)	285	62.5	94	41.0	191	84.1	
	Non-radical (R1/R2)	129	28.3	93	40.6	36	15.9	
	Unknown	35	7.7	35	15.3	0	0	
	No resection	7	1.5	7	3.1	0	0	
Tumor size (cm), median (IQR)		15 (10-21)		15 (10.5-21)		15 (10-20)		0.486
Neoadjuvant therapy	None	239	52.4	185	80.8	54	23.8	<0.001 ^{b,c}
	RTx	168	36.8	14	6.1	154	67.8	
	ILP	28	6.1	28	12.2	0	0	
	CTx	5	1.1	0	0	5	2.2	
	CTx and RTx	14	3.1	0	0	14	6.2	
Adjuvant therapy	Unknown	2	0.4	2	0.9	0	0	<0.001 ^{b,c}
	None	370	81.1	160	69.9	210	92.5	
	RTx	82	18.0	65	28.4	17	7.5	
	CTx	1	0.2	1	0.4	0	0	
Local recurrence (LR)	Unknown	3	0.7	3	1.3	0	0	<0.001 ^b
	Yes	88	19.3	61	26.6	27	11.9	
	No	368	80.7	168	73.4	200	88.1	
	Time to LR (mo.), median (IQR)	22.5 (10.3-59.3)		43 (14-70.5)		13 (8-35)		0.006 ^b
Distant metastasis (DM)	Yes	92	20.2	40	17.5	52	22.9	0.148
	No	364	79.8	189	82.5	175	77.1	
	Time to DM (mo.), median (IQR)	21 (9.3-34.3)		24.5 (9.3-58.8)		17 (9.3-27)		

		Total (N=456)		Rotterdam (N=229)		Warsaw (N=227)		p-value ^a
		N	% of total	N	% of total	N	% of total	
Survival	Alive	357	78.3	170	74.2	187	82.4	0.035 ^b
	Dead	99	21.7	59	25.8	40	17.6	
	- Death of disease	-	-	38	16.6	-	-	
	- Death of other/ unknown causes	-	-	21	9.2	-	-	
	Follow-up time (mo.), median (IQR)	61 (32-109)		68 (31-126)		57 (32-84)		

Abbreviations: WDLPS: well-differentiated liposarcoma,DDLPS: dedifferentiated liposarcoma, MLPS: myxoid liposarcoma, PLPS: pleomorphic liposarcoma, RTx: radiotherapy, ILP: isolated limb perfusion, CTx: chemotherapy

^a P-values calculated by χ^2 -tests (categorical data) and Mann-Whitney *U* tests (continuous data), unless otherwise stated

^b Significant at level of $\alpha < 0.05$

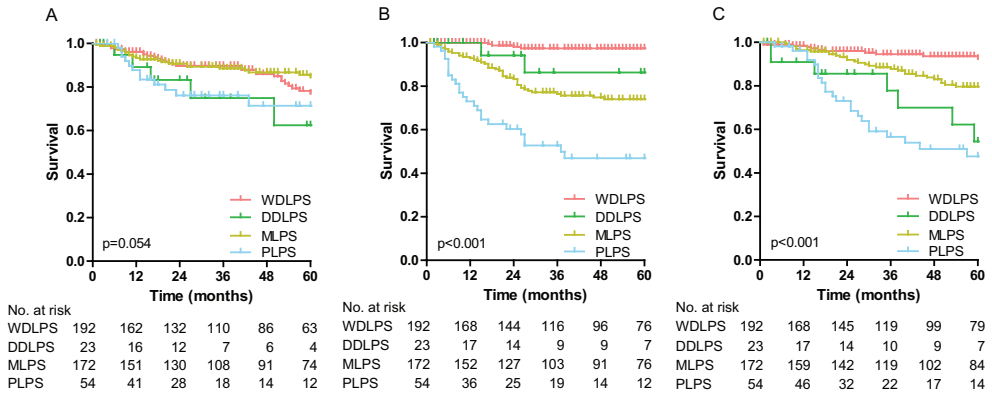
^c Calculated by Fisher's Exact Test

^d Calculated by log-rank test (because median follow-up time was calculated by the reversed Kaplan-Meier method)

Recurrence patterns

Of the remaining 441 patients, 57 patients (12.9%) developed a local recurrence (LR), 57 patients (12.9%) developed distant metastasis (DM), 31 patients experienced both LR and DM (7.0%), while 296 patients (67.1%) had no evidence of recurrent disease at last follow-up. LR was most commonly observed in DDLPS (5/23 patients) with a 5y-LRFS of 62.4% (SE 14.7%), followed by PLPS (12/54, 71.4%, SE 7.5%), WDLPS (29/192, 77.0%, SE 4.0%) and MLPS (22/172, 84.5%, SE 3.2%, $p = 0.054$, Fig. 1A). Median time to LR was 22.5 months (IQR 10.3-59.3).

DM was most frequently observed in patients with PLPS (25/54 patients) with a 5y-DMFS of 46.9% (SE 7.6%). MLPS was the second most common subtype experiencing DM (40/172, 74.0%, SE 3.6%), followed by DDLPS (2/23, 86.3%, SE 9.2%). DM in patients with WDLPS was rare (4/192, 97.3%, SE 1.3%, $p < 0.001$, Fig. 1B). Of the four patients with metastatic WDLPS, at least two patients had dedifferentiated disease, one patient had non-dedifferentiated disease (i.e. WDLPS) and of one patient the dedifferentiation status is unknown. However, this patient died one month after diagnosis of extensive metastatic disease, suggesting differentiated disease. Median time to DM was 21 months (IQR 9.3-34.3).



▲ **Fig. 1.** 5-year local recurrence-free survival per liposarcoma subtype (**A**), 5-year distant metastasis-free survival per liposarcoma subtype (**B**) and 5-year overall survival per liposarcoma subtype (**C**).

Lastly, 5y-OS also differed significantly between subtypes ($p < 0.001$, Fig. 1C). The 5y-OS was poorest for patients with PLPS (23/54, 47.6%, SE 7.9%), followed by patients with DDLPS (7/23, 54.4%, SE 13.4%), patients with MLPS (29/172, 79.7%, SE 3.4%) and patients with WDLPS (11/192, 92.4%, SE 2.3%).

Because of the long time period during which patient data was collected, an additional analysis was performed to determine whether there was a difference in the outcome measures (5y-LRFS, 5y-DMFS or 5y-OS) over time. There were no significant differences in either of these outcomes over the years from 1986 to 2015 (5y-LRFS: $p = 0.731$, 5y-DMFS: $p = 0.696$, 5y-OS: $p = 0.690$, data not shown).

Differences between centres

Between centres, a significant difference in 5y-LRFS for all subtypes was observed (Rotterdam: 73.1%, SE 3.7% vs. Warsaw: 85.5%, SE 2.8%, $p = 0.030$, Table 2). When analysing the subgroups, this difference was almost completely attributable to the difference in 5y-LRFS in patients with WDLPS (Rotterdam: 65.0%, SE 5.9% vs. Warsaw: 94.1%, SE 3.4%, $p < 0.001$), whereas in the other subtypes no significant difference in 5y-LRFS was found between the centres (DDLPS: $p = 0.608$, MLPS: $p = 0.873$, PLPS: $p = 0.184$, Table 2).

There was no significant difference in 5y-DMFS between the two centres (Rotterdam: 84.6%, SE 2.7% vs. Warsaw: 77.2%, SE 3.1%, $p = 0.056$), nor in 5y-OS (Rotterdam: 78.5%, SE 3.2% vs. Warsaw: 80.9%, SE 3.1%, $p = 0.561$, Table 2). However, when analysing differences in 5y-OS rates per subtype between the centres, we observed a significant difference in 5y-OS between Rotterdam and Warsaw in patients with WDLPS (Rotterdam: 10/113, 88.2%, SE 3.6% vs. Warsaw: 1/79, 98.5%, SE 1.5%, $p = 0.027$), but not in patients with one of the other subtypes (DDLPS: $p = 0.570$, MLPS: $p = 0.243$, PLPS: $p = 0.360$, Table 2).

Table 2. 5-year local recurrence-free survival, 5-year distant metastasis-free survival and 5-year overall survival per centre and per subtype.

	5y-LRFS		p-value	5y-DMFS		p-value	5y-OS		p-value
	Rotterdam	Warsaw		Rotterdam	Warsaw		Rotterdam	Warsaw	
Overall	73.1% (3.7%)	85.5% (2.8%)	0.030^a	84.6% (2.7%)	77.2% (3.1%)	0.056	78.5% (3.2%)	80.9% (3.1%)	0.561
WDLPS	65.0% (5.9%)	94.1% (3.4%)	<0.001 ^a	96.3% (2.1%)	98.5% (1.5%)	0.438	88.2% (3.6%)	98.5% (1.5%)	0.027 ^a
DDLPS	56.6% (17.2%)	83.3% (15.2%)	0.608	100% (-)	62.5% (21.3%)	0.050	59.9% (16.2%)	44.4% (22.8%)	0.570
MLPS	84.0% (4.8%)	84.9% (4.1%)	0.873	76.3% (5.2%)	72.2% (4.9%)	0.566	75.6% (5.4%)	83.1% (4.4%)	0.243
PLPS	78.6% (11.5%)	67.7% (9.0%)	0.184	50.3% (12.3%)	44.5% (9.7%)	0.623	57.5% (11.6%)	38.8% (10.9%)	0.360

Values in parentheses are the Standard Errors (SE) corresponding to the noted 5-year survival rates. Differences between the Rotterdam-cohort and Warsaw-cohort were tested for their significance by the log-rank test. 5y-LRFS: 5-year local recurrence free survival, 5y-OS: 5-year overall survival, WDLPS: well-differentiated liposarcoma, DDLPS: dedifferentiated liposarcoma, MLPS: myxoid liposarcoma, PLPS: pleomorphic liposarcoma

^aSignificant at level of $\alpha < 0.05$

To explain this difference in 5y-OS in WDLPS patients between the two centres, the additional data on cause of death in the Rotterdam-cohort was assessed and 5y-DSS was calculated. For the Warsaw-cohort, we assumed that the one death occurring in the WDLPS subgroup was indeed death of disease, since the patient had metastatic disease. It turned out that two out of ten deaths in the Rotterdam-cohort were disease-related, and that the majority of deaths in patients with WDLPS (eight out of ten deaths) were due to other/unknown causes that were not attributed or unlikely related to LPS or LPS treatment. This results in a 5y-DSS of 98.2% (SE 1.2%) for WDLPS in the Rotterdam-cohort, compared to 98.5% (SE 1.5%) in Warsaw ($p = 0.765$, Appendix table B and Figure S1). The same pattern was observed for patients with DDLPS, where one out of four deaths was disease-related (in a total of 13 patients), resulting in a 5y-DSS of 90.9% (SE 8.7%). This is in contrast to the patients with MLPS and PLPS, where most deaths in the Rotterdam-cohort were disease-related (MLPS: 14/16 deaths in 77 patients, PLPS: 7/8 deaths in 20 patients), with a 5y-DSS of 77.7% (SE 5.3%) and 60.6% (SE 11.8%) respectively ($p < 0.001$, Appendix table B).

Differences in treatment

In the Rotterdam-cohort, 34.5% of LPS patients received radiotherapy, mostly adjuvant. In contrast, 78.4% of LPS patients in Warsaw received radiotherapy, mostly neoadjuvant. The difference in use of radiotherapy was observed in all four subtypes, although this difference is not significant for the subtypesDDLPS and PLPS, probably because of the small subgroups (Table 3).

Table 3. Number of patients receiving (neo)adjuvant radiotherapy per centre and subtype.

	Rotterdam (N=223)		Warsaw (N=218)		p-value
	N	% of total	N	% of total	
Well-differentiated liposarcoma	13/113	11.5%	44/79	55.7%	<0.001 ^a
Neoadjuvant RTx	1	0.9%	42	53.2%	
Adjuvant RTx	12	10.6%	1	1.3%	
Both	0	0.0%	1	1.3%	
Dedifferentiated liposarcoma	7/13	53.9%	9/10	90.0%	0.089 ^b
Neoadjuvant RTx	2	15.4%	9	90.0%	
Adjuvant RTx	5	38.5%	0	0%	
Both	0	0%	0	0%	
Myxoid liposarcoma	43/77	55.8%	88/95	92.6%	<0.001 ^a
Neoadjuvant RTx	10	13.0%	78	82.1%	
Adjuvant RTx	33	42.9%	6	6.3%	
Both	0	0.0%	4	4.2%	
Pleomorphic liposarcoma	14/20	70.0%	30/34	88.2%	0.147 ^b
Neoadjuvant RTx	1	5.0%	25	73.5%	
Adjuvant RTx	13	65.0%	4	11.8%	
Both	0	0.0%	1	2.9%	
Overall	77/223	34.5%	171/218	78.4%	<0.001 ^a
Neoadjuvant RTx	14	6.3%	154	70.6%	
Adjuvant RTx	63	28.3%	11	5.0%	
Both	0	0.0%	6	2.8%	

RTx: radiotherapy. The numbers are in bold to indicate that these are the numbers belonging to the entire group, while the numbers that are not bold represent the subgroups (neoadjuvant/ adjuvant/both RTx).
^aχ²-test, ^b Fisher's Exact test

Except for the difference in neoadjuvant/adjuvant treatment with radiotherapy, also a difference in the number of radical (R0) versus non-radical resections (R1/R2) was observed. After excluding patients of whom the tumour was not resected (N = 6) and patients with unknown status of the resection margins (N = 34), 49.7% (N = 183) of the resections in the Rotterdam-cohort had negative resection margins and were radical, while in the Warsaw-cohort this percentage was 83.5% (N = 218, p < 0.001). When analysing the different subtypes, only a significant difference in the WDLPS subgroup is observed (Rotterdam: 23.5% vs. Warsaw: 81.0%, p < 0.001), but not in the other subgroups (DDLPS: p = 0.198, MLPS: p = 0.130, PLPS: p = 0.194, Table 4).

Table 4. Radical/non-radical resections per subtype and centre.

		Rotterdam	Warsaw	p-value
Well-differentiated liposarcoma	R0	19 (23.5%)	64 (81.0%)	<0.001 ^a
	R1/R2	62 (76.5%)	15 (19.0%)	
Dedifferentiated liposarcoma	R0	3 (27.3%)	6 (60.0%)	0.198 ^b
	R1/R2	8 (72.7%)	4 (40.0%)	
Myxoid liposarcoma	R0	56 (78.9%)	84 (88.4%)	0.094 ^a
	R1/R2	15 (21.1%)	11 (11.6%)	
Pleomorphic liposarcoma	R0	13 (65.0%)	28 (82.4%)	0.194 ^b
	R1/R2	7 (35.0%)	6 (17.6%)	
Overall	R0	91 (49.7%)	182 (83.5%)	<0.001a
	R1/R2	92 (50.3%)	36 (16.5%)	

^aχ²-test, ^bFisher's Exact test

Prognostic factors for recurrence and survival

In multivariable Cox regression analysis, male gender (HR 1.686, 95%CI 1.032-2.754, $p = 0.037$) was the only factor of significant influence for the risk of LR (Table 5). The variable centre tested significant in univariable analyses (Appendix table C), but not in multivariable analysis (HR 0.696, 0.401-1.208, $p = 0.198$).

For DM, the subtypes MPLS and PLPS were significant negative prognostic factors (MLPS: HR 8.540, 2.895-25.194; PLPS: HR 25.792, 8.402-79.168; both $p < 0.001$), as well as male gender (HR 2.079, 1.245-3.470, $p = 0.005$, Table 5). Although not significant in univariable analysis (Appendix table C), the variable centre was added to correct for differences in baseline characteristics, but was not of significant influence (HR 1.125, 0.592-2.138, $p = 0.720$).

Lastly, the factors age at time of diagnosis (HR 1.029, 1.012-1.047, $p = 0.001$) and all three subtypes compared to WDLPS (DDLPS: HR 4.755, 1.803-12.540, $p = 0.002$; MLPS: HR 3.596, 1.745-7.398, $p = 0.001$; PLPS: HR 8.609, 4.177-17.747, $p < 0.001$) tested significant in multivariable analysis for OS (Table 5). Again, the variable centre was added, but was not of significant influence (HR 0.835, 0.517-1.347, $p = 0.460$).

Discussion

This study represents the largest cohort of extremity LPS patients published to date and demonstrates clear differences in recurrence and survival patterns between the different LPS subtypes. Patients with DDLPS, MLPS and PLPS show similar patterns of recurrence and survival in both cohorts, and in our multivariable analyses we could confirm some already known risk factors, such as LPS subtype and age [5, 6, 8], but not factors such as positive

resection margins [7, 9-12, 14]. The survival rates found in this study are comparable to the survival rates described in smaller series in literature [8, 12, 19].

Table 5. Results of the multivariable Cox regression analyses for local recurrence, distant metastasis and overall survival.

		HR	CI (95%)	p-value
Local Recurrence				
Gender	Male vs. female	1.686	1.032-2.754	0.037*
Resection margins	R1/R2 vs. R0	1.212	0.688-2.135	0.506
	Unknown vs. R0	2.024	0.979-4.183	0.057
Centre	Warsaw vs. Rotterdam	0.696	0.401-1.208	0.198
Distant Metastasis				
Gender	Male vs. female	2.079	1.245-3.470	0.005*
Subtype	DDLPS vs. WDLPS	4.950	0.884-27.708	0.069
	MLPS vs. WDLPS	8.540	2.895-25.194	<0.001*
	PLPS vs. WDLPS	25.792	8.402-79.168	<0.001*
Resection margins	R1/R2 vs. R0	0.960	0.512-1.802	0.900
	Unknown vs. R0	1.380	0.299-6.370	0.680
Neoadj. treatment	RTx vs. none	1.665	0.801-3.458	0.172
	Other vs. none	1.609	0.779-3.324	0.199
Centre	Warsaw vs. Rotterdam	1.125	0.592-2.138	0.720
Overall Survival				
Age (in years)		1.029	1.012-1.047	0.001*
Gender	Male vs. female	1.573	0.966-2.562	0.069
Subtype	DDLPS vs. WDLPS	4.755	1.803-12.540	0.002*
	MLPS vs. WDLPS	3.596	1.745-7.398	0.001*
	PLPS vs. WDLPS	8.609	4.177-17.747	<0.001*
Centre	Warsaw vs. Rotterdam	0.835	0.517-1.347	0.460

WDLPS: well-differentiated liposarcoma, DDLPS: dedifferentiated liposarcoma, MLPS: myxoid liposarcoma, PLPS: pleomorphic liposarcoma, RTx: radiotherapy

* Significant at level of $\alpha < 0.05$.

A few valuable nomograms to predict overall/disease-specific survival and distant metastasis in soft tissue sarcoma patients have been developed, including for patients with liposarcoma, but focus either on one subtype and multiple primary tumour localisations [8], or on one localisation and multiple STS subtypes [6]. The added value of this study is that it specifically focuses on one type of STS (i.e. liposarcoma and its four distinct subtypes) and one localisation (i.e. the extremity), and describes not only survival and metastases, but also patterns of local recurrence for all the different subtypes in depth.

Despite having the most favourable prognosis of all subtypes, some remarkable differences were noticed in the subgroup of patients with WDLPS. Between the two centres, a large and significant difference in 5y-LRFS in patients with WDLPS was observed (Warsaw: 94.1% vs. Rotterdam: 65.0%, Table 2). As mentioned, in the Warsaw-cohort, more than half

of the WDLPS patients received neoadjuvant/adjuvant radiotherapy, compared with 11.5% in the Rotterdam-cohort, possibly explaining this difference in 5y-LRFS. However, since WDLPS is generally not considered very radiosensitive, it is unlikely that this is the only explanation. Another contributing explanation can be the significantly more non-radical resections in the Rotterdam-cohort, which is most likely due to differences in surgical approach (enucleation vs. wide excision). Nevertheless, these differences in treatment do not lead to a significant difference in 5y-DMFS or 5y-DSS, although the 5y-OS was significantly higher in the Warsaw-cohort. However, this difference was presumably not attributable to LPS or differences in LPS treatment, because there was no significant difference in 5y-DSS for the WDLPS subgroup. Furthermore, even with 20-40% more patients receiving radiotherapy in the Warsaw-cohort in theDDLPS, MLPS and PLPS subgroups than in the Rotterdam-cohort, no significant difference in LRFS, DMFS and survival between the centres is observed for these subgroups. So, in addition to the WDLPS subgroup, more radiotherapy does not seem to lead to significant less distant metastasis and better overall survival, but in these subgroups also not to significant less local recurrences, as one would expect.

Radiotherapy has proven to be effective in preventing LR in LPS patients, as shown in this study in WDLPS, as well as in literature in all LPS subtypes, and especially in MLPS [7, 20-25]. Nevertheless, radiotherapy has some well-known, serious side effects and disadvantages, such as wound complications, fibrosis, pathological fractures, functional impairment, oedema and secondary tumours [26-33]. The results of this study point out that radiotherapy as a local therapy should be applied very selectively, for example only in those patients in whom a possible local recurrence will lead to treatment issues and in whom re-resection is not feasible, so that the risk of having a LR should be minimized. In doing so, the toxicities of radiotherapy should be taken into consideration, especially in patients of young age, when joints are involved, and so on. The same arguments more or less apply for extent of resection in patients with extremity WDLPS, choosing between enucleating the tumour (R1 resection) and possibly a higher chance of LR, or resecting the tumour with wide margins (R0 resection) and lower risk of LR, but with higher chance of inducing morbidities, depending on local conditions during surgery. For the other and more aggressive subtypes, there is more consensus on resecting the primary tumour with wide resections margins, reflected in the non-significant difference in R0 and R1/R2 resections in these subgroups and the more frequent use of neoadjuvant/adjuvant radiotherapy. In summary, the presented data show that in WDLPS an aggressive approach of neoadjuvant/adjuvant radiotherapy and radical surgery leads to excellent local control rates. A more conservative approach carries a significantly higher risk of LR. As this is not influencing DMFS/survival, the local treatment strategy for WDLPS should be tailored to the need of the individual patient, balancing risks and benefits of both approaches.

A limitation of our study is that a bias might have been introduced by the long time period during which patients were included. Due to changes and improvements in practice over the years, results of early years of inclusion and treatment might not be representative for outcomes and treatment in the more recent years. However, these changes in practice did not have a substantial effect on the outcomes, since we did not observe any significant differences in either 5y-LRFS, 5y-DMFS or 5y-OS over the years. Second, this study has a relatively short follow-up time of five years, particularly for the WDLPS subgroup. Censoring at five years of follow-up was chosen based on the median follow-up time (61 months) and because numbers at risk would become too small to perform reliable tests, for example, at 10 years follow-up. Additionally, we observed that approximately 75% of all WDLPS patients with a local recurrence developed their recurrence within five years of follow-up. So, despite the relatively short follow-up, we believe this follow-up period is sufficient and useful in daily clinical practice. Third, in the Warsaw-cohort, more than 55% of the WDLPS patients received neoadjuvant/adjuvant radiotherapy. It should be mentioned that the high number of patients receiving radiotherapy is exceptional and that in most STS expertise centres as well as in most cohorts described in literature, this is not standard practice. Most cohorts that include patients with extremity WDLPS report percentages ranging from 18.7% to 47% of patients receiving radiotherapy [7, 34-36].

Importantly, this study is based on retrospective data, which therefore may bring some bias inherent to such analyses, such as selection bias, and depends on accurate record keeping. Patients receiving neoadjuvant/adjuvant therapy were probably fit patients with poor tumour characteristics, such as aggressive LPS subtype and a large tumour. This selection bias is probably present in both datasets, neutralizing each other in the comparisons between the centres. Additionally, we tried to minimize the selection bias by including all patients treated for primary LPS of the extremity, without any exclusion criteria except for metastatic disease at time of diagnosis and insufficient data available. In addition to using the largest cohort of extremity LPS patients reported to date, the strength of this study is that the data and results are based on daily clinical practice representative for all extremity LPS patients in the Netherlands, Poland and probably other European/western countries as well, and gives insight into the value and effectiveness of current treatment policies outside the context of a clinical trial. Furthermore, it can give guidance during treatment decision making, for example in determining the extent of surgery or in opting for neoadjuvant/adjuvant radiotherapy.

Conclusion

Patients with the four liposarcoma subtypes in this large cohort show distinct patterns of local recurrence, distant metastasis and survival, with liposarcoma subtype also being one of the most important prognostic factors for these outcomes. This indicates that 'extremity liposarcoma' is not a single entity. Radiotherapy can reduce the risk of local recurrence in WDLPS, but its benefits should be carefully balanced against its disadvantages. Despite the differences in recurrence and treatment, five year (disease-specific) survival did not differ significantly between the two expertise centres. These prognostic patterns and characteristics may be used to further tailor treatment regarding surgery and neoadjuvant/ adjuvant radiotherapy.

Conflicts of interest statement

None of the authors have any conflicts of interest.

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None.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.ejso.2018.03.028>.

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Supplementary Data

Appendix table A. Follow-up schedules of the Erasmus MC Cancer Institute, Rotterdam, the Netherlands and the Maria Skłodowska-Curie Institute-Oncology Center, Warsaw, Poland

Rotterdam	Grade I	Grade II-III
<i>Physical examination</i>		
Year 1-2	Every 4 months	Every 4 months
Year 3-5	Every 6 months	Every 6 months
Year 6-10	Every 12 months	Every 12 months
<i>Imaging</i>		
Local evaluation	4 months after surgery, afterwards only on indication	4 months after surgery, afterwards only on indication
Chest	X-ray every 12 months ^a	X-ray at every follow-up visit ^b
Warsaw	Grade I	Grade II-III
<i>Physical examination</i>		
Year 1-3	Every 3-6 months	Every 3-4 months
Year 4-5	Every 12 months	Every 6 months
Year 6-10	Every 12 months	Every 12 months
<i>Imaging</i>		
Local evaluation	6 months after surgery, afterwards only on indication	3-6 months after surgery, afterwards only on indication
Chest	X-ray every 6-12 months	X-ray or CT every visit

^a except for WDLPS. In case of WDLPS chest X-rays are not indicated.

^b in case of MLPS, also CT-abdomen

Appendix table B. 5-year overall survival and 5-year disease specific survival in the Rotterdam-cohort

	5y-OS^a	SE	5y-DSS^b	SE
Well-differentiated liposarcoma	88.2%	3.6%	98.2%	1.2%
Dedifferentiated liposarcoma	59.9%	16.2%	90.9%	8.7%
Myxoid liposarcoma	75.6%	5.4%	77.7%	5.3%
Pleomorphic liposarcoma	57.5%	11.6%	60.6%	11.8%
Overall	78.5%	3.2%	85.9%	2.7%

^a Difference between subtypes significant with $p=0.005$

^b Difference between subtypes significant with $p<0.001$

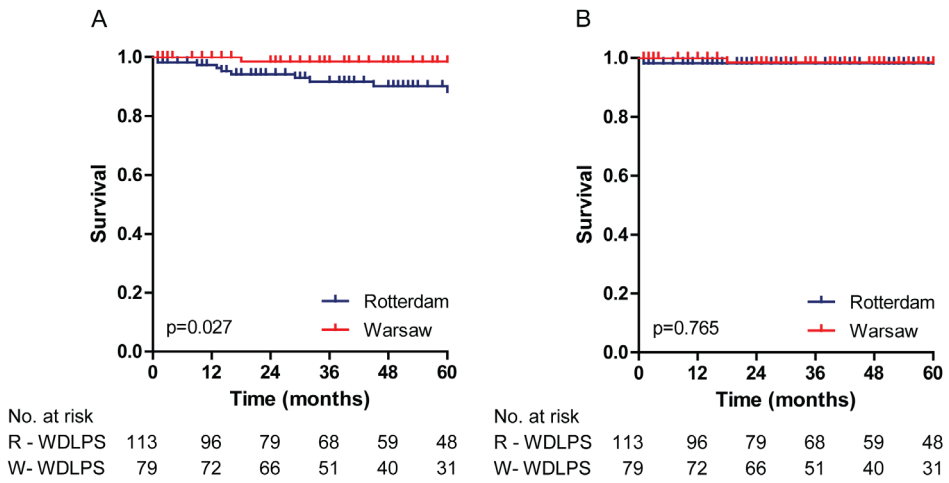
5y-OS: 5-year overall survival, 5y-DSS: 5-year disease specific survival, SE: Standard Error

Appendix table C. Univariable Cox regression analysis for local recurrence, distant metastasis and overall survival.

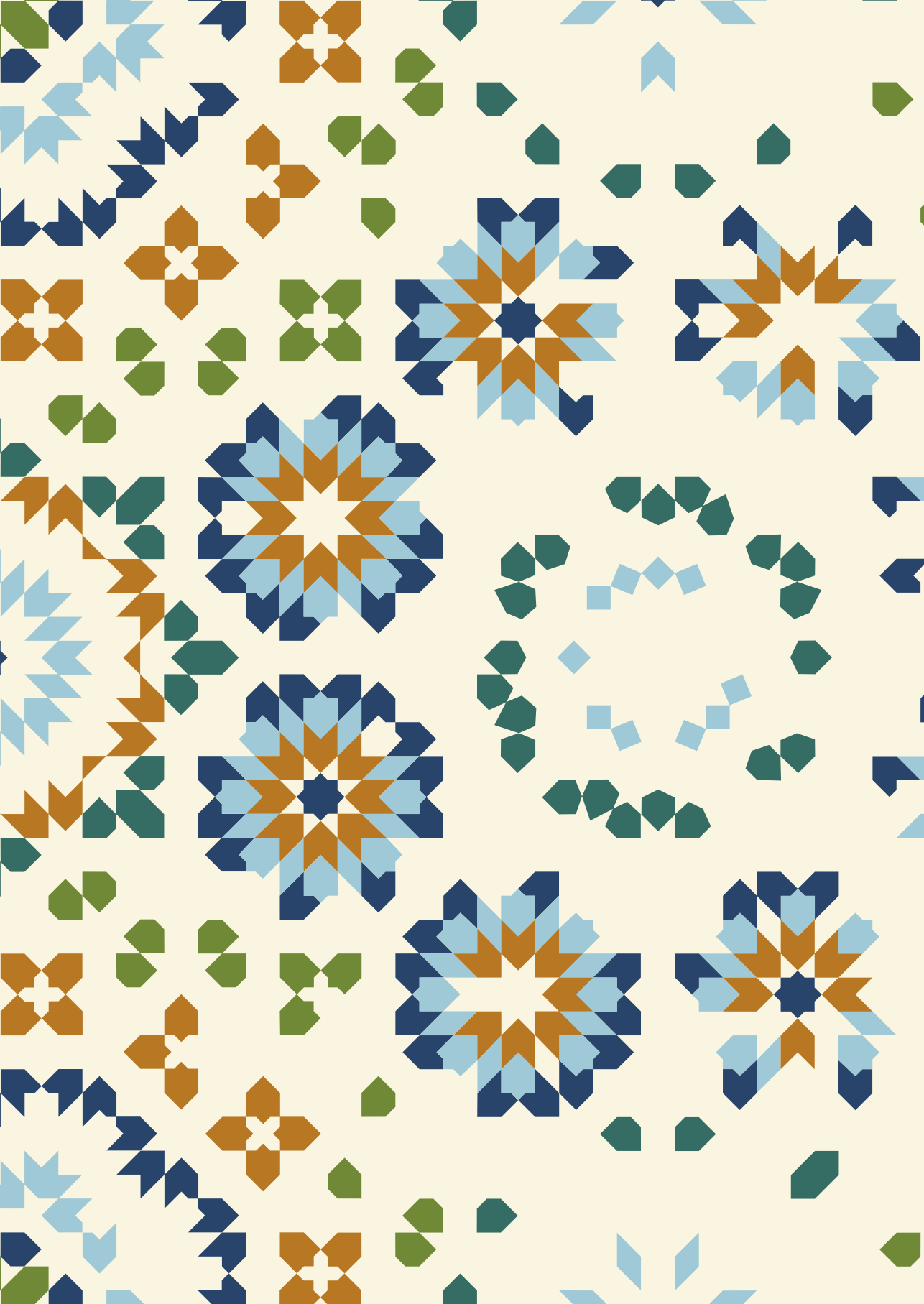
	Local recurrence				Distant metastasis				Overall survival			
	N	HR	CI (95%)	p-value	N	HR	CI (95%)	p-value	N	HR	CI (95%)	p-value
Age at diagnosis (in years)	441	1.016	0.999-1.033	0.069	434	0.996	0.980-1.012	0.600	441	1.031	1.014-1.048	<0.001*
Gender												
Female	215	1			215	1			215	1		
Male	226	1.725	1.057-2.816	0.029*	226	2.017	1.233-3.299	0.005*	226	1.635	1.010-2.648	0.045*
Subtype												
WDLPS	192	1			192	1			192	1		
DDLPS	23	1.938	0.749-5.011	0.172	23	5.128	0.939-27.998	0.059	23	6.652	2.578-17.164	<0.001*
MLPS	172	0.770	0.442-1.340	0.354	172	11.153	3.990-31.171	<0.001*	172	2.67	1.334-5.345	0.006*
PLPS	54	1.773	0.904-3.480	0.096	54	30.144	10.481-86.700	<0.001*	54	9.173	4.467-18.839	<0.001*
Grade												
I	219	1			219	1			219	1		
II	55	1.064	0.509-2.224	0.868	55	6.024	2.575-14.094	<0.001†	55	2.684	1.246-5.784	0.012†
III	87	1.673	0.951-2.942	0.074	87	11.856	5.684-24.730	<0.001†	87	5.765	3.162-10.509	<0.001†
Unknown	80	0.611	0.270-1.381	0.236	80	5.257	2.300-12.017	<0.001†	80	2.112	0.980-4.552	0.056
Site												
Lower extremity	392	1			392	1			392	1		
Upper extremity	49	1.619	0.827-3.168	0.160	49	0.674	0.272-1.673	0.395	49	0.855	0.370-1.974	0.713
Size (in cm)												
R0	402	1.010	0.977-1.043	0.553	402	1.020	0.989-1.051	0.202	407	1.029	0.999-1.061	0.060
R1/R2	273	1			273	1			273	1		
Unknown	128	1.404	0.817-2.412	0.219	128	0.542	0.296-0.992	0.047*	129	1.027	0.606-1.738	0.922
None	34	2.621	1.360-5.051	0.004*	34	0.249	0.061-1.023	0.054	34	0.142	0.020-1.029	0.053
RTx	234	1			234	1			234	1		
Other	160	0.696	0.408-1.185	0.182	160	2.661	1.540-4.597	<0.001*	160	1.491	0.883-2.519	0.136
None	47	0.648	0.276-1.525	0.320	47	3.765	1.928-7.355	<0.001*	47	2.324	1.223-4.414	0.010†
None	357	1			357	1			357	1		
RTx	80	0.487	0.232-1.020	0.056	80	1.453	0.859-2.458	0.163	80	1.197	0.692-2.070	0.521
Other	4	1.564	0.216-11.292	0.658	4	0	0	0.973	4	2.056	0.284-14.881	0.475
Centre												
Rotterdam	223	1			223	1			223	1		
Warsaw	218	0.583	0.356-0.955	0.032*	218	1.583	0.983-2.547	0.059	218	0.870	0.544-1.393	0.562

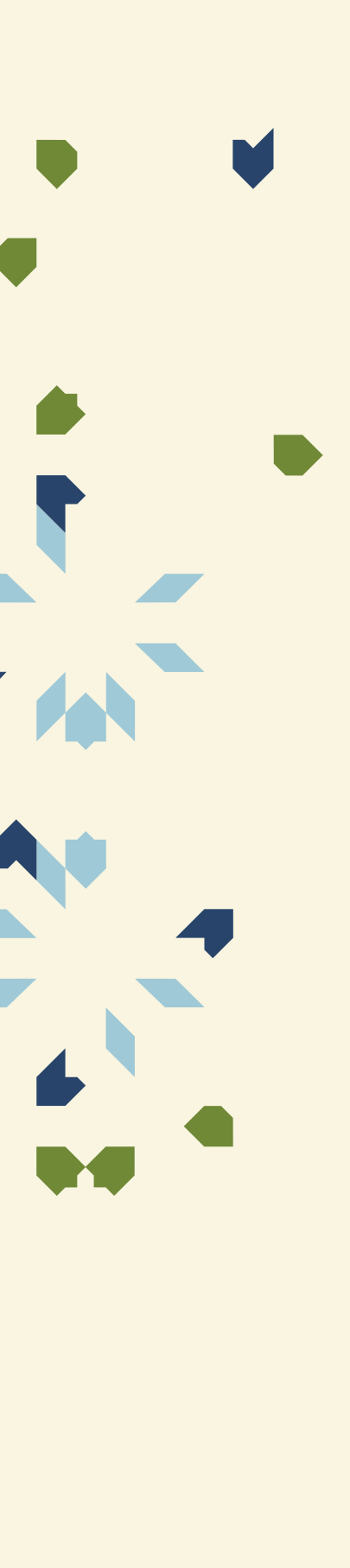
*Variables included in multivariate analysis. † Significant in univariable analysis, but not included in multivariable analysis, because of strong coherence with subtype and limited degrees of freedom.

WDLPS: well-differentiated liposarcoma, DDLPS: dedifferentiated liposarcoma, MLPS: myxoid liposarcoma, PLPS: pleomorphic liposarcoma, RTx: radiotherapy



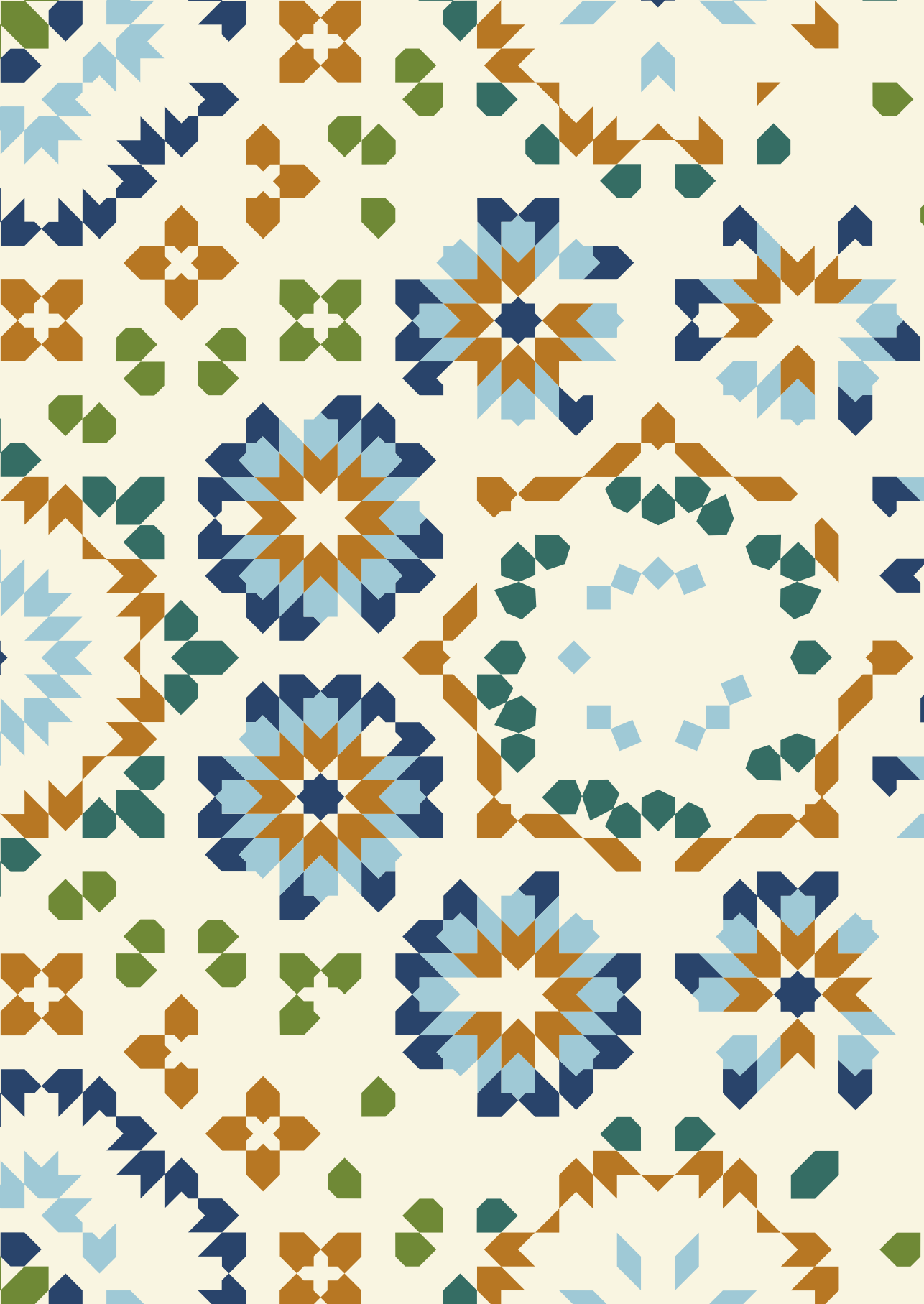
▲Figure S1. 5-year overall survival (A) and 5-year disease specific survival (B) compared between the two centres for the subgroup of patients with WDLPS.





PART III

EVALUATION OF THE SURGICAL TREATMENT OF LOCALIZED SOFT TISSUE SARCOMA





CHAPTER 8

NATURAL HISTORY OF WELL-DIFFERENTIATED LIPOSARCOMA OF THE EXTREMITY COMPARED TO PATIENTS TREATED WITH SURGERY

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Abstract

Background: Patients with well-differentiated liposarcoma (WDLPS) of the extremity are mostly treated surgically, thereby possibly inducing severe morbidities. Despite the excellent prognosis, the natural history is barely studied. The aim of this study was to evaluate the natural history of extremity WDLPS by evaluating the outcome of patients treated with active surveillance (AS), who thereby exhibited the natural history of extremity WDLPS, and of patients treated surgically.

Methods: A large retrospective database of patients with extremity WDLPS was assessed to evaluate treatment, dedifferentiation and disease-specific survival. Lastly, our experience with patients treated with AS was explored.

Results: Distant metastases (5/191 patients, 2.6%) were mainly seen after a dedifferentiated local recurrence. Death of disease occurred in 4/191 patients (2.1%); two patients died from metastatic disease (although not pathologically proven), two patients died of treatment-related complications. In our center, 24 patients are treated with AS. Time of AS varied from 0.1 to 8.9 years (median 1.8). Four patients eventually underwent surgery after a period of AS (range 14–52 months) because of symptoms and/or tumor growth. No areas of dedifferentiation were found in these resection specimens. The other patients are still under active surveillance.

Conclusion: Since surgical treatment might induce morbidity and even mortality, there might be overtreatment of these patients. Evaluation of the natural history of extremity WDLPS showed that AS could be a reasonable option for selected patients. Prospective studies in patients with extremity WDLPS are needed to assess the safety of AS as a treatment option.

Keywords: Well-differentiated liposarcoma; Extremity; Natural history; Active surveillance; Surgery

Introduction

Well-differentiated liposarcoma (WDLPS) is the most common subtype of liposarcoma, accounting for approximately half of all liposarcoma patients. Most WDLPS patients present with a deep-seated, slowly growing and painless mass, most frequently located in one of the extremities. WDLPS are low-grade tumors and have very little to no metastatic potential. However, they can dedifferentiate into a more aggressive subtype, thereby gaining the ability to metastasize [1]. Patients with extremity WDLPS have a good prognosis with very low dedifferentiation rates and excellent survival rates of 90-100% after 10 years of follow-up [1]. Because of this indolent disease course, extremity WDLPS is considered borderline malignant, and is therefore also called an atypical lipomatous tumor [1-3].

Despite of these disease characteristics, almost all patients undergo (extensive) surgery, optionally preceded or followed by radiotherapy. Although consensus has shifted from radical amputation to wide excision – and even marginal excision now is considered appropriate and adequate more often in case of localization in one of the extremities – patients still have to deal with the morbidities and complications induced by surgery, such as loss of limb function and wound infections [4-6].

To date, the natural history of extremity WDLPS has rarely been described in these patients. While this is much more studied in other borderline malignant tumors, such as desmoid-type fibromatosis [7-11], no study has ever been published yet in extremity WDLPS evaluating its natural history. Therefore, the aim of this study was to describe the natural history of patients with extremity WDLPS and to initiate and open a discussion on the treatment of patients with extremity WDLPS by discussing active surveillance as a treatment option for these patients. With this purpose we looked in detail into the disease course of both treated and untreated patients with extremity WDLPS.

Methods

Data collection

Data of surgically treated patients with primary WDLPS located in one of the extremities were extracted from the database previously described by Vos et al. [12]. This database was revolved around patients diagnosed with primary liposarcoma in the extremity in the Erasmus MC Cancer Institute in Rotterdam, the Netherlands, and the Maria Skłodowska-Curie Institute-Oncology Center in Warsaw, Poland, between 1986 and 2015. Both centers are designated as tertiary referral and expertise centers for soft tissue sarcoma. One patient in this cohort eventually did not undergo surgery because of minimal complaints, although at start there was the intent to operate, and was excluded. Imaging, in particular an MRI scan,

was part of the standard diagnostic work-up in both expertise centers. In some patients the diagnosis was confirmed by fluorescent in situ hybridization (FISH) for *MDM2* amplification, but in most of the patients the diagnosis was based on histological, morphological and/or immunohistochemical criteria. Patients who presented with a local recurrence or metastatic disease were excluded in this dataset. Although these patients underwent surgery, the results from this cohort gave rise to the current study and the discussion on treatment of these patients.

In the Erasmus MC Cancer Institute, Rotterdam, the Netherlands, already a few selected patients are being treated with active surveillance, thereby exhibiting the natural history of extremity WDLPS. These patients were identified during weekly multidisciplinary tumor board meetings, by the treating physicians and through the institutional database on liposarcomas. This group also includes patients who initially started with active surveillance but eventually underwent surgery after a period of active surveillance because of anxiety, occurrence of/ increase in symptoms and/or tumor growth. Frequency of follow-up and imaging during active surveillance was in accordance with the national soft tissue sarcoma guideline [13] and was similar to the follow-up schedule of low-grade sarcomas, or more often if indicated: first two years every 4 months, year three to five every 6 months and after five years once a year, with an X-ray of the thorax yearly and a MRI scan if indicated. Of these patients, data on characteristics such as primary or recurrent tumor, age at start of active surveillance, symptoms, time of active surveillance and vital status (death/alive) were obtained. If patients opted to undergo surgery after a period of active surveillance, the resection specimen was examined for (areas of) dedifferentiation. Time of active surveillance was defined as time between start of active surveillance and last follow-up or date of surgery.

This study was approved by the local ethics committee and performed in accordance with local ethics committee guidelines and national legislation.

Statistical analyses

All statistical analyses were performed by using SPSS (IBM SPSS Statistics for Windows, Version 24.0, IBM Corporation, Armonk, NY, USA). Categorical variables are shown as numbers with percentages in parentheses and continuous variables as medians with the interquartile ranges (IQRs) in parentheses. χ^2 -tests, Fisher's Exact tests and Mann-Whitney U tests were used to test for differences in clinicopathological variables between groups when appropriate. Two-sided p-values < 0.05 were considered statistically significant.

Results

Course of disease of the surgically treated patients – distant metastasis

In total, 191 patients with primary WDLPS located in the extremity who were treated surgically were identified: 112 in the Rotterdam-cohort and 79 in the Warsaw-cohort. As described and discussed before [12], there was a difference in the number of radical resections, use of neoadjuvant and adjuvant radiotherapy and percentage of patients experiencing a local recurrence between the two centers (Table 1). In brief this study showed that an aggressive approach with radical surgery and neoadjuvant/adjuvant radiotherapy led to excellent local control, while a more conservative approach with enucleation of the tumor (i.e. R1-resection) and without radiotherapy led to higher local recurrence rates, but also that these differences in treatment did not lead to a difference in disease-specific survival for patients with extremity WDLPS [12]. Distant metastases were scarcely observed, neither were dedifferentiation and death of disease, with a median follow-up time of 49 months (IQR 24–75.5, Table 1). In total, five patients out of 191 patients developed metastatic disease (Table 2). Three of these five patients first developed a dedifferentiated local recurrence before developing metastatic disease, the fourth patient developed a local recurrence and a distant metastasis simultaneously. The local recurrent tumor was confirmed by biopsy, showing WDLPS without any signs of dedifferentiation, but no material from the metastatic site was obtained for pathological examination. The patient is still alive, after 'palliative' radiotherapy with a total of 24Gy, with a follow-up period of 60 months (42 months after diagnosis of metastatic disease). The last patient developed massive distant metastases in lungs and liver as a first manifestation of recurrent disease, within four months since the prior follow-up visit with a 'clean' chest X-ray, and died one month later. No data on confirmation of the LPS diagnosis and dedifferentiation in the metastases were available, although the aggressive course of disease suggests either dedifferentiation or that these lesions were metastases from another unknown primary tumor. So, it is questionable whether the last two patients with 'metastases', without a dedifferentiated local recurrence, really had metastatic WDLPS.

Course of disease of the surgically treated patients - survival

Death of disease was also rarely observed in this group of patients (4 out of 191 patients), with a 5-year disease-specific survival of 98.3% [12]. Two of the deceased patients were with metastatic disease described above; the other two deaths were both one month after diagnosis of the primary tumor and turned out to be treatment-related, instead of disease-related. One patient died a few days after neoadjuvant treatment with isolated limb perfusion, and the second patient a few days after surgical resection of the primary tumor due to acute myocardial infarction.

Table 1. Patient characteristics

		Total (N=191)		Rotterdam (N=113)		Warsaw (N=79)		p-value [§]
		N	%	N	%	N	%	
Sex	Female	103	53.9	61	54.5	42	53.2	0.859
	Male	88	46.1	51	45.5	37	46.8	
Age at diagnosis (years) [§]		59 (49-67)		60 (50-68.5)		58 (48-64.5)		0.104
Site	Lower extremity	163	85.3	97	86.6	66	83.5	0.556
	Upper extremity	28	14.7	15	13.4	13	16.5	
Size (cm) [§]		17 (12-23)		18.5 (13-23)		16 (10.5-20.3)		0.106
Resection margins	R0	83	43.7	19	17.1	64	81.0	<0.001
	R1/R2	78	41.1	63	56.8	15	19.0	
	Unknown	29	15.3	29	26.1	0	0.0	
Neoadjuvant treatment	None	143	74.9	107	95.5	36	45.6	<0.001 [‡]
	Radiotherapy	44	23.0	1	0.9	43	54.4	
	ILP	2	1.0	2	1.8	0	0.0	
	Unknown	2	1.0	2	1.8	0	0.0	
Adjuvant treatment	None	175	91.6	98	87.5	77	97.5	0.042 [‡]
	Radiotherapy	14	7.3	12	10.7	2	2.5	
	Unknown	2	1.0	2	1.8	0	0.0	
Local recurrence	None	154	80.6	79	70.5	75	94.9	<0.001
	Yes	37	19.4	33	29.5	4	5.1	
	Time to local recurrence (months) [§]	41 (15-57)		41 (15-57)		43 (21.5-64)		
Dedifferentiation	None	186	97.4	109	97.3	77	97.5	0.448 [‡]
	Yes	4	2.1	3	2.7	1	1.3	
	Unknown	1	0.5	0	0.0	1	1.3	
Distant metastasis	None	186	97.4	109	97.3	77	97.5	1.000 [‡]
	Yes	5	2.6	3	2.7	2	2.5	
	Time to metastasis (months) [§]	24 (18-26)		24 (21-25)		68.5 (17-120)		
Survival	Alive	174	91.1	98	87.5	76	96.2	0.110 [‡]
	Death of disease	4	2.1	3	2.7	1	1.3	
	Death of other/unknown cause	13	6.8	11	9.8	2	2.5	
Follow-up (months) [§]		49 (24-75.5)		49.5 (19-82.5)		48 (27-74)		0.949

[§] Presented as median (interquartile range). [§] Calculated by χ^2 -tests (categorical data) or Mann-Whitney U tests (continuous data). [‡] Fisher's Exact test

Natural history of extremity WDLPS in untreated patients

These observations raised questions on the treatment of patients with extremity WDLPS. Since patients only die of the disease after dedifferentiation, dedifferentiation rates are low, dedifferentiation only occurred in local recurrences after surgical removal of the primary tumor, and surgery might induce morbidity and even mortality, we could be overtreating these patients. This might especially apply for patients with inconveniently localized or deep seated and large tumors (i.e. surgeries where chances of inducing morbidity are substantial) without any debilitating symptoms, elderly patients and/or patients with significant comorbidities.

Therefore, in the Erasmus MC Cancer Institute Rotterdam, the Netherlands, already a few patients with extremity WDLPS have been treated with active surveillance, in whom the natural history of extremity WDLPS can be studied. In all these patients, a conscious decision for active surveillance was taken. Most of these patients have local recurrent WDLPS without any (debilitating) symptoms (19 out of 24 patients treated with AS), in a smaller number of patients the primary tumor is treated with AS (5 out of 24 patients). Reasons for choosing active surveillance included one, or a combination, of the following motives: the absence of any debilitating symptoms, no/minimal tumor growth, an inconvenient localization (i.e. minimal chance of radical resection), and/or a high risk of inducing severe morbidity during surgery. Follow-up of the patients treated with active surveillance ranges from a few months to almost 9 years (median 22 months, IQR 10–51 months, total range 1–107 months) and the median age at time of start of active surveillance was 70 years (IQR 62–74.5) (Table 4). Of these 24 patients, four patients opted to undergo surgery after a period of active surveillance, because of symptoms and/or tumor growth. Time of active surveillance in these four patients was 14, 16, 24 and 52 months. After surgery, no areas of dedifferentiation were found in any of the tumor specimens. The tumors of the remaining 20 patients are still in situ (with a median follow up of 26 months, IQR 5–51 months). These patients are monitored according to the follow-up schedule of low-grade sarcomas as stated in the national soft tissue sarcoma guidelines, including imaging on indication, except for two patients (one patient died to a cause unrelated to WDLPS, one patient is lost to follow-up). Although some of the patients only have been treated with active surveillance for a few months so far, there is no/minimal growth of these tumors and they do not have any signs of dedifferentiation up to date, even not in the patient treated with active surveillance for almost nine years.

Table 2. Patients with metastatic disease.

No.	Age ^a	R/W	Primary tumor			Local Recurrence			Distant Metastases			Follow-up		
			Margins	RTx	No RTx	LR	TLR	Dediff.	Treatment	TSD	Metastatic sites	Treatment	Survival	Total FU [†]
1	48	R	R1	No RTx	Yes	Yes	Surgery + RTx	26	Lungs, later multiple	CTx + RTx	DOD	75	49	
2	42	R	Unknown	No RTx	Yes	Yes	Surgery + RTx	24	Multiple sites (subcutaneous) (CR)	Surgery + CTx	Alive	198	174	
3	47	W	R0	Neoadj. RTx	Yes	Yes	Surgery	120	Lungs	Surgery, CTx, RTx	Alive	145	25	Second local recurrence in 2009, treated with surgery + RTx
4	80	R	Unknown	No RTx	Yes	No	None	18	Paravertebral	RTx (24Gy)	Alive	60	42	No biopsy of metastatic site
5	64	W	R0	Neoadj. RTx	No	NA	NA	17	Lungs and liver	None	DOD	18	1	No biopsy of metastatic site. Aggressive course suggests dedifferentiated disease

TSD: time to systemic disease (in months), dediff.: dedifferentiation, RTx: radiotherapy, LR: local recurrence, TLR: time to local recurrence (in months), FU: follow-up time (in months), DOD: death of disease, R/W: Rotterdam-cohort (R) or Warsaw-cohort (W), CR: Complete Response.

^aAge at time of diagnosis (of primary tumor), [†]Follow-up in months from date of diagnosis to date of death or last date of follow-up. [‡]Follow-up in months from date of diagnosis of distant metastases to date of death of last follow-up.

Table 3. Patients with disease-related death.

Age ^a	RW	Primary tumor			Local Recurrence			Distant metastasis			Follow-up		Remarks	
		Margins (Neo)adj.	treatment	LR	TLR	Dediff.	Treatment	DM	TSD	Treatment	Survival	FU since DM ^b		Total FU ^c
1	64	W	R0	Neoadj. RTx	No	-	-	Yes	17	None	DOD	1	18	Unfit for CTx
2	48	R	R1	None	Yes	Yes	Surgery + RTx	Yes	26	CTx + RTx	DOD	49	75	
3	91	R	-	(Neoadj.) ILP	-	-	-	-	-	-	TRD	-	1	Died few days after (neoadj.) ILP
4	59	R	R1	None	-	-	-	-	-	-	TRD	-	1	Died few days after surgery

RW: Rotterdam-cohort (R) or Warsaw-cohort (W). ILP: isolated limb perfusion, RTx: radiotherapy, LR: local recurrence, TLR: time to local recurrence (in months), NA: not applicable, dediff.: dedifferentiation, DM: distant metastasis, TSD: time to systemic disease (in months), CTx: chemotherapy, DOD: death of disease, TRD: treatment-related death, FU: follow-up time (in months)

^a Age at time of diagnosis (of primary tumor). ^b Follow-up in months from date of diagnosis to date of death or last date of follow-up. ^c Follow-up in months from date of diagnosis of distant metastases to date of death of last follow-up.

Table 4. Patients characteristics of the patients treated with active surveillance

Sex	Female	9 (37.5)
	Male	15 (62.5)
Age at time of start AS (years) ^a		70 (62-74.5)
Site	Upper extremity	1 (4.2)
	Lower extremity	23 (95.8)
Presentation	Primary tumor	5 (20.8)
	Local recurrence	19 (79.2)
Surgery	No, tumor still in situ	20 (83.3)
	Yes	4 (16.7)
Follow-up/duration of AS (months) ^a		21.7 (9.5-51.1)

^a Presented as median (interquartile range). AS: active surveillance

Discussion

The cases described above in a large dataset of 191 surgically treated patients with extremity WDLPS outline that patients do not or seldom die because of extremity WDLPS, unless the recurrent tumor has dedifferentiated, while two deaths were induced by its treatment. These observations raised questions about the possible overtreatment of this patient group, and led to a discussion on whether a more conservative treatment or even no treatment at all (active surveillance) is more appropriate and justified in selected cases.

In line with the results of our study, other studies of extremity WDLPS have reported low rates of dedifferentiation [1, 6, 14, 15], metastatic disease [4, 16] and mortality [17-19]. Despite these excellent outcomes, treatment of these patients remains almost similar to that of patients with more aggressive subtypes, such as dedifferentiated, myxoid or pleomorphic liposarcoma. The extent of treatment of extremity WDLPS is already under debate, with ongoing discussions regarding the harms and benefits of wide excision versus marginal excision [4, 6, 20], and the use of neoadjuvant/adjuvant radiotherapy [21, 22]. To this debate, we can now add the question whether excision – or treatment in general – is indicated at all. The morbidity and risks of marginal resections (i.e. R1 resections) are generally quite low, but they are still present and should be taken into account. The results of this study, together with those of other studies reporting excellent survival rates, indicate that not all cases of extremity WDLPS may require surgical removal. In selected cases, especially in elderly patients, patients with comorbidities and/or patients with inconveniently localized, large and deep-seated tumors without any symptoms in whom surgical resection most probably will lead to substantial morbidity, it may be appropriate and adequate to pursue conservative treatment in the form of active surveillance. The appropriateness of active surveillance was further underscored by the observation that it has been safe so far to apply this approach in selected patients with extremity WDLPS who do not experience any debilitating symptoms and who have inconveniently localized tumors at the Erasmus MC Cancer Institute, although follow-up is still short. In these patients, the natural history of extremity WDLPS showed no/minimal growth of the tumors. In the few patients (4/24) who did experience growth/symptoms after a period of active surveillance and therefore opted to undergo surgery, no dedifferentiation was found in the specimens. However, it remains unknown whether it is preferable to remove these large and inconveniently localized WDLPS quickly after diagnosis, or to observe them for a period of time for possible tumor growth and/or dedifferentiation.

During treatment decision making numerous factors have to be taken into consideration, balancing the risks and benefits of the treatment for each patient. Radical local treatment leads to better local control, but comes at the costs of morbidity, impaired functional outcome and even mortality, but does not affect disease-specific survival [12]. Factors that influence this balance include patient-related factors, such as age, performance

status, comorbidities and the patient's own wish, and tumor-related factors, such as symptoms, localization, tumor size and signs of dedifferentiation. A tumor localized in the extremity is particularly suitable for an active surveillance approach, since tumor growth can be monitored by physical examination – even by the patient him/herself – and does not completely rely on imaging alone [23, 24]. For example, for the 91-year old patient in our study who died due to the treatment (Table 3), the risk of dying of an age-related disease (e.g. cardiovascular disease, stroke, dementia) was probably higher than the risk of developing dedifferentiated metastatic disease and dying as a result of extremity WDLPS. In retrospect, we feel that active surveillance with a natural course of disease might have been both feasible and adequate in this case. In patients who are younger and fitter or who have smaller and more favorable localized tumors (i.e. less complex surgery), this consideration is likely to be different and surgery might be the preferred option.

Active surveillance in WDLPS has been discussed and suggested before, but such discussions have mainly focused on specific situations, such as after resection of recurrent tumors, after surgical treatment of the primary tumor, or for large inoperable primary tumors [22, 25-27]. There is a distinct lack of studies and data regarding patients with extremity WDLPS who actually have been treated with active surveillance. A further problem with previous studies is that overall or disease-specific survival alone might not be the most appropriate outcome measurements for this subgroup of patients, since survival rates approach 100% [1, 17-19]. Other outcome measurements, such as quality of life, are becoming more and more relevant. To date, no studies on quality of life have been conducted in patients with extremity WDLPS. On the one hand, quality of life of patients with active surveillance might be better than those undergoing surgery, because they would avoid the necessity of surgery and its related complications and morbidities. On the other hand, their quality of life might be poorer than those undergoing surgery, because living with a tumor in situ might lead to tumor-related symptoms and cause anxiety.

A limitation of our study is that it was based on retrospective data – inherent when studying rare diseases – which relies on accurate recordkeeping and which has induced bias. We acknowledge that there is most probably a selection bias in the patients currently treated with active surveillance, although we believe that this type of treatment will always be subject to some extent of selection bias, since patients with symptoms most likely will refuse active surveillance and prefer surgical treatment. Notwithstanding these assumptions and bias, this study was set up to initiate a discussion regarding the (over)treatment of these patients and to generate hypotheses for further research to test the safety and feasibility of active surveillance in a larger prospective trial. A second limitation was that not all diagnoses were confirmed by FISH for *MDM2* amplification. Furthermore, data regarding imaging was missing, although imaging is part of the standard diagnostic work-up in both expertise centers.

Since the life expectancy is high and unaffected by local treatment [12], we believe that active surveillance is feasible for selected cases, in particular for elderly patients with comorbidities and minimal symptoms and/or for patients with a large, deep-seated or otherwise inconveniently localized tumor without symptoms in whom surgical resection most probably will lead to substantial morbidity. However, further research is needed before active surveillance can be safely applied in daily clinical practice. Therefore, we propose a prospective observational study to investigate the differences between surgical treatment and active surveillance in patients with extremity WDLPS regarding disease-specific survival, dedifferentiation rates, tumor growth (using the RECIST criteria [28]) and quality of life, comparable to the prospective studies in patients with desmoid-type fibromatosis treated with active surveillance [10, 29, 30]. This future prospective trial should include regular MRI imaging, allowing for timely intervention in case of tumor growth and/or signs of dedifferentiation.

Conclusion

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Although the numbers are small and the follow-up relatively short, the evaluation of the natural history of extremity WDLPS illustrated that active surveillance could be a reasonable option for selected patients. This highlights the observation that there might be an overtreatment of these patients, since surgical treatment might lead to morbidity and even mortality in patients with this borderline malignant tumor. The harms and benefits of surgical treatment and active surveillance should be carefully balanced, taking the extension and localization of the tumor (i.e. complexity of the surgery), comorbidities and the indolent disease course into account. This especially applies for elderly patients with comorbidities and/or patients with large, deep-seated or otherwise inconveniently localized tumors without symptoms. We propose to conduct a prospective observational study to assess the safety and outcomes of active surveillance in this patient group.

Conflicts of interest

None of the authors have any disclosures or conflicts of interest.

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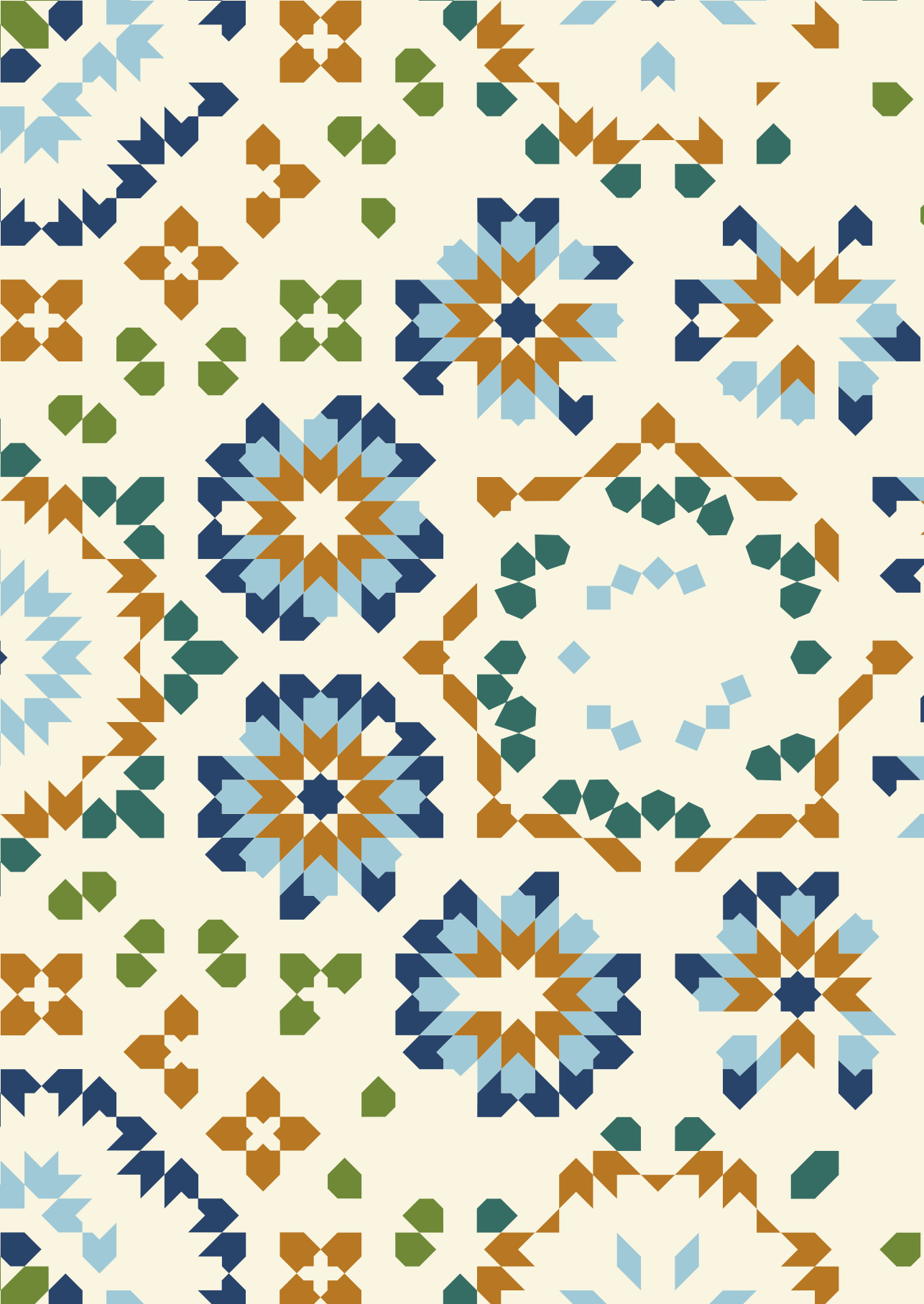
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CHAPTER 9

INCREASED SURVIVAL OF NON LOW-GRADE AND DEEP-SEATED SOFT TISSUE SARCOMA AFTER SURGICAL MANAGEMENT IN HIGH- VOLUME HOSPITALS: A NATIONWIDE STUDY FROM THE NETHERLANDS

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Abstract

Background: Diagnosing and treating soft tissue sarcomas (STSs) remains challenging, stressing the urgency for centralisation. This nationwide survey aimed to evaluate the centralisation of STS surgery and its effect on survival.

Methods: Patients operated for primary STS from 2006 to 2015 were queried from the Netherlands Cancer Registry. Hospitals in which STS surgery was performed were allocated into three categories: low-volume (1-9 resections per year), medium-volume (10-19 resections) or high-volume (≥ 20 resections). Differences in tumour characteristics and outcome were calculated. A multivariable regression analysis was performed to adjust for case-mix.

Results: Of the 5282 identified patients, 42% was treated in low-volume hospitals, 7.7% in medium-volume hospitals and 51% in high-volume hospitals, with a significant trend over time towards treatment in a high-volume hospital ($p < 0.01$). In high-volume hospitals, more often patients with non low-grade, large and deep-seated tumours were treated than in low-volume hospitals. For the whole group, there was no survival benefit for patients treated in high-volume hospitals, with 10-year net survival rates of 76% (low-volume), 68% (medium-volume) and 68% (high-volume). However, subgroup analysis for patients with non low-grade and deep-seated tumours did reveal a benefit from treatment in a high-volume hospitals with 10-year survival rates of 54% (high-volume), 49% (low-volume) and 42% (medium-volume) and a relative risk of 1.3 (high-volume versus low-volume, $p = 0.03$).

Conclusion: Centralisation of STS surgery has increased in the past decade. Surgery in a high-volume hospital improved survival of patients with non low-grade and deep-seated tumours, and therefore these patients should be referred to such a hospital.

Keywords: Soft tissue sarcoma; Centralisation; Survival; Surgery

Introduction

Soft tissue sarcomas (STSs) are a group of rare mesenchymal tumours and comprise approximately 1% of all adult malignancies. Within the group of STS, over 50 different malignant histological subtypes have been described, with a broad variety in biological behaviour, presentation, treatment approach and prognosis. Owing to the rarity of these tumours, it is estimated that a general practitioner in the Netherlands only sees one patient with STS every 20 years and a surgeon in a general hospital only once every 4 years, which makes it difficult to gain sufficient clinical experience in diagnosing and treating these patients [1, 2].

These observations highlight the urgency for centralisation of sarcoma care, in both diagnosis and treatment. Within the Netherlands, we strive to centralise sarcoma care into dedicated STS expertise centres, but centralisation until 2011 was limited and in need of improvement [3].

The aim of this nationwide study was to determine whether centralisation of STS care has increased over time and whether this has affected survival and other surgical outcomes, such as the proportion of 'whoops' resections, by using data of the Netherlands Cancer Registry (NCR).

Methods

Data collection

All patients diagnosed with primary STS and who underwent surgery during the time interval 2006-2015 were identified and queried from the NCR. Gastrointestinal stromal tumours (GISTs), visceral sarcomas, Kaposi sarcomas and children (age at diagnosis <18 years) were excluded. Data on patient characteristics, tumour characteristics and primary treatment were obtained directly from patients' medical records by data managers of the Netherlands Comprehensive Cancer Organisation, which hosts the NCR.

The STS were categorised according to the World Health Organisation-classification and graded according to the Fédération Nationale des Centres de Lutte Contre Le Cancer (FNCLCC) [1]. Grade I tumours were labelled as low-grade tumours. Grade II tumours, grade III tumours and tumours in which grading is not applicable were pooled and labelled as non low-grade tumours. Tumour subtypes and localisations were recorded in the NCR following the International Classification of Diseases for Oncology (third version) (ICD-O-3) morphology and ICD-O-3 topography codes. There was no central pathology review. Tumours were classified as superficial when located entirely above the fascia or as deep-seated when located beneath the fascia or with invasion of the fascia.

For assessing patients' survival, information on their vital status during follow-up was obtained through linkage with the Municipal Personal Records Database. The most recent linkage for the current study was performed in February 2018.

Potential 'whoops' resections were identified by coinciding dates of first pathological confirmation and surgical resection. They were named 'potential' because not all these resections may have been unplanned but instead deliberately be performed without prior biopsy (i.e. diagnostic excision). Resection margins were classified as R0 (microscopically negative margins), R1 (microscopically positive margins), R2 (macroscopically positive margins) or Rx (unknown/margins not assessed). The proportion of patients requiring multiple procedures (i.e. re-resections) included patients who underwent more than one operation as part of their primary treatment.

Owing to the nature of our data source, no data were available regarding comorbidities/medical history, local recurrence rate, distant metastasis rate or causes of death/disease-specific survival (DSS).

Hospitals performing STS surgery

The hospitals in which the patients were treated were allocated into three categories based on the number of STS resections performed annually: 1-9 resections (low-volume), 10-19 resections (medium-volume) or ≥ 20 resections per year (high-volume).

Statistical analyses

Trends in STS treatment and in centralisation of STS surgery over the study period were tested for significance using the np-trend test [4]. Age of the different subgroups was presented as medians with corresponding interquartile ranges (IQRs). To estimate the impact of surgical volume on survival, net survival rates were calculated as an approximation of – and perhaps more robust alternative to [5] – DSS. Accordingly, crude survival rates were adjusted for the expected survival in the general population according to persons' age, sex and birth year by applying the lifetable approach. In other words, the crude survival rates were adjusted for mortality in a comparable 'healthy' population of equal age and gender as a proxy for DSS, since the NCR does not register information on recurrence and DSS. For high-volume and low-volume hospitals, the Pohar Perme method [6] was used to estimate the net survival rates, while for medium-volume hospitals the Ederer-II method [7] was chosen to prevent overcorrection because of the low number of patients in this subgroup. The univariable impact of surgical volume was displayed graphically, and a multivariable Poisson regression model was developed to assess the effect of surgical volume adjusted for established prognostic factors (age, STS subtype, grade, depth and size). Subsequently, the same analyses were performed for the subgroup of patients with non low-grade and deep-

seated tumours. All tests were two-sided, and p-values <0.05 were considered statistically significant. Statistical analyses were performed using Stata version 14.1 (StataCorp, College Station, Texas).

Results

Patient characteristics

In total 5282 patients who were diagnosed with primary STS and who underwent STS surgery between 2006 and 2015 were identified, with a median age of 61 year (IQR 47-73). The most common subtypes were liposarcoma, leiomyosarcoma and fibrosarcoma, and the extremity and trunk were the most frequently observed localisations. Most tumours were non low-grade, superficially located and larger than 5 cm (Supplementary table S1). Most patients underwent surgery only (61%), and approximately a third of the patients received radiotherapy. A small subgroup received systemic therapy as part of their primary treatment (5.9%) (Table 1).

Hospitals performing STS surgery

On annual average, in 76 hospitals STS surgery was performed. This number decreased from 82 hospitals in 2006-2007 to 66 in 2014-2015 ($p = 0.05$), mainly because of a decrease in the number of low-volume hospitals (72 to 56) (Table 1). Of the hospitals in which STS surgery was performed, 88% of the hospitals were low-volume hospitals in which 42% of all STS patients were treated, 3.9% were medium-volume hospitals in which 7.7% of all STS patients were treated, and 7.9% were high-volume hospitals in which 51% of all STS patients were treated (Table 1, Fig. 1A, Fig. 1B). Patients treated in low-volume hospitals had a median age of 64 years (IQR 49-77), patients treated in medium-volume hospitals had a median age of 62 years (IQR 46-72), and patients treated in high-volume hospitals had a median age of 59 years (IQR 46-70). During the study period, there was a significant trend over time towards treatment in a high-volume hospital, from 43% of the patients in 2006-2007 to 62% of the patients in 2014-2015 being treated in a high-volume hospital ($p < 0.01$) (Table 1, Fig. 1B).

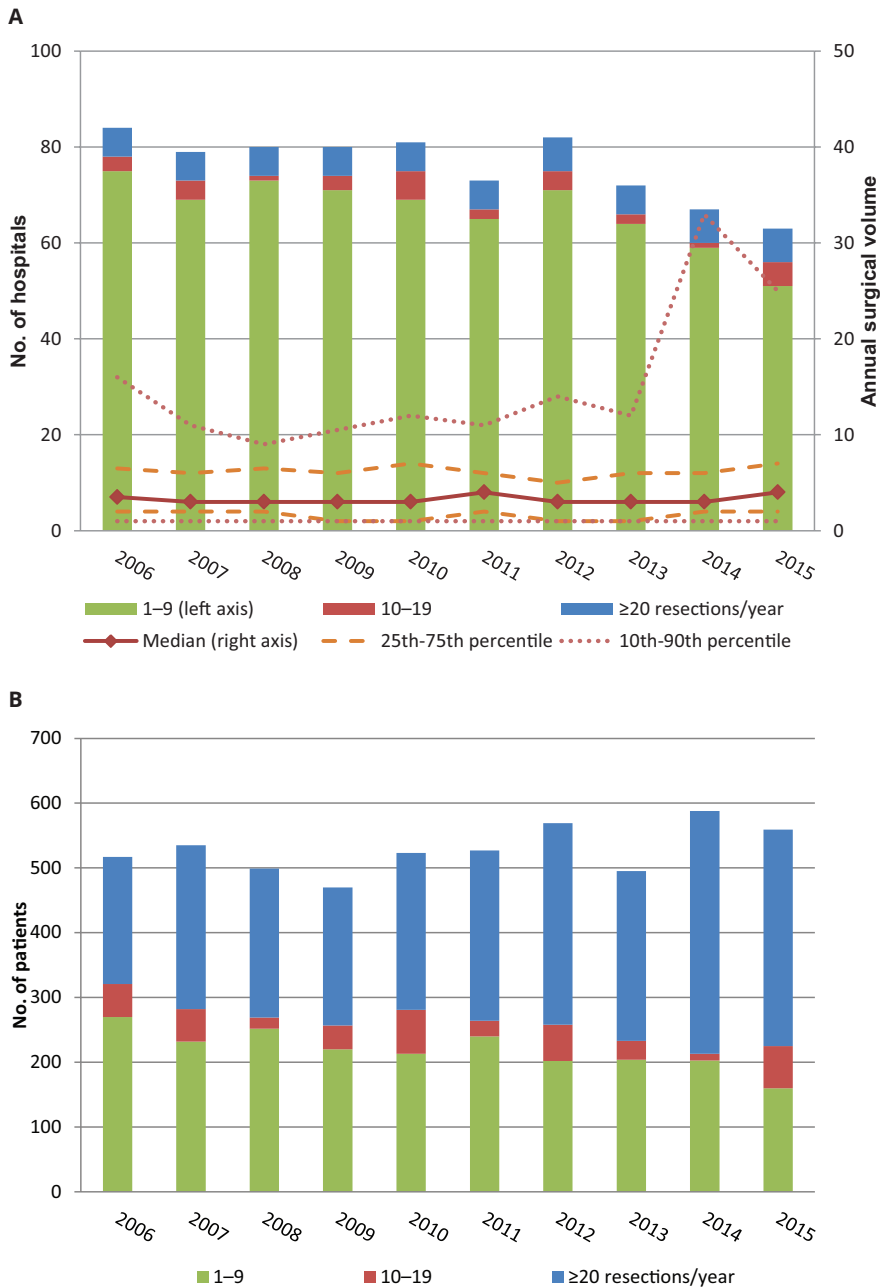
We observed a skewed distribution of patients across the hospitals in which STS surgery was performed, although a significant change over time was observed ($p < 0.01$) (Fig. 2). While in 2006-2007 10.3% of all hospitals accounted for half of all STS resections, this proportion decreased to 6.0% in 2014-2015. In 2014-2015, 75% of the STS resections were performed in 21% of the hospitals (35% in 2006-2007), and 90% of the resections in 46% of the hospitals (59% in 2006-2007). The last 10% of resections are widely spread over the remaining 40-55% of the hospitals.

Table 1. Trends in the treatment and centralisation of patients diagnosed with soft tissue sarcoma and undergoing surgery in the Netherlands during the study period (2006–2015).

Factor	2006– 2007	2008– 2009	2010– 2011	2012– 2013	2014– 2015	Total period	p- value ^c
No. of STS patients with primary surgery	1052	969	1050	1064	1147	5282	
Primary treatment regimen, n (%)							p < 0.01
Surgery only	611 (58%)	596 (62%)	643 (61%)	660 (62%)	735 (64%)	3245 (61%)	
Surgery + RTx	364 (35%)	305 (32%)	352 (34%)	337 (32%)	367 (32%)	1725 (33%)	
Surgery + RTx + CTx	37 (3.5%)	24 (2.5%)	31 (3.0%)	37 (3.5%)	22 (1.9%)	151 (2.9%)	
Surgery + CTx	40 (3.8%)	44 (4.5%)	24 (2.3%)	30 (2.8%)	23 (2.0%)	161 (3.0%)	
No. of patients treated per surgical volume, n (%)							p < 0.01
1–9 resections/year (low-volume)	502 (48%)	472 (49%)	453 (43%)	406 (38%)	363 (32%)	2196 (42%)	
10–19 resections/year (medium-volume)	101 (9.6%)	54 (5.6%)	92 (8.8%)	85 (8.0%)	75 (6.5%)	407 (7.7%)	
≥20 resections/year (high-volume)	449 (43%)	443 (46%)	505 (48%)	573 (54%)	709 (62%)	2679 (51%)	
Mean no. of hospitals performing STS surgery ^a	82	80	77	77	66	76	p = 0.05 ^d
Total no. of hospitals performing STS surgery ^b	87	89	86	88	83	105	p = 0.09
Mean no. of hospitals performing STS surgery per surgical volume, n (%)							p = 0.29
1–9 resections/year (low-volume)	72 (88%)	72 (90%)	67 (87%)	67 (87%)	56 (86%)	67 (88%)	
10–19 resections/year (medium-volume)	4 (4.9%)	2 (2.5%)	4 (5.2%)	3 (3.9%)	3 (4.6%)	3 (3.9%)	
≥20 resections/year (high-volume)	6 (7.3%)	6 (7.5%)	6 (7.8%)	7 (9.1%)	7 (11%)	6 (7.9%)	
Proportion of operations in top quartile of hospitals	69%	71%	74%	80%	77%	76%	p < 0.01

STS, soft tissue sarcoma; RTx, radiotherapy; CTx, chemotherapy

^a Mean over period^b Including mergers^c Tested for trend over total study period using the np-trend test^d Tested for trend over total study period using a linear regression analysis



▲**Fig. 1.** Trends in centralisation of STS surgery of patients diagnosed in the Netherlands from 2006 to 2015 stratified by surgical volume (low-volume: 1-9 resections, medium-volume: 10-19 resections, high-volume: ≥ 20 resections). **(A)** Number of hospitals performing STS surgery. **(B)** Number of patients undergoing STS surgery. STS, soft tissue sarcoma.

Case-mix in hospitals performing STS surgery

In high-volume and medium-volume hospitals, mainly patients with non low-grade STS (73% and 75%) were treated, while the proportion of patients with non low-grade tumours treated in low-volume hospitals was 56%. In high-volume centres also mainly patients with large tumours (70%) were treated, whereas this number was lower in medium-volume hospitals (61%) and low-volume hospitals (46%). At last, in low-volume hospitals, mainly patients

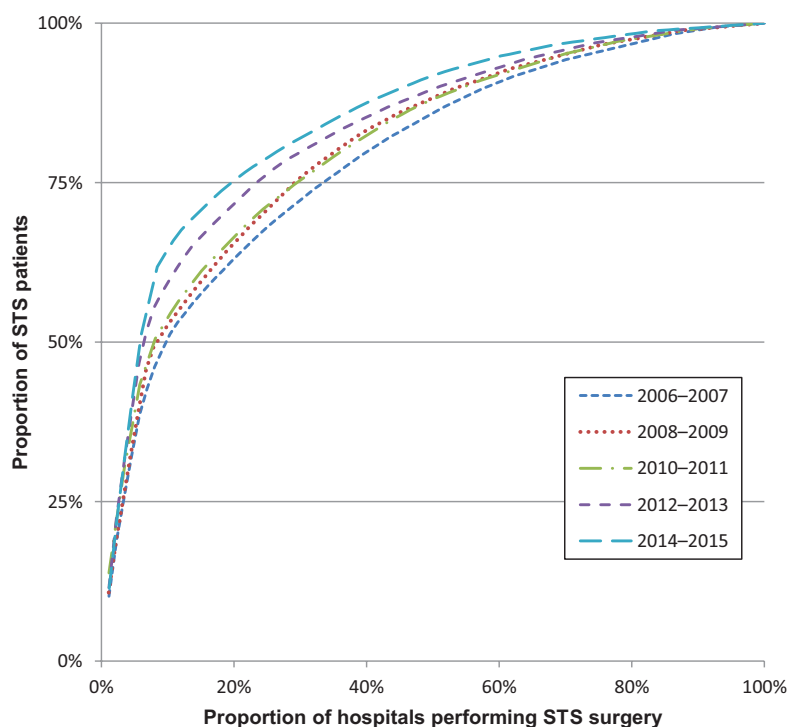
Table 2. Tumour characteristics and surgical characteristics of patients undergoing surgery for soft tissue sarcoma in the Netherlands from 2006 to 2015 stratified by hospital volume.

Factor	2006–2007	2008–2009	2010–2011	2012–2013	2014–2015	Total period	p-value ^d
Surgery in low-volume hospitals (1–9 resections/year)	502	472	453	406	363	2196	
Tumour grade ^a , n (%)							p = 0.93
Low grade	184 (44%)	154 (40%)	177 (49%)	143 (46%)	104 (40%)	762 (44%)	
Non low-grade	236 (56%)	228 (60%)	184 (51%)	165 (54%)	156 (60%)	969 (56%)	
Tumour size ^b , n (%)							p < 0.01
≤5 cm	148 (50%)	140 (47%)	161 (53%)	164 (56%)	181 (64%)	794 (54%)	
>5 cm	149 (50%)	159 (53%)	140 (47%)	130 (44%)	102 (36%)	680 (46%)	
Tumour depth ^c , n (%)							p < 0.01
Superficial	333 (75%)	289 (70%)	333 (77%)	290 (76%)	271 (79%)	1516 (75%)	
Deep	110 (25%)	122 (30%)	98 (23%)	93 (24%)	70 (21%)	493 (25%)	
Potential 'whoops' resections, n (%)	293 (58%)	282 (60%)	298 (66%)	256 (63%)	239 (66%)	1368 (62%)	p = 0.02
Potential 'whoops' resections for large, deep-seated and non-low grade tumours, n(%)	20 (40%)	28 (48%)	25 (48%)	17 (36%)	16 (55%)	106 (45%)	p = 0.83
Patients with R1/R2 resection, n (%)	72 (14%)	65 (14%)	66 (15%)	53 (13%)	57 (16%)	313 (14%)	p = 0.76
Patients requiring multiple procedures, n (%)	175 (35%)	163 (35%)	182 (40%)	151 (37%)	119 (33%)	790 (36%)	p = 0.99
Surgery in medium-volume hospitals (10–19 resections/year)	101	54	92	85	75	407	
Tumour grade ^a , n (%)							p = 0.74
Low grade	20 (22%)	10 (24%)	20 (26%)	24 (32%)	11 (18%)	85 (25%)	
Non low-grade	72 (78%)	32 (76%)	56 (74%)	50 (68%)	49 (82%)	259 (75%)	
Tumour size ^b , n (%)							p < 0.01
≤5 cm	17 (22%)	14 (33%)	28 (40%)	32 (54%)	32 (50%)	123 (39%)	
>5 cm	62 (78%)	29 (67%)	42 (60%)	27 (46%)	32 (50%)	192 (61%)	

Factor	2006–2007	2008–2009	2010–2011	2012–2013	2014–2015	Total period	p-value ^d
Tumour depth ^c , n (%)							p = 0.01
Superficial	37 (40%)	26 (52%)	60 (69%)	58 (77%)	33 (47%)	214 (57%)	
Deep	55 (60%)	24 (48%)	27 (31%)	17 (23%)	37 (53%)	160 (43%)	
Potential 'whoops' resections, n (%)	37 (37%)	23 (43%)	42 (46%)	43 (51%)	33 (44%)	178 (44%)	p = 0.17
Potential 'whoops' resections for large, deep-seated and non-low grade tumours, n(%)	7 (20%)	2 (13%)	4 (27%)	3 (30%)	5 (31%)	21 (23%)	p = 0.74
Patients with R1/R2 resection, n (%)	26 (26%)	10 (19%)	8 (8.7%)	8 (9.4%)	12 (16%)	64 (16%)	p = 0.01
Patients requiring multiple procedures, n (%)	25 (25%)	17 (32%)	27 (29%)	30 (35%)	18 (24%)	117 (29%)	p = 0.70
Surgery in high-volume hospitals (≥20 resections/year)							
	449	443	505	573	709	2679	
Tumour grade ^a , n (%)							p = 0.48
Low grade	107 (26%)	101 (26%)	112 (25%)	144 (29%)	164 (27%)	628 (27%)	
Non low-grade	301 (74%)	292 (74%)	336 (75%)	357 (71%)	444 (73%)	1730 (73%)	
Tumour size ^b , n (%)							p = 0.35
≤5 cm	109 (30%)	106 (30%)	114 (28%)	139 (30%)	197 (32%)	665 (30%)	
>5 cm	257 (70%)	247 (70%)	299 (72%)	330 (70%)	410 (68%)	1543 (70%)	
Tumour depth ^c , n (%)							p < 0.01
Superficial	218 (56%)	193 (51%)	260 (56%)	303 (57%)	352 (52%)	1326 (54%)	
Deep	171 (44%)	184 (49%)	204 (44%)	232 (43%)	330 (48%)	1121 (46%)	
Potential 'whoops' resections, n (%)	128 (29%)	132 (30%)	147 (29%)	170 (30%)	198 (28%)	775 (29%)	p = 0.76
Potential 'whoops' resections for large, deep-seated and non-low grade tumours, n(%)	19 (18%)	16 (14%)	9 (8%)	18 (14%)	24 (12%)	86 (13%)	p = 0.51
Patients with R1/R2 resection, n (%)	98 (22%)	86 (19%)	103 (20%)	118 (21%)	105 (15%)	510 (19%)	p = 0.01
Patients requiring multiple procedures, n (%)	132 (29%)	133 (30%)	141 (28%)	164 (29%)	204 (29%)	774 (29%)	p = 0.70

^a Excluding unknown grade. ^b Excluding unknown size ^c Excluding unknown depth. ^d Tested for trend over total study period using the np-trend test.

with superficial tumours (76%) were treated, whereas in medium-volume and high-volume hospitals, the distribution between superficial and deep-seated tumours was more equal (medium-volume: 57% superficial versus 43% deep; high-volume: 54% superficial versus 46% deep) (Table 2).



▲ **Fig. 2.** Allocation of patients undergoing STS surgery in the Netherlands from 2006 to 2015 across the hospitals performing STS surgery. Trend over time was tested by a test for equality of the regression coefficients of the fitted values. STS, soft tissue sarcoma.

Over the years, in low-volume and medium-volume hospitals, significantly less patients with deep-seated tumours were operated (low-volume: 25% in 2006-2007 to 21% in 2014-2015, $p < 0.01$; medium-volume: 60% to 53%, $p = 0.01$), and significantly less patients with large tumours were operated (low-volume: 50% to 36%, $p < 0.01$; medium-volume: 79% to 50%, $p < 0.01$). On the contrary, in high-volume hospitals, the proportion of patients with deep-seated tumours increased (44% to 48%, $p < 0.01$), while the proportion of patients with large tumours remained stable (70% to 68%, $p = 0.35$). There were no significant changes over time in the proportions of patients with non low-grade and low-grade tumours (low-volume: $p = 0.93$, medium-volume: $p = 0.74$, high-volume: $p = 0.48$) (Table 2).

Shift in use of treatment modalities

Most probably related to this case-mix, the use of neoadjuvant/adjuvant radiotherapy and chemotherapy raised as the annual surgical volume increased ($p < 0.001$). Whereas in low-volume hospitals, 75% of the patients were treated with surgery alone, this proportion was 61% in medium-volume hospitals and 50% in high-volume hospitals. Subsequently, the

proportion of patients receiving neoadjuvant/adjuvant radiotherapy increased from 23% in low-volume hospitals to 35% in medium-volume hospitals and 46% in high-volume hospitals. The proportion of patients receiving chemotherapy also increased from 2.1% in low-volume hospitals to 6.1% in medium-volume hospitals and 9.0% in high-volume hospitals (Table 3).

Table 3. Use of the different treatment modalities of patients undergoing surgery for soft tissue sarcoma in the Netherlands from 2006 to 2015, stratified by surgical volume.

Treatment	Low-volume, n (%)	Medium-volume, n (%)	High-volume, n (%)	Total, n (%)
Surgery alone	1656 (75%)	250 (61%)	1339 (50%)	3245 (61%)
Surgery + RTx	493 (22%)	132 (32%)	1100 (41%)	1725 (33%)
Surgery + RTx + CTx	14 (0.6%)	11 (2.7%)	126 (4.7%)	151 (2.9%)
Surgery + CTx	33 (1.5%)	14 (3.4%)	114 (4.3%)	161 (3.0%)

χ^2 -test: $p < 0.001$

RTx: radiotherapy, CTx: chemotherapy

STS surgery – Potential ‘whoops’ resections, resection margins and multiple procedures

The proportion of patients undergoing a potential ‘whoops’ resection was lower as the annual surgical volume increased: 62% in low-volume hospitals, 44% in medium-volume hospitals and 29% in high-volume hospitals ($p < 0.01$). For medium-volume and high-volume hospitals, there were no significant changes in this proportion over time ($p = 0.17$ and $p = 0.76$), but for low-volume hospitals, this proportion increased from 58% in 2006-2007 to 66% in 2014-2015 ($p = 0.02$) (Table 2).

Considering R1/R2 resections, the number of non-radical resections was higher when patients were treated in high-volume hospitals (19%) than in medium-volume (16%) and low-volume (14%) hospitals ($p < 0.01$). Over time, the amount of non-radical resections was stable for low-volume hospitals ($p = 0.76$) but decreased for medium-volume (26% to 16%, $p = 0.01$) and high-volume hospitals (22% to 15%, $p = 0.01$) (Table 2).

The number of patients requiring multiple procedures varied from 29% in high-volume hospitals and in medium-volume hospitals to 36% in low-volume hospitals ($p < 0.01$). These proportions remained stable over time (low-volume: $p = 0.99$, medium-volume: $p = 0.70$, high-volume: $p = 0.70$) (Table 2).

Effects on survival

Univariable net survival rates were significantly higher for patients treated in low-volume hospitals than for those treated in medium-volume and high-volume hospitals, with 10-year net survival rates of 76% versus 68% and 68% respectively and relative rates of 1.5 (medium-volume versus low-volume, 95% confidence interval [CI] 1.2-2.0, $p = 0.001$) and 1.5 (high-

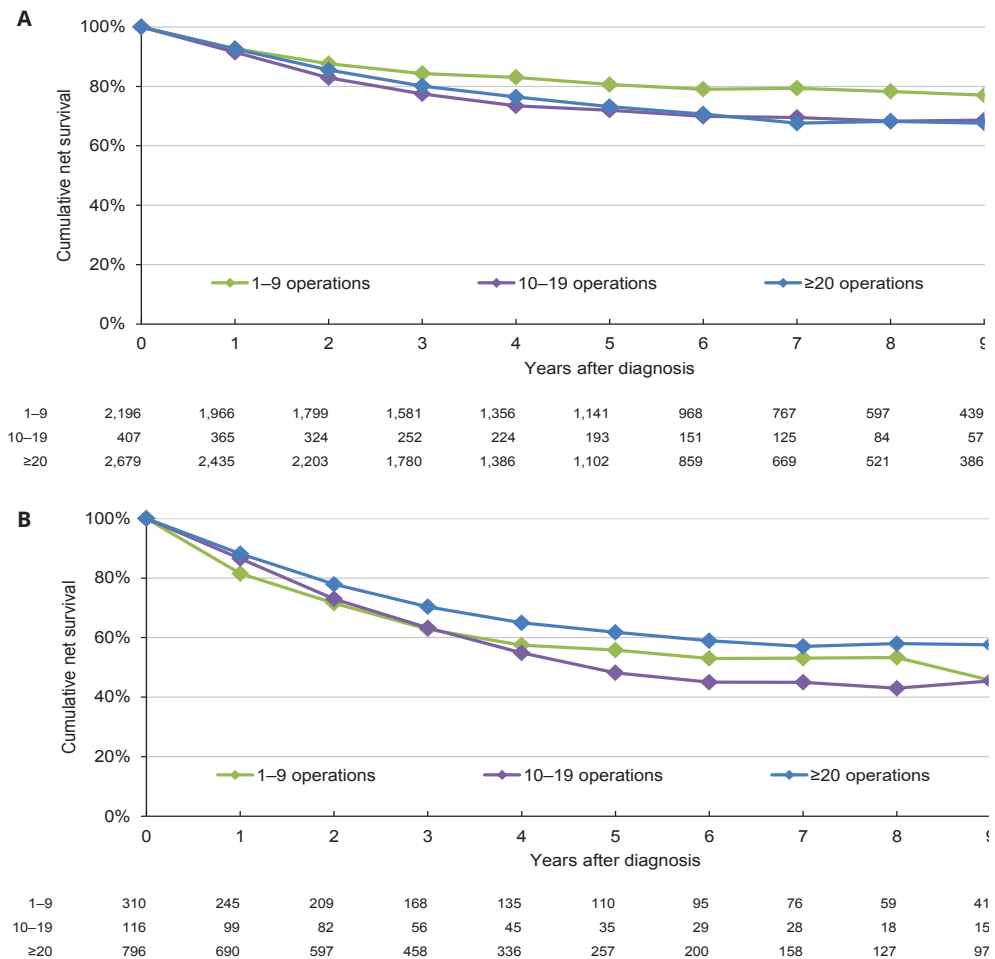
volume versus low-volume, 95% CI 1.3-1.7, $p < 0.001$) (Fig. 3A). However, after adjustment for other prognostic factors, a multivariable Poisson regression analysis did not show any impact of surgical volume on net survival (medium-volume versus high-volume: relative rate [RR] 1.2, 95% CI 0.93-1.4, $p = 0.20$; low-volume versus high-volume: RR 1.01, 95% CI 0.87-1.2, $p = 0.91$) (Table 4A).

Table 4. Effect of surgical volume on survival after adjustment for case-mix (age, STS subtype, size, grade and depth) in a multivariable Poisson regression analysis. Results of the total study cohort **(A)** and of the subgroup analysis including only patients with non low-grade and deep-seated tumours **(B)**. Full results with all covariates are shown in Supplementary Table S2.

Hospital volume	RR	95% CI	p-value
(A)			
≥20 (high)	ref		
10-19 (medium)	1.2	0.93-1.4	0.20
1-9 (low)	1.01	0.87-1.2	0.91
(B)			
≥20 (high)	ref		
10-19 (medium)	1.3	0.98-1.8	0.07
1-9 (low)	1.3	1.02-1.6	0.03*

STS, soft tissue sarcoma; RR, relative risk; 95% CI, 95% confidence interval
* means statistically significant/ $p < 0.05$.

Since in high-volume hospitals more often patients with non low-grade, large and deep-seated tumours were treated (Table 2), which is associated with more complex surgery, a subgroup analysis was performed including only patients with non low-grade and deep-seated STS (median age 61 years, IQR 48-71). The univariable analysis on patients with non low-grade and deep-seated tumours ($n = 1222$) did not show a difference in survival anymore, with net survival rates of 49%, 42% and 54%, respectively and relative rates of 1.03 (medium-volume versus low-volume, 95% CI 0.76-1.4, $p = 0.84$) and 0.83 (high-volume versus low-volume, 95% CI 0.69-1.01, $p = 0.06$) (Fig. 3B). However, in multivariable analysis, surgery in a high-volume hospital did show a significant and beneficial effect on net survival compared with surgery in a low-volume hospital (RR 1.3, 95% CI 1.02-1.6, $p = 0.03$). The same impact was observed in comparison with medium-volume hospitals, although this failed to reach statistical significance (RR 1.3, 95% CI 0.98-1.8, $p = 0.07$) (Table 4B). The full results of the multivariable Poisson regression analysis are shown in Supplementary Table S2.



▲ **Fig. 3. Net survival** curves of patients undergoing STS surgery in the Netherlands from 2006 to 2015 stratified by surgical volume (low-volume: 1-9 resections, medium-volume: 10-19 resections, high-volume: ≥ 20 resections). (A) Net survival rate of all patients undergoing STS surgery (medium-volume versus low-volume: RR 1.5, 95%CI 1.2-2.0, $p = 0.001$; high-volume versus low-volume: RR 1.5, 95%CI 1.3-1.7, $p \leq 0.001$). (B) Net survival rate of patients undergoing STS surgery for non low-grade and deep-seated tumours ($n = 1222$) (medium-volume versus low-volume: RR 1.03, 95%CI 0.76-1.4, $p = 0.84$; high-volume versus low-volume: RR 0.83, 95%CI 0.69-1.01, $p = 0.06$). STS, soft tissue sarcoma; RR, relative rate; 95% CI, 95% confidence interval.

Discussion

We observed a significant effect of surgery in a high-volume hospital on net survival rate for patients with non low-grade and deep-seated tumours (i.e. tumours for which more complex surgery and more multidisciplinary treatment is required) and an increase in referring and treating STS patients to/in high-volume hospitals, although STS surgery is still highly fragmented across the country. This increase in centralisation was mainly the result of the

more frequent referral of patients with deep-seated and large tumours from low-volume and medium-volume hospitals to high-volume hospitals, whereas there was no increase in the referral of non low-grade STS.

Previous studies regarding centralisation of STS care mainly reported not only on improvements in surgical outcomes [3, 8-10] and disease-free, relapse-free or progression-free survival [11-15], but also on improvements in overall survival after treatment at expert sites/high-volume hospitals [10, 14-16]. The added value of this study to these studies on centralisation of STS care is the nationwide set-up, and the use of net survival rates as an alternative to and proxy for DSS [5], especially since many patients with low-grade STS are included who most probably will not die from their STS. In the current study, we could confirm an effect on net survival, but only in the subgroup of patients with poor prognostic characteristics, such as non low-grade and deep-seated tumours.

Notably, we observed a large proportion of and an increase in the number of potential 'whoops' resections in low-volume hospitals, although the absolute number of 'whoops' resections decreased over time. It should be remarked that this proportion includes resections of large and deep-seated tumours as well as resections of small and superficial tumours. Especially the latter category, 'whoops' resections of a small and superficial STS, cannot be prevented at all times and are 'all-in-the-game', considering that benign soft tissue lesions are 100 times more prevalent [1]. Furthermore, some of these resections might deliberately have been performed without prior histological confirmation by biopsy (i.e. diagnostic excision). The increase in the proportion of 'whoops' resections in low-volume hospitals even might be the result of centralisation itself. The proportion of tumours that are unrecognised as an STS probably will be stable over time. When low-volume hospitals perform less surgeries, the proportion of these 'whoops' resections will increase. Nonetheless, in a considerable part of these patients suboptimal surgical approaches are chosen [17, 18], with unclear/inadequate resection margins [17-19], and these patients will need to undergo a re-resection to remove residual tumour and obtain adequate margins [19, 20]. This is also reflected in the current study, where patients treated in low-volume hospitals significantly more often needed to undergo re-resection than patients treated in high-volume hospitals.

The higher number of irradical resections in high-volume hospitals, although decreasing over time, is most probably because these hospitals more often operate on large and deep-seated tumours. They might perform planned R1-resections more often, after neoadjuvant radiotherapy, to spare surrounding vital structures, such as the neurovascular bundle, or even to prevent amputation. This hypothesis is also supported by the observation that patients treated in high-volume hospitals more often receive neoadjuvant/adjvant radiotherapy, instead of surgery alone.

It has been shown that simple referral guidelines (referral of all deep-seated tumours and all superficial tumours ≥ 5 cm) can result in nearly complete referral of STS patients to

expertise centres, with an acceptable surplus referral of benign tumours [21]. Currently, the guidelines in the Netherlands list a set of requirements that hospitals have to meet in order to treat STS patients, rather than indicate when to refer patients to an STS expertise centre regarding size, localisation or depth of the tumour [22]. Three of these requirements state that hospitals have to perform at least 10 primary STS resections annually, that all patients have to be discussed in an STS multidisciplinary team regarding diagnostic and treatment procedures and that the formulated advice during these multidisciplinary meetings is mandatory to follow. These referral guidelines allow general surgeons to consult an STS expertise centre for diagnostic and treatment advice without necessarily referring the patient in person. Remarkably, over 85% of the hospitals in which STS surgery is performed do not meet the specific requirement of at least 10 STS resections per year. Unfortunately, the rationales behind deviating from the guidelines are not registered in the NCR. For various reasons, such as travel distance, the patient's own wish, unawareness of the existence of STS expertise centres or based on the (binding) advice of the multidisciplinary tumour board, patients are treated in low-volume hospitals.

On top of the beneficial effect on survival of patients with non low-grade and deep-seated STS and the lower number of 'whoops' resections and re-resections, treatment in a high-volume and expertise centre also might improve STS care on other levels. For example, patients will be treated by more experienced clinicians with more insight into the heterogeneity of the disease, its diagnosis and the rapidly evolving treatment options. Other examples include patient counselling regarding treatment decision-making and inclusion in clinical trials, which might be more optimal in high-volume multidisciplinary hospitals than in low-volume hospitals. However, in order to establish the best possible care for these patients, centralisation of STS care into high-volume hospitals should be paired with improving the diagnostic work-up for soft tissue tumours of unknown origin and creating more awareness, since centralisation is not only a result of high-volume hospitals recruiting these patients but mostly relies on the alertness and willingness of physicians in low-volume and medium-volume hospitals to refer their patients.

Conclusion

Centralisation of STS surgery has increased in the past 10 years, although it is still highly fragmented across the country. Treatment in a high-volume hospital had a beneficial effect on net survival rates for patients with non low-grade and deep-seated STS on a population-level, and it most probably also does reduce surgery-related morbidities reflected by the lower number of potential 'whoops' resections and re-resections. Therefore, we plea for centralisation of STS care into dedicated multidisciplinary expertise centres and for more strict referral guidelines, stating that all patients with suspected or confirmed STS have to

be at least discussed in an expertise centre. Patients with suspected non low-grade and deep-seated STS based on imaging – and subsequently more complex surgeries and more multidisciplinary treatment required – have to be referred to a high-volume hospital for a imaging-guided biopsy prior to start of treatment.

Acknowledgements

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Conflicts of interest statement

None of the authors has any conflicts of interest.

9

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ejca.2019.01.005>.

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Supplementary data

Supplementary table S1. Baseline characteristics of all patients undergoing surgery for soft tissue sarcoma in the Netherlands from 2006-2015 by subtype, grade, size, depth and primary tumour site.

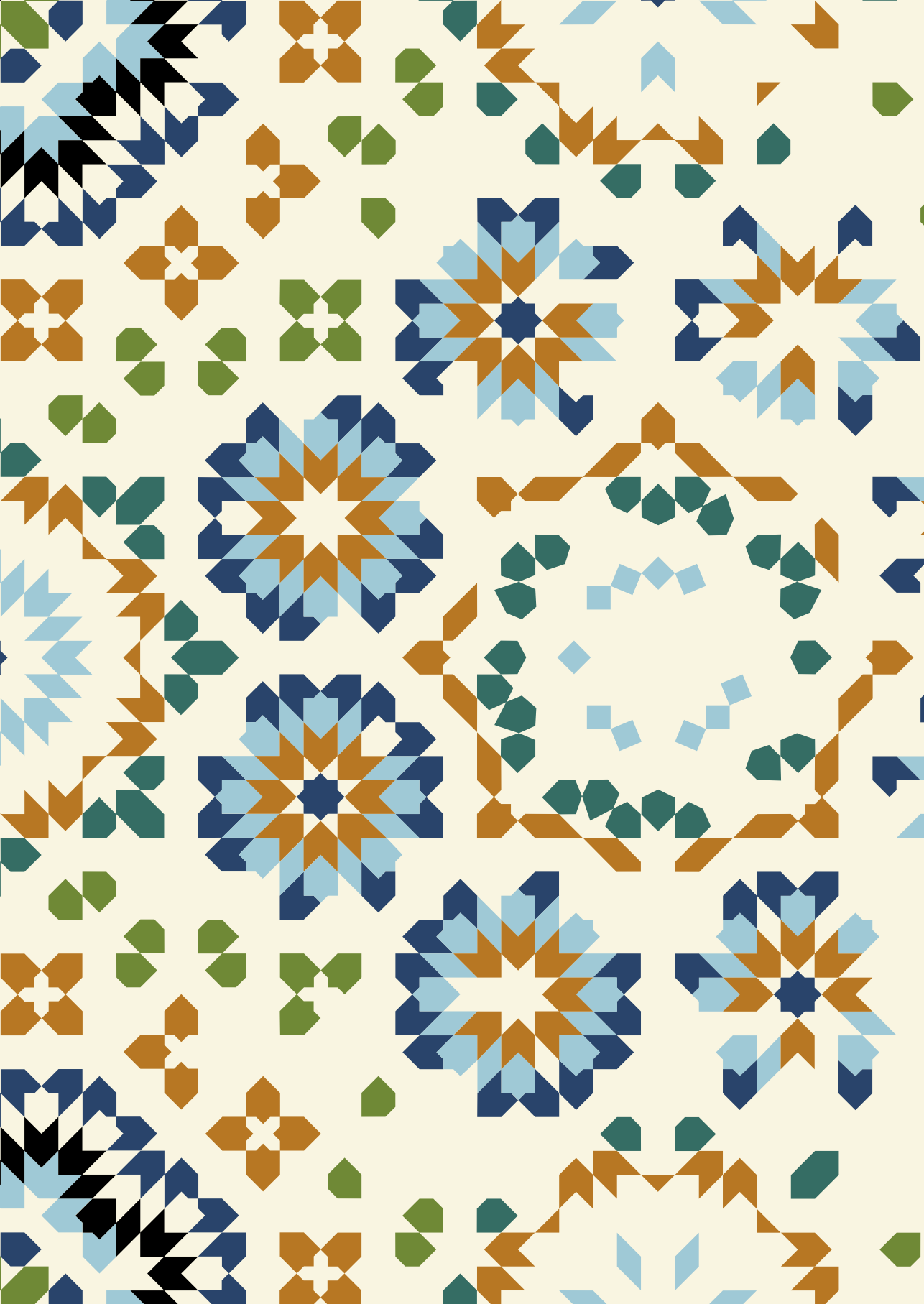
STS subtype	Total		Grade			Size			Depth			Tumor site		
	Low	High	Unknown	≤5 cm	>5 cm	Unknown	Superficial	Deep	Unknown	H&N	Trunk	Extremity	Retro	peritoneum
Liposarcoma	1,087	415	634	38	163	873	51	417	610	60	28	349	501	209
Fibrosarcoma	653	160	343	150	265	363	25	373	245	35	44	157	449	3
Leiomyosarcoma	865	203	423	239	421	308	136	552	265	48	88	285	419	73
PUS	499	12	294	193	221	229	49	324	139	36	150	106	233	10
Angiosarcoma	271	0	271	0	2	3	266	137	6	128	48	180	42	1
Rhabdomyosarcoma	72	0	72	0	24	39	9	30	39	3	14	23	35	0
Synovial sarcoma	155	0	155	0	62	88	5	60	74	21	12	25	117	1
MPNST	235	0	235	0	85	137	13	98	108	29	24	94	115	2
DFSP	632	632	0	0	0	0	632	632	0	0	64	327	241	0
Other	813	53	531	229	339	375	99	433	288	92	189	251	357	16
Total	5,282	1,475	2,958	849	1,582	2,415	1,285	3,056	1,774	452	661	1,797	2,509	315

Abbreviations: STS: soft tissue sarcoma, PUS: pleomorphic undifferentiated sarcoma, H&N: Head & Neck

Supplementary table S2. Results of the multivariable Poisson regression analysis of the total study cohort of patients undergoing surgery for soft tissue sarcoma in the Netherlands from 2006-2015 **(A)** and of the subgroup analysis including only patients with non-low grade and deep-seated tumors **(B)**.

(A)		RR	95% CI	p-value	(B)	RR	95% CI	p-value
Hospital volume	≥20 (high)	ref				ref		
	10-19 (medium)	1.22	0.93-1.4	0.20		1.3	0.98-1.8	0.07
	1-9 (low)	1.01	0.87-1.2	0.91		1.3	1.02-1.6	0.03
Age		1.01	1.01-1.02	<0.01	*	1.01	1.00-1.02	<0.01
STS subtype	Liposarcoma	ref				ref		
	Fibrosarcoma	0.82	0.62-1.1	0.16		0.75	0.50-1.1	0.15
	Leiomyosarcoma	1.5	1.2-1.8	<0.01	*	1.3	0.97-1.7	0.08
	PUS (MFH)	1.7	1.3-2.2	<0.01	*	1.5	1.1-2.1	0.02
	Angiosarcoma	3.4	2.4-4.8	<0.01	*	1.9	0.62-6.0	0.26
	Rhabdomyosarcoma	2.1	1.4-3.1	<0.01	*	1.6	1.00-2.7	0.05
	Synovial sarcoma	1.9	1.4-2.5	<0.01	*	1.3	0.86-2.0	0.22
	MPNST	2.0	1.6-2.6	<0.01	*	1.9	1.3-2.6	<0.01
	DFSP	0.09	0.01-0.97	0.05	*	-	-	-
	Other	1.9	1.5-2.3	<0.01	*	1.1	0.81-1.5	0.59
Size	≤5cm	Ref				Ref		
	>5cm	3.1	2.5-3.8	<0.01	*	2.3	1.7-3.2	<0.01
	Unknown	1.8	1.3-2.5	<0.01	*	2.6	1.5-4.4	<0.01
Grade	Low grade	Ref						
	Non-low grade	4.1	2.9-5.8	<0.01	*			
	Unknown	3.1	2.1-4.6	<0.01	*			
Depth	Superficial	Ref						
	Deep	1.6	1.4-1.8	<0.01	*			
	Unknown	1.5	1.2-1.8	<0.01	*			

RR, relative rate; 95% CI, 95% confidence interval; PUS (MFH), pleomorphic undifferentiated sarcoma (malignant fibrous histiocytoma)





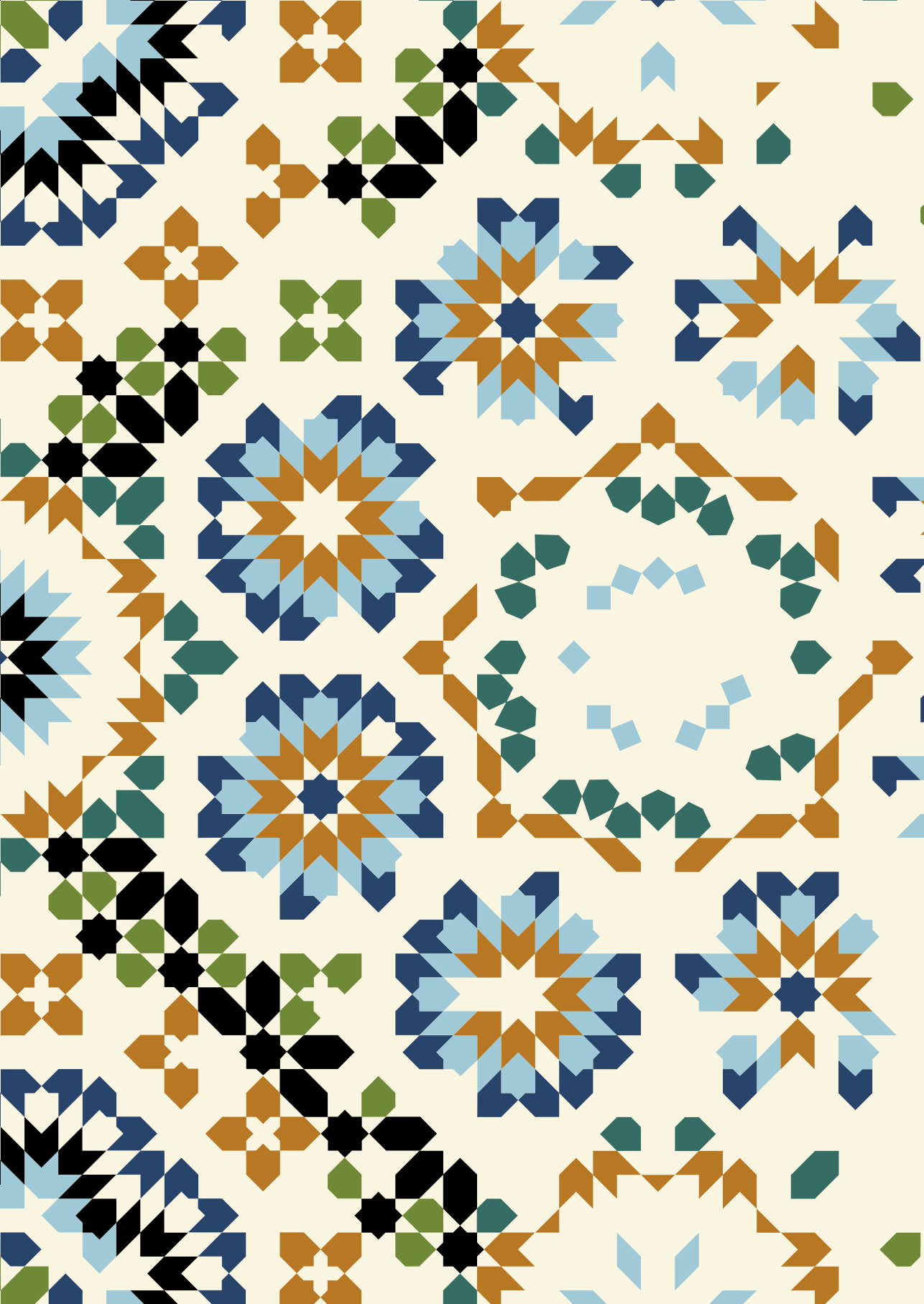
CHAPTER 10

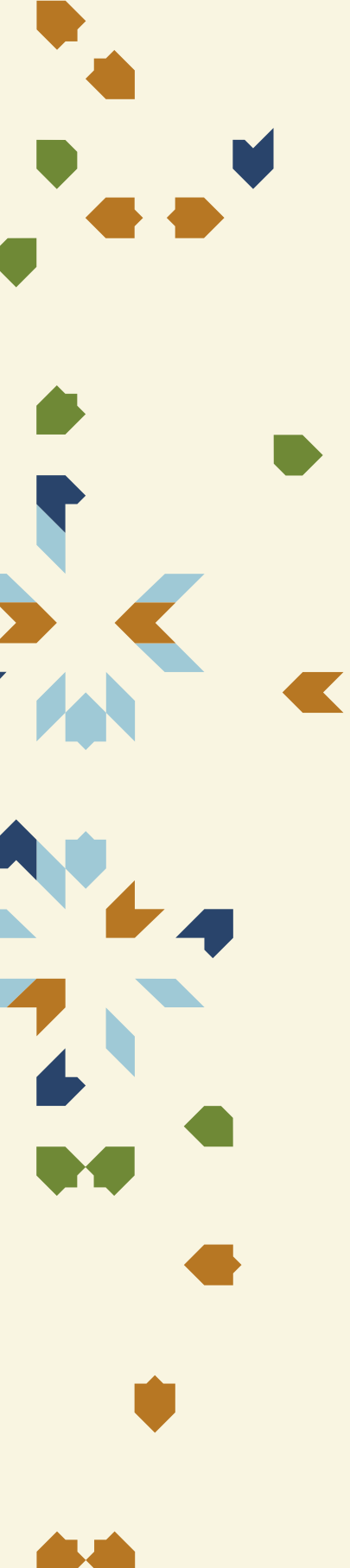
UNPLANNED RESECTIONS OF SOFT TISSUE SARCOMAS

A.S. Melis, M. Vos, M.S. Schuurman, T. van Dalen,
W.J. van Houdt, J.A. van der Hage, Y.M. Schrage, L.B. Been,
J.B. Bonenkamp, M.H.A. Bemelmans, D.J. Grünhagen,
C. Verhoef, V.K.Y. Ho; on behalf of the Dutch Sarcoma Study
Group (DSSG)

Manuscript in preparation.

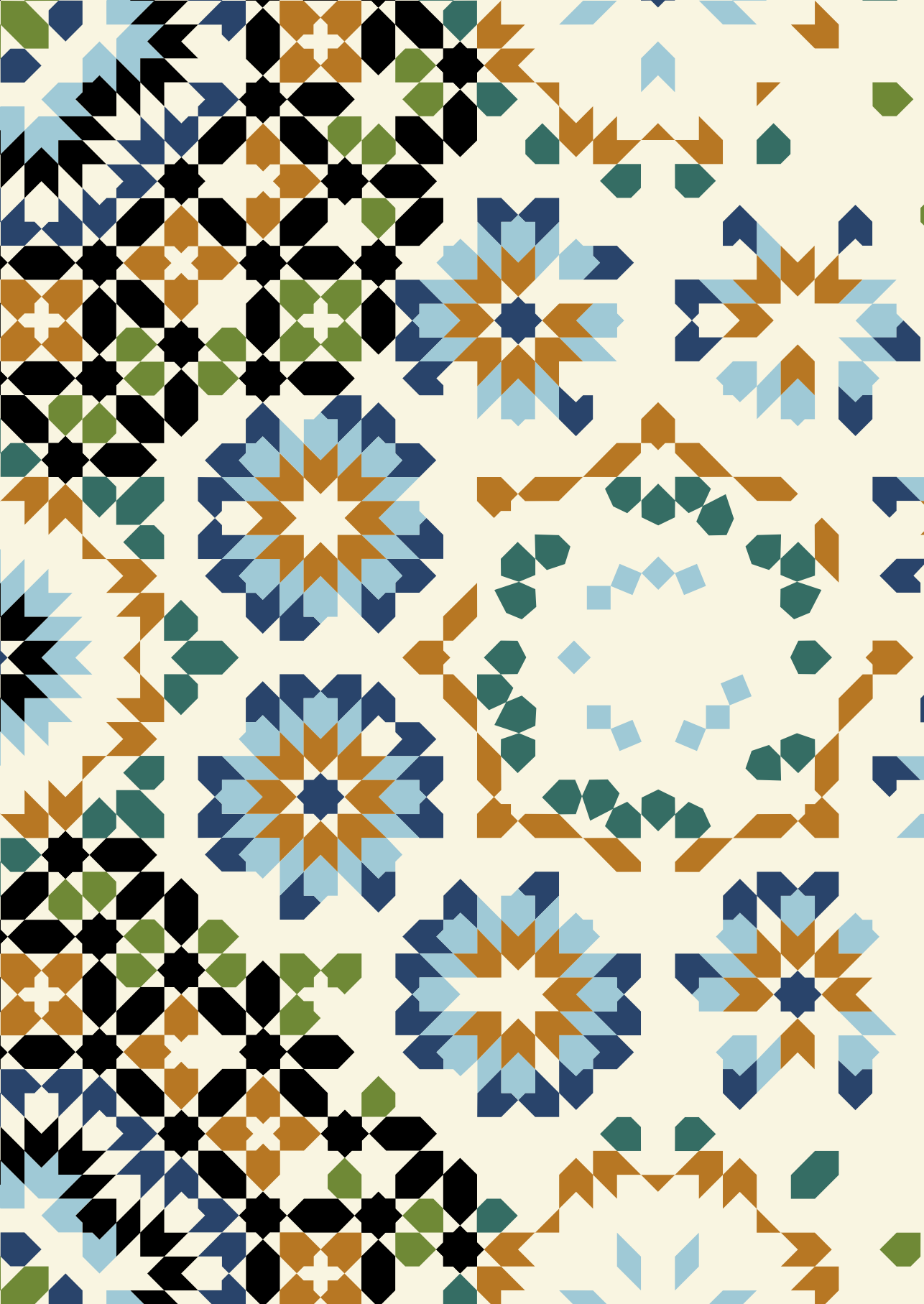
Manuscript in preparation, not included in this PDF.

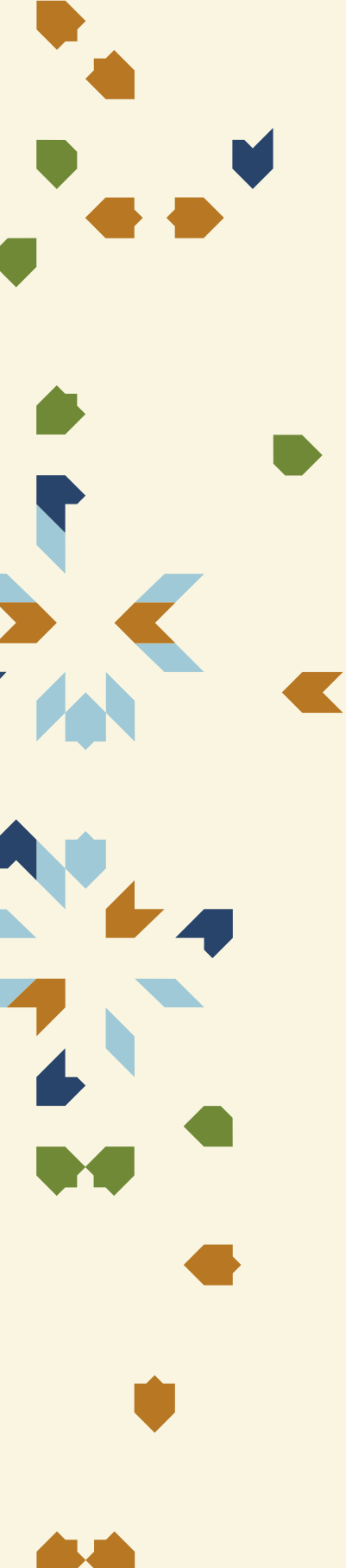




PART IV

EVALUATION OF THE SYSTEMIC TREATMENT OF METASTATIC SOFT TISSUE SARCOMA





CHAPTER 11

EJC'S BIENNIAL REPORT ON METASTATIC SOFT TISSUE SARCOMA: STATE OF THE ART AND FUTURE PERSPECTIVES

M. Vos, S. Sleijfer

Eur J Cancer. 2018 Jan;88:87-91.

Abstract

In the last decade the limited treatment options for patients with metastatic soft tissue sarcoma have expanded considerably. With the addition of olaratumab to first-line treatment with doxorubicin, the introduction of several new agents in second-line treatment and beyond and other promising agents in the pipeline, perspectives of patients with metastatic soft tissue sarcoma are improving. Due to increasing insight into the biology of the different soft tissue sarcoma subtypes, choice of treatment has become much more histology-driven, although more prognostic and predictive factors are needed to further personalise therapy. This report summarises the current state of the art and discusses the promising developments in the treatment of patients with metastatic soft tissue sarcoma.

Keywords: Soft tissue sarcoma; Metastatic soft tissue sarcoma; Chemotherapy; Systemic treatment; Immunotherapy

Introduction

Soft tissue sarcomas (STSs) form a heterogeneous group of rare, mesenchymal tumours, accounting for approximately 1% of all adult malignancies and comprising over 50 different histological subtypes. Roughly they can be divided into three groups; small blue round cell tumours (SBRCTs), gastrointestinal stromal tumours (GISTs) and adult-STTs. The latter group, adult-STTs, forms the topic of the current overview.

Most adult-STT patients are diagnosed at an early stage and can be treated with curative intent by local treatment options such as surgery and radiotherapy. Unfortunately, a substantial proportion of patients presents with metastatic disease at time of initial diagnosis (10-15%) or will develop metastases over time (up to 40%). Here, we summarise the different aspects of the treatment of metastatic STTs while putting the recent developments into perspective.

First-line treatment of widespread metastatic disease

Despite the large heterogeneity in terms of pathogenesis, clinical course and sensitivity to systemic agents across the different STS entities, almost all STS subtypes are treated in the first-line treatment with doxorubicin-based regimens (Table 1). The majority of patients receive doxorubicin monotherapy, yielding a response rate of approximately 10-14%, a median progression-free survival (PFS) of 4.5 months and median overall survival (OS) of 12-18 months in most recent trials [1-5]. Aiming to improve outcome, multiple different regimens have been assessed during the last decades. These efforts were all in vain, apart from the combination of doxorubicin and ifosfamide. This combination resulted in a higher response rate and PFS, but failed to prolong OS and is therefore mainly considered for fit patients in need of a response [1]. Other randomized studies assessing the combinations of doxorubicin plus evofosfamide or palifosfamide, both ifosfamide derivatives, failed to demonstrate clinically relevant superior outcomes over doxorubicin alone [4,5]. Recently, another phase III trial compared the combination of gemcitabine and docetaxel to doxorubicin as first-line treatment. No benefit for the combination was seen over doxorubicin in this case either, also not in specific subtypes in which this combination was thought to be active. Additionally, the combination induced more toxicity and was at the expense of a lower quality-of-life, which only reconfirmed the role of doxorubicin monotherapy in advanced STS [3].

A doxorubicin-based combination that might have a huge impact on the outcome of advanced/metastatic STS patients is the combination with olaratumab, a monoclonal antibody directed towards the platelet-derived growth factor receptor-alpha (PDGFR- α). Based on a phase II trial, randomising patients to either receive doxorubicin plus olaratumab or doxorubicin alone, olaratumab was conditionally approved by the European Medicines

Agency (EMA) as first-line treatment in combination with doxorubicin. The primary goal of the study, a 50% improvement in median PFS, was met (6.6 versus 4.1 months in the combination group versus the doxorubicin alone group; hazard ratio (HR) 0.67; 95% confidence interval (CI) 0.44-1.02). Remarkably, a much larger difference in median OS was seen favouring the combination therapy (26.5 versus 14.7 months in the doxorubicin alone group; HR 0.46; 95% CI 0.30-0.71). This gain was at the expense of an increase in adverse events, such as neutropenia and mucositis, although the rate of treatment discontinuation due to adverse events was lower in the combination group [2]. Recently, accrual of the phase III trial with a similar design has been completed and results are expected in 2020. Obviously, confirmation of these results would mean a substantial improvement for these patients.

Table 1. Overview of the different agents commonly given in first-line treatment, second-line treatment and beyond in advanced STS patients.

Agent	STS subtype	Ref.	Level of evidence and additional remarks
First-line treatment			
Doxorubicin monotherapy	All	[1, 2]	IA
Doxorubicin + olaratumab	All	[2]	IB, conditionally approved pending results of the phase III trial
Doxorubicin + ifosfamide	All	[1]	IA, mainly given to fit patients in need of a response
Second-line treatment and beyond			
Trabectedin	All	[6, 7, 22]	IIB, only level IA available in LPS and LMS
Pazopanib	All but LPS	[8, 9]	IB
Eribulin	LPS	[12]	IA
Taxanes	AS	[15]	IIIB
VEGF-TKIs (cediranib, sunitinib) ASPS		[16, 17]	IIA
Gemcitabine-based regimens	LMS	[13, 14]	IIC

STS, soft tissue sarcoma; LPS, liposarcoma; AS, angiosarcoma; LMS, leiomyosarcoma; ASPS, alveolar soft part sarcoma

Second-line treatment and beyond of widespread metastatic disease

Given the great heterogeneity among the STS subtypes, over 50 of them, it is likely that different systemic treatment approaches will have to be used in diverse STS entities. Whereas this is rarely the case in first-line therapy yet, in second-line therapy, a histology-driven choice is already much more common (Table 1). In the last decade, trabectedin (a synthetic compound derived from a Caribbean sea squirt) was EMA approved for all adults with advanced STS failing first-line treatment. Based on phase II studies, it is mostly used in patients with leiomyosarcomas or liposarcomas ('L-sarcomas'). A recent phase III study in advanced L-sarcoma patients showed superior disease control and clinical benefit, but no significant

survival benefit, over dacarbazine [6,7]. Pazopanib, a multikinase angiogenesis inhibitor, is another second-line treatment option for patients with advanced STS failing to doxorubicin-based regimens, and also here histology matters. Following the results of the preceding phase II trial showing no efficacy of pazopanib in liposarcoma patients, these patients were not included in the phase III trial that formed the basis for registration of pazopanib [8,9]. This was confirmed by a large retrospective cohort study [10], but contradicted by another phase II trial in advanced intermediate/high-grade liposarcomas showing potential activity of pazopanib in this cohort [11]. These apparently conflicting results on the efficacy in liposarcomas are probably associated with the heterogeneity within the liposarcoma entity, consisting of five different subtypes, and the variation in distribution of these subtypes across the various studies. Another agent active in second-line is eribulin, which significantly increased OS compared to dacarbazine in patients with advanced L-sarcomas, although the effect seems to be restricted to the liposarcoma subgroup [12]. Therefore, eribulin was recently approved in 2016, but for advanced liposarcoma only. In addition to these agents for which data from phase III studies are available, several other agents are being used in the second-line treatment for specific STS entities, including vascular endothelial growth factor receptor-tyrosine kinase inhibitors (VEGFR-TKIs) in alveolar soft part sarcoma, gemcitabine-based regimens in leiomyosarcoma and taxanes in angiosarcoma [13-17]. However, evidence from randomised studies (in second line treatment) showing that these options truly result in better outcomes compared to the above mentioned treatment strategies is currently lacking.

Future perspectives

Although treatment of metastatic STS has improved over the years and options have expanded, there is much to be gained. A considerable proportion of patients are not eligible to receive any of the relatively toxic drugs due to poor performance status or comorbidities. As for now, best supportive care is the only option for these patients, underlining the urgent need for novel treatment strategies.

Despite multiple clinical trials in search for a more effective and/or less toxic drug, doxorubicin has remained the mainstay of first-line treatment. Its treatment can be accompanied by severe side-effects, such as myelosuppression and mucositis, but its use is mostly limited due to cumulative cardiotoxicity. At present, there is ongoing research on aldoxorubicin, a prodrug of doxorubicin [18]. Based on preliminary data of a phase III trial, aldoxorubicin, compared to investigator's choice of treatment, seems to have a slightly longer PFS, but only in patients with L-sarcomas. There was no significant improvement in response rate or OS, nor a significant difference in any of these outcomes in other STS subtypes. Remarkably, minimal cardiotoxicity was observed, suggesting it might be worthwhile to assess this compound against doxorubicin-based regimens [19].

Moreover, several studies have been conducted to assess the feasibility of numerous agents and regimens in elderly patients. For example, metronomic cyclophosphamide and trabectedin showed efficacy as well as favourable toxicity profiles and safety in elderly patients, indicating that there are certainly treatment options available for this fragile patient population [20-22].

Furthermore, there are some promising agents in the pipeline. Regorafenib is one of those agents, demonstrating antitumour activity in multiple non-adipocytic STS subtypes with a significant improvement in PFS. There was no significant difference in OS, although the study design (allowing cross-over from placebo to regorafenib after progression) made it impossible to adequately assess OS, but the results are promising enough to consider a phase III trial with this drug [23].

Additionally, numerous clinical trials investigating different immunotherapies and in different combinations are currently ongoing. Especially the combination of nivolumab and ipilimumab with a response rate of 16% compared to 5% for nivolumab alone, showed favourable and promising results in multiple STS subtypes in phase II, though its toxicity profile is substantial [24]. Also pembrolizumab, albeit evaluated in small patient groups, demonstrated clinical activity in undifferentiated pleomorphic sarcoma (response rate 40%) and dedifferentiated liposarcoma (20%). The expansion cohorts are enrolling to confirm the antitumour activity in these subtypes [25]. Another discovery is the expression of the NY-ESO-1-antigen in approximately 20% of all STS subtypes, with higher expression levels in specific subtypes (88.0% in myxoid liposarcoma, 49.3% in synovial sarcoma) [26]. The cancer-testes antigen NY-ESO-1 represents an attractive target in STS establishment. CMB305 is an immune modulating agent, aiming to generate and expand anti-NY-ESO-1 T-cells and antibodies. The sequential and alternating administration of the two components leads to priming of the immune system followed by a boost, resulting in a robust and prolonged immune response against NY-ESO-1. Trials with CMB305, but also genetically engineered anti-NY-ESO-1 T-cells are ongoing and show promising effects in early phase trials in synovial sarcoma and myxoid liposarcoma [27-29].

Although the treatment decision making in STS is increasingly histology driven, more specific and sensitive predictive factors to tailor therapy are warranted. Increasing insight into the biology of the different STS subtypes will hopefully lead to the identification of predictive markers beyond conventional histology. For example, in the context of a phase II trial, a subset of miRNAs, regardless of underlying histology, was revealed that might identify patients benefiting from eribulin [30]. Likewise, studies are investigating whether programmed cell death ligand-1 (PD-L1) expression, mutational load or composition of immune infiltrates can be used to predict outcome to anti-PD(L)1-antibodies [24,31].

Conclusions

The treatment of metastatic STS has changed considerably over the last few years, with addition of olaratumab to the first-line treatment probably being the biggest development, provided that its preliminary efficacy can be confirmed. Moreover, the limited treatment options in second-line treatment and beyond have expanded, introducing trabectedin, pazopanib and eribulin amongst others. Despite these innovations, overall survival of metastatic STS patients remains poor, underlining the great need for novel agents and strategies to further personalise treatment.

Conflict of interest statement

M. Vos: no conflicts of interest.

S. Sleijfer: advisory board EliLilly (all funding for institute).

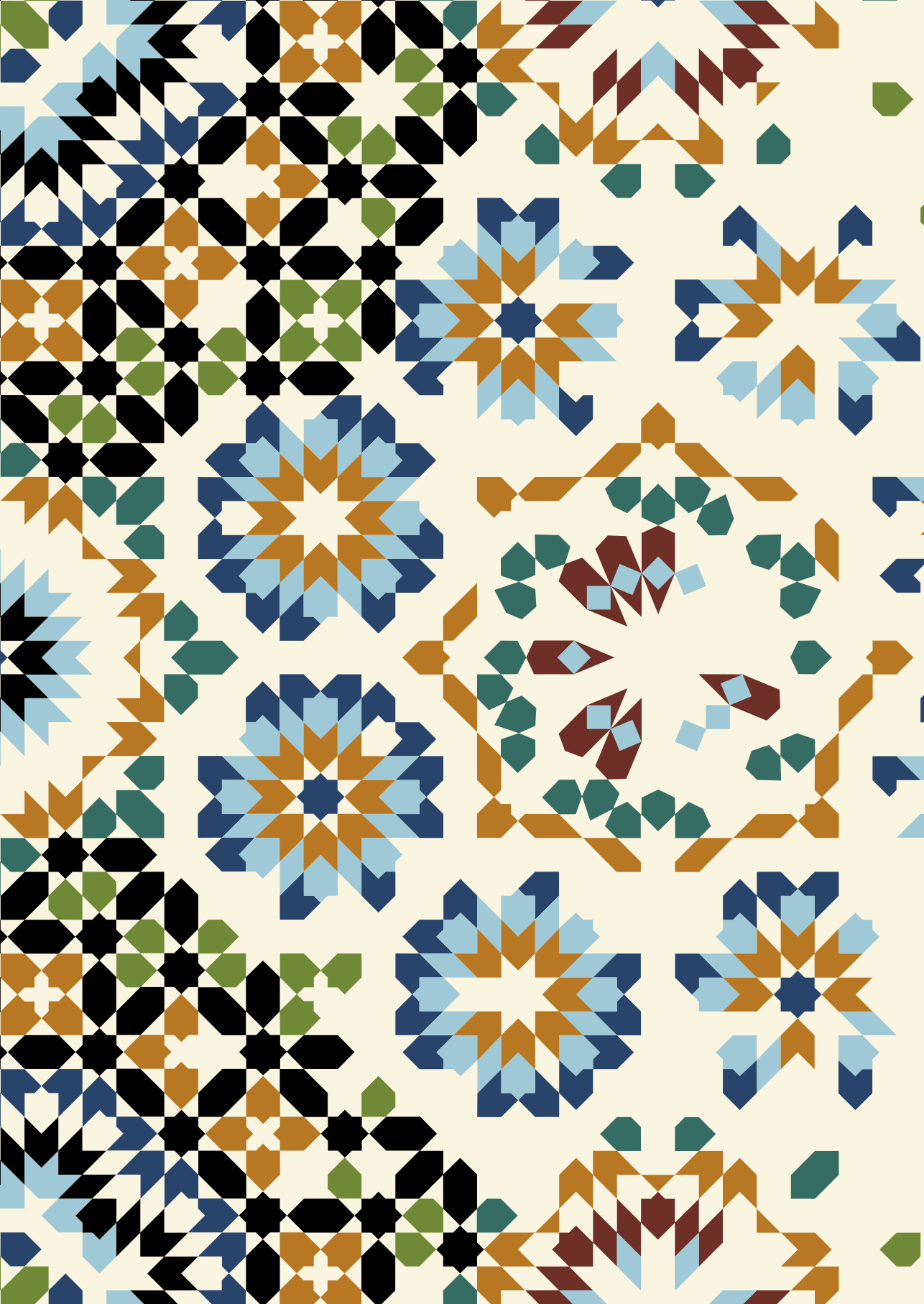
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CHAPTER 12

MINIMAL INCREASE IN SURVIVAL THROUGHOUT THE YEARS IN PATIENTS WITH SOFT TISSUE SARCOMA WITH SYNCHRONOUS METASTASES: RESULTS OF A POPULATION-BASED STUDY

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Abstract

Background: Treatment options for patients with metastatic soft tissue sarcoma (STS) have increased in the last decade. We aimed to examine whether this is associated with improved overall survival (OS) in patients with STS with synchronous metastases.

Patients and Methods: Patients diagnosed with STS and synchronous metastases from 1989 to 2014 were queried from The Netherlands Cancer Registry. Trends in OS were assessed by the Kaplan-Meier method and log-rank test in time intervals of 5 years, for the whole study population and in subgroups for liposarcomas, leiomyosarcoma, and other STS subtypes. A multivariable Cox regression analysis was performed to identify characteristics prognostic for OS.

Results: Median OS of the 1,393 identified patients did not improve significantly over the years from 5.8 months in 1989–1994 to 8.1 months in 2010–2014, but there was an evident trend. Median OS was prolonged in the subgroups of liposarcomas (3.6 to 9.3 months), leiomyosarcomas (11.3 to 14.6 months), and other STS subtypes (5.7 to 6.3 months), although there were no significant improvements in OS over the years. Primary tumor site in one of the extremities and surgery in an academic center had a favorable effect on OS, whereas significant negative predictors were no treatment, elderly age, STS subtype other than liposarcoma or leiomyosarcoma, high or unknown grade, and nodal involvement.

Conclusion: Although overall survival of patients with STS with synchronous metastases in this nationwide and “real-life” population has improved over the years, the improvement was not statistically significant, despite new treatment options.

Keywords: Soft tissue sarcoma; Synchronous metastases; Overall survival; Population-based study

Implications for Practice: Treatment of patients with metastatic soft tissue sarcoma (STS) has changed in the past years, with new drugs such as trabectedin (2007) and pazopanib (2012) becoming available. By using data from the nationwide Netherlands Cancer Registry, the impact of these changes in treatment policies on survival is analyzed in a “real-life” population of patients with STS with synchronous metastases, rather than in a strictly selected trial population. Unfortunately, overall survival improved only minimally and not significantly for these patients diagnosed from 1989 to 2014. Hopefully, the advent of novel treatment options, such as eribulin and olaratumab, will further improve the outcome of this patient group.

Introduction

Soft tissue sarcomas (STSs) are a group of rare and heterogeneous tumors of mesenchymal origin, comprising over 50 different histological subtypes and accounting for approximately 1% of all adult malignancies [1, 2]. Roughly 650 to 700 new patients are diagnosed annually with STS in the Netherlands, with a slightly increasing incidence over the years [3]. Leiomyosarcomas and liposarcomas form the two most prevalent subtypes, each representing approximately 20% of patients with STS [1]. When STS is diagnosed at an early stage and complete resection of the tumor can be carried out, cure may be achieved in up to 90% of patients [4-6]. Unfortunately, cure is generally not attainable in case of metastatic disease, a situation for which only palliative treatment remains. Most of these cases occur as a relapse after primary treatment, which may take several years of follow-up. Approximately 10% to 15% of patients present with synchronous metastases, that is, metastases that are diagnosed before or simultaneously with the primary tumor [7]

In the majority of patients with advanced STS, the disease cannot be cured, and only treatment with palliative intent remains. However, in selected cases with oligometastatic disease, mostly isolated and solitary lung metastases, long-term survival and in rare cases even cure can be achieved by surgical resection of the metastasis, also called metastasectomy [8-11]. Besides surgical resection, other local treatment options can be applied. Examples include radiofrequency ablation, isolated limb perfusion and (stereotactic) radiotherapy [12]. In contrast to metastasectomy of solitary (lung) metastases, these treatment options are used rarely with therapeutic intent but rather with a palliative intent. Although they might prolong remission or prevent or slow down progression [13], these treatment modalities are usually used to reduce symptoms and thereby improve quality of life.

In recent years, the number of systemic palliative treatment options for patients with metastatic STS has increased. Whereas doxorubicin-based chemotherapy is the mainstay of first-line treatment, mostly as a single agent and sometimes in combination with ifosfamide [14, 15], two new agents have been approved in the last decade for patients in whom doxorubicin-based chemotherapy fails or is unsuitable. In the Netherlands, the alkylating agent trabectedin became available in 2007 for adults who have advanced STS and who fail on treatment with anthracyclines and/or ifosfamide or who are unsuited to receive these agents. Although registered for all STS subtypes, it is mostly applied in patients with liposarcomas or leiomyosarcomas, as efficacy was proved most pronounced in these entities [16, 17]. Secondly, the tyrosine kinase inhibitor pazopanib was introduced in 2012. In the Netherlands, it is registered for advanced STS after prior chemotherapy for metastatic disease or advanced STS with progressive disease within 12 months after neoadjuvant/ adjuvant therapy, irrespective of subtype except for patients with liposarcoma [18, 19].

Besides these two new agents, other chemotherapeutic drugs are increasingly used in daily clinical practice, although these have not been formally registered for STS. Examples include gemcitabine in leiomyosarcoma and taxanes in angiosarcoma, which are also mentioned in international guidelines [13, 20]. These drugs can be used in multiple lines of therapy or in combination with other locoregional treatment options [13].

For this study, a population-based analysis was performed to determine the impact of the changes in treatment policies on overall survival in patients with STS presenting with metastatic disease at time of diagnosis (also known as synchronous metastases) and to establish whether the survival has improved over the years. Because our data source, the nationwide database of The Netherlands Cancer Registry (NCR), does not include information on patients who relapsed after initial treatment for non-metastatic disease, the focus in this study is on the 10% to 15% of patients with soft tissue sarcoma with synchronous metastases.

Materials and methods

Data Collection

From the NCR, data on all patients with STS diagnosed with synchronous metastases between 1989 and 2014 were identified and extracted. Synchronous metastases were defined as metastases detected prior to or during screening in the diagnostic workup before start of (neoadjuvant) treatment of the primary tumor. Children (age at diagnosis <18 years), gastrointestinal stromal tumors (GIST) and small blue round cell tumors (SBRCTs: Ewing's sarcomas, mesenchymal chondrosarcomas, peripheral neuroectodermal tumors, and rhabdomyosarcomas) were excluded because of the different tumor biologies, different treatment regimens, and outcomes.

Information in the NCR on patient characteristics, disease characteristics, and primary treatment was retrieved from patients' medical records by trained registration employees of The Netherlands Comprehensive Cancer Organisation (IKNL). Follow-up information on vital status was obtained through yearly linkage with the Municipal Personal Records Database (MPRD). For our study, the last linkage was performed in February 2016.

Primary tumors were staged according to the TNM classification and STS subtypes were classified following the World Health Organization classification [2, 21]. No central pathology review was performed. Primary tumor site as well as metastatic site(s) and STS subtype were coded under the International Classification of Diseases of Oncology topography and morphology codes, respectively. STS subtypes that are acknowledged for exhibiting aggressive behavior but usually not graded (malignant peripheral nerve sheath tumors, angiosarcoma, extraskeletal chondrosarcoma, epithelioid sarcoma, clear cell sarcoma not otherwise specified, alveolar soft part sarcoma) were pooled with grade III tumors and

classified as high-grade tumors. Grade I and II tumors were pooled and classified as low-grade tumors. For the study period, the NCR database lacked information on the type of systemic therapy (cytostatic drugs, kinase inhibitors, etc.) or intent of surgery (resection of primary tumor, palliative debulking, etc.). In addition, no data were available on patients' performance score or comorbidities.

The study was performed in accordance with local ethics committee guidelines and national legislation.

Statistical Analysis

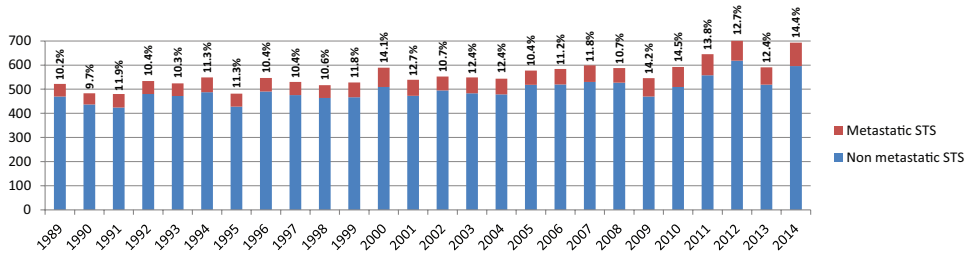
Median follow-up time with corresponding interquartile range (IQR) was calculated by the reversed Kaplan–Meier method [22]. Overall survival (OS) was defined as time in months between diagnosis (first pathological confirmation) and death or last follow-up. Patients alive at date of last linkage to the MPRD were censored. To assess trends in overall survival over the study period, the data were analyzed in intervals of 5 years, using the Kaplan–Meier method. Overall survival was assessed for the total study population, as well as in subgroups for patients with liposarcomas, leiomyosarcomas, and other STS subtypes. Survival times are noted as median survival times in months, together with the corresponding IQRs. Differences between subgroups were tested by the log-rank test. An additional survival analysis focusing on patients who received systemic therapy was performed to explore the effect of the new agents. Because of small subgroups, liposarcomas and leiomyosarcomas were combined (“L-sarcomas”) for this analysis.

A multivariable Cox proportional hazards regression analysis for OS was performed to identify relevant patient, tumor or treatment related characteristics. Also, we explored possible influences on survival of the type of hospital (academic vs. nonacademic) where patients were diagnosed and treated. Factors that tested significantly at an α -level of 0.05 in univariable analyses were included in the multivariable Cox regression analysis. The definitive model was obtained with a backward stepwise elimination method. Results are described as hazard ratios (HRs) with corresponding 95% confidence intervals (95% CI). P values ≤ 0.05 were considered statistically significant. SPSS was used for the statistical analyses (SPSS Statistics for Windows; IBM, Armonk, NY).

Results

Between 1989 and 2014, 1,689 patients were diagnosed with STS and synchronous metastases, including children and those diagnosed with GIST and SBRCTs, representing roughly 12% of all patients diagnosed with STS. Over the years, an increase in incidence was noticed from 52 (10.2%) to 97 (14.4%) patients a year (Fig. 1). After exclusion of all children

and patients with GISTs and SBRCTs, 1,393 patients with STS and synchronous metastases were included in the analyses. There was a slight male predominance, and the trunk was the primary tumor site in approximately half of the patients (Table 1). The most common localizations of metastases were the lungs (42.9% of patients), liver (13.3%), bones (11.6%), and lymph nodes (7.4%). In 30.8% of the patients, the sites of metastases were unknown or unspecified.



▲**Figure 1.** Incidence of STS and metastatic STS at time of initial diagnosis in the Netherlands. The blue fractions of the bars represent patients with non-metastatic/localized STS. The red fractions and the numbers above the bars represent the proportion of patients with metastatic STS at initial diagnosis. STS: soft tissue sarcoma.

Trends in Survival

Median OS over the whole period and for all subtypes was 6.3 months (IQR 2.4–15.5). Throughout the years, median OS did not improve significantly; it increased from 5.8 months (IQR 2.3–14.8) in 1989–1994 to 8.1 months (IQR 2.7–17.1) in 2010–2014 ($p = .095$), although there was an evident trend (log-rank trend test, $p = .015$; Fig. 2A).

When analyzing the different STS subtypes, median OS for patients with liposarcoma did not change significantly from 3.6 months (IQR 1.7–18.5) in 1989–1994 to 9.3 months (IQR 3.8–28.8) in 2010–2014 ($p = .180$; Fig. 2B). Neither did the median OS for patients with leiomyosarcomas improve significantly (11.3 months, IQR 3.5–19.5, to 14.6 months, IQR 5.5–21.0, $p = .449$; Fig. 2C). Also, for patients with one of the other STS subtypes, median OS remained stable from 5.7 months (IQR 2.1–12.7) in 1989–1994 to 6.3 months (IQR 2.2–13.5) in 2010–2014 ($p = .559$; Fig. 2D).

In our study population, almost one third of patients did not receive any treatment. This subgroup had a poor median OS of 2.1 months (IQR 0.9–5.8) compared with 9.5 months (IQR 4.2–20.0) for patients who received any type of treatment ($p < .001$; Table 2). Among the latter group, those who underwent multimodality treatment had a better OS, and patients treated with both radiotherapy and surgery had the most favorable median OS (19.9 months, IQR 8.0–45.2).

Table 1. Characteristics of all 1,393 patients with soft tissue sarcoma with metastatic disease at time of initial diagnosis

Characteristics	N	%
Gender		
Male	742	53.3
Female	651	46.7
Age (years), median (range)	64 (18-96)	
Subtype		
Liposarcoma	129	9.3
Well differentiated liposarcoma	9	7.0
Dedifferentiated liposarcoma	34	26.4
Myxoid liposarcoma (including round cell liposarcomas)	42	32.6
Pleomorphic liposarcoma	24	18.6
Liposarcoma NOS	19	14.7
Mixed type liposarcoma	1	0.8
Leiomyosarcoma	348	25.0
Other	916	65.8
Site		
Retroperitoneum & peritoneum	132	9.5
Trunk	712	51.1
Head & neck	73	5.2
Upper extremity	83	6.0
Lower extremity	393	28.2
Grade		
Low (G I/II)	181	13.0
High (G III/NA)	680	48.8
Unknown (Gx)	532	38.2
Depth		
Superficial	136	9.8
Deep	571	41.0
Unknown	686	49.2
Size (T-stadium)		
≤ 5 cm (T1)	121	8.7
> 5 cm (T2)	913	65.5
Unknown (Tx)	359	25.8
Lymph Node Involvement		
N0	475	34.1
N1	189	13.6
Nx (lymph nodes not assessed)	729	52.3
Pulmonary metastases		
No	796	57.1
Yes	597	42.9

Characteristics	N	%
Treatment (in any order)		
Chemotherapy (CTx)	316	22.7
Radiotherapy (RTx)	159	11.4
Surgery	206	14.8
CTx and RTx	52	3.7
CTx and surgery	107	7.7
RTx and surgery	79	5.7
CTx, RTx and surgery	28	2.0
No therapy	446	32.0
FU time (months), median (IQR)	136.2 (53.1-198.0)	

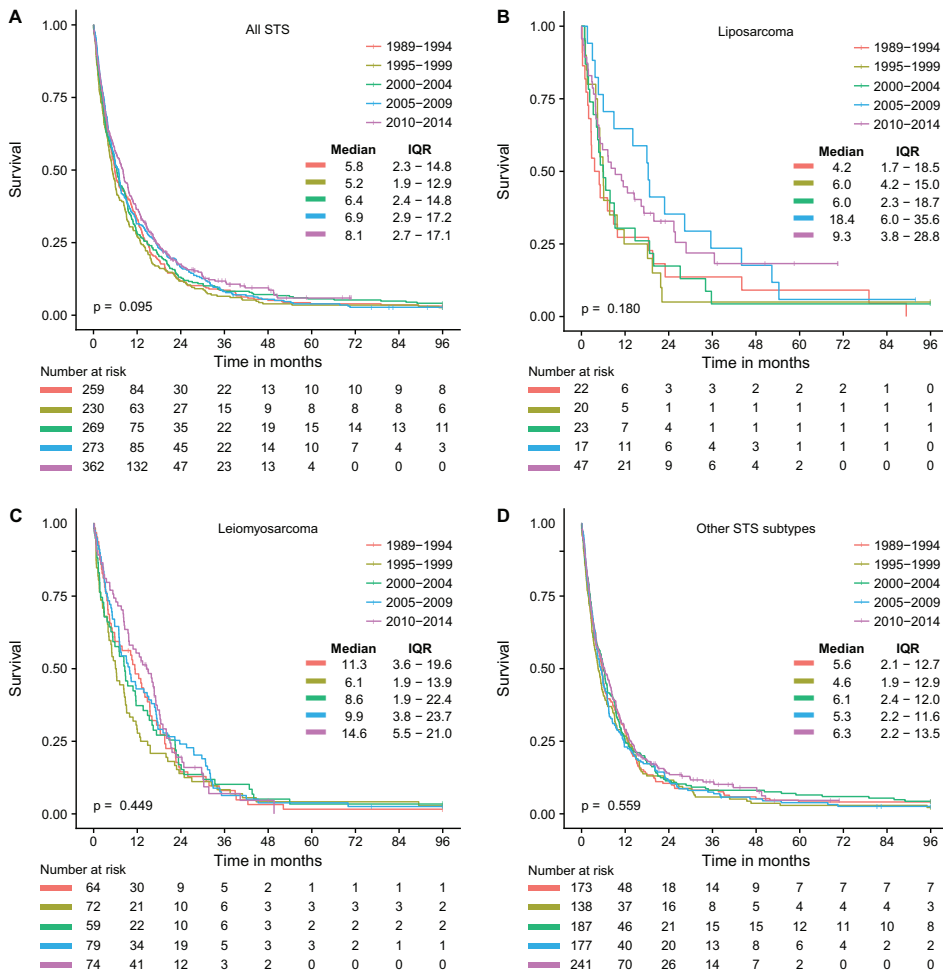
Abbreviations: CTx, chemotherapy; FU, follow-up; IQR, interquartile range; NA, not applicable; NOS, not otherwise specified; RTx, radiotherapy.

Table 2. Median overall survival in months of patients with metastatic STS at initial diagnosis per (combination of) treatment modality

Treatment modalities	n (%)	median OS (IQR), months
No therapy	446 (32.0%)	2.1 (0.9-5.8)
Therapy	947 (68.0%)	9.5 (4.2-20.0)
RTx	159 (11.4%)	5.8 (2.8-12.2)
Surgery	206 (14.8%)	6.9 (3.3-18.3)
CTx	316 (22.7%)	9.4 (4.4-16.1)
CTx-RTx	52 (3.7%)	10.8 (5.6-23.0)
CTx-Surgery	107 (7.7%)	15.6 (7.1-29.8)
CTx-RTx-Surgery	28 (2.0%)	16.1 (9.5-37.5)
RTx-Surgery	79 (5.7%)	19.9 (8.0-45.2)
Overall	1,393 (100%)	6.3 (2.4-15.5)

Combinations of treatment modalities can be in any order, i.e., RTx-CTx is pooled with CTx-RTx, surgery-RTx is pooled with RTx-surgery, etc. P-values were calculated by using the log-rank test ($p < .0001$). Abbreviations: CTx, chemotherapy; IQR, interquartile range; OS, overall survival; RTx, radiotherapy.

A subanalysis of the subgroup of patients who received chemotherapy ($n = 503$) was performed to explore the effect of the new systemic therapies. Median OS over the total period in the chemotherapy subgroup was 10.8 months (IQR 5.5–20.4) and improved minimally from 10.5 months (IQR 4.9–17.1) in 1989–1994 to 13.0 months (IQR 5.8–24.3) in 2010–2014 ($p = .446$; Fig. 3A). In the different STS subgroups, median OS also did not improve for the L-sarcomas (13.0 months, IQR 4.9–34.5, in 1989–1994 to 18.1 months, IQR 8.4–29.7, in 2010–2014, $p = .485$, Fig. 3B) or for the other STS subtypes (10.1 months, IQR 4.9–14.1, in 1989–1994 to 10.6 months, IQR 4.5–20.8, in 2010–2014, $p = .789$; Fig. 3C).



▲Figure 2. Overall survival of all patients with STS with synchronous metastases per 5-year time intervals (A), specified for liposarcomas (B), leiomyosarcomas (C), and other STS subtypes (D). P values were calculated by the log-rank test. For (A), an additional log-rank trend test was performed, showing a significant trend over the years ($p = .015$).

Prognostic Factors for Overall Survival in Metastatic STS at Initial Diagnosis

In the univariable Cox regression analyses almost all factors tested significantly, except for the variables gender, socioeconomic status, pulmonary metastases, and the annual volume of the hospital where patients received their (first-line) chemotherapy (Table 3). In multivariable analysis seven factors remained independently prognostic (Table 4). Whereas an elderly age, STS subtype other than liposarcoma or leiomyosarcoma, high or unknown grade, and nodal involvement had a negative effect on survival, a primary tumor located

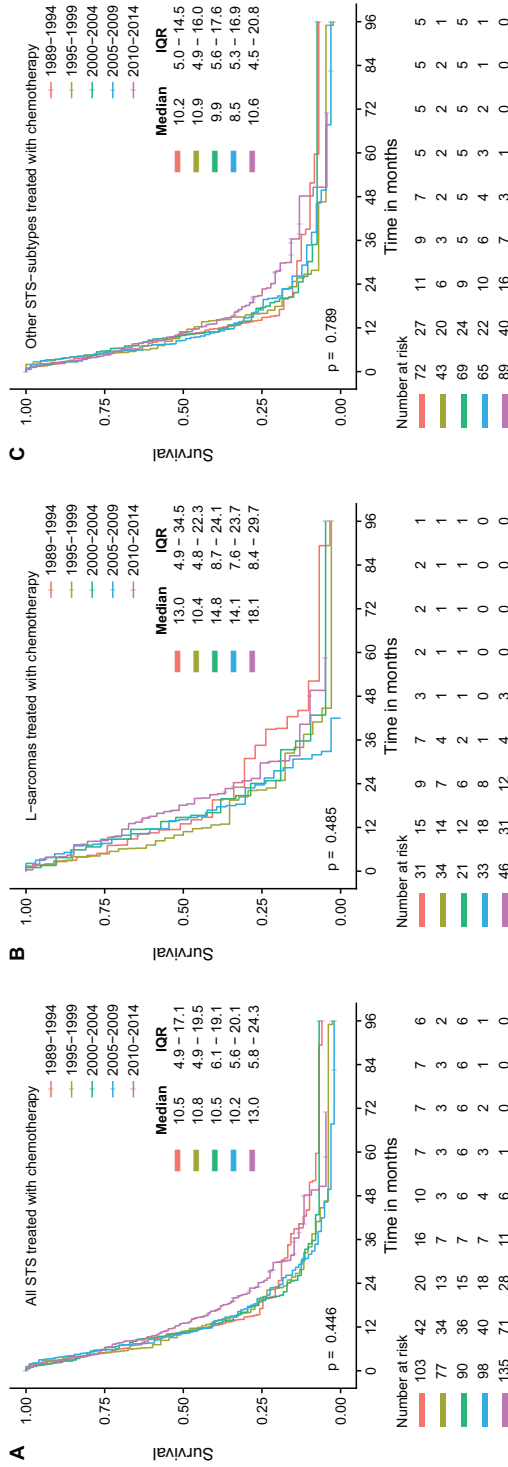
in the upper or lower extremity, any type of treatment (chemotherapy, radiotherapy, and/or surgery), and undergoing surgery in an academic center (compared with a nonacademic center) had a favorable effect on survival.

Discussion

Several new therapeutic agents and/or regimens for the treatment of patients with advanced STS have been introduced in the last decade, including trabectedin in 2007 and pazopanib in 2012. Despite these new options, the overall survival of patients with STS with synchronous metastases has improved only minimally and not statistically significantly over the years.

As probably only a small proportion of patients received one of the new agents, the survival benefit of trabectedin reported in several trials apparently has not translated to patients with advanced disease at initial diagnosis on a population level [16, 17, 23-25]. A possible contributing factor could be the different composition of STS subtypes included in our study population compared with the study populations of clinical trials. For instance, the efficacy of trabectedin was most pronounced in the L-sarcomas and especially myxoid liposarcomas, and whereas in most trabectedin trials 50% to 100% of the included patients had liposarcoma or leiomyosarcoma, only approximately 35% of the patients in our study population were diagnosed with an L-sarcoma, of whom only 3.0% had myxoid liposarcoma. In line with the results from the PALETTE trial, in which a significant difference in progression-free survival but not in overall survival was observed between the pazopanib and placebo arm [19], we did not observe a significant difference in overall survival. It must be noted that a possible beneficial effect might be masked because of short follow-up, with 2012 being the year of introduction of pazopanib [26].

Throughout the years, we observed a median overall survival across all STS subtypes of 6.3 months, which is poorer than reported in clinical trials for patients with metastatic STS, in which median OS times of 12 to 24 months have been described [9, 27-35]. However, these studies have included only patients who received any kind of treatment, whereas this study also included a substantial number of patients who did not receive any treatment. As expected, patients who did not receive therapy, probably because of a poor performance status or multiple comorbidities, have a poorer prognosis than patients receiving (any kind of) treatment. Unfortunately, no data on comorbidities or performance status were available to confirm this hypothesis or to correct for possible interactions in our analyses. When focusing only on the group receiving any kind of treatment, we observed a median OS of 9.5 months, which is still poorer than described in literature. It is likely that the patients who received treatment in our cohort did not meet the strict eligibility criteria that patients included into clinical studies have to fulfil. Remarkably, patients who made it to a combination of local treatment only (i.e., surgery and radiotherapy) had the most favorable median OS of 19.9



▲ **Figure 3.** Overall survival of all patients with STS with synchronous metastases receiving chemotherapy per 5-year time interval (**A**), specified for L-sarcomas (liposarcomas and leiomyosarcomas) (**B**) and other subtypes of metastatic STS (**C**). P values were calculated by the log-rank test. Abbreviations: IQR, interquartile range; STS, soft tissue sarcoma.

Table 3. Results of univariable Cox proportional hazards regression analysis for overall survival of patient-, tumor-, and treatment-related characteristics

Characteristics		N	HR	95% CI	p ^a
Patient-related characteristics					
Age		1393	1.016	1.012-1.019	<.001
Gender	Male	742	1		
	Female	651	0.977	0.877-1.089	.673
Socioeconomic status	High	400	1		
	Medium	544	1.137	0.995-1.299	.059
	Low	449	1.091	0.950-1.253	.219
Tumor-related characteristics					
Year of diagnosis		1393	0.991	0.984-0.998	.010
Site	Trunk	712	1		
	(Retro)peritoneum	132	0.826	0.682-0.999	.049
	Head and neck	73	0.766	0.598-0.982	.036
	Upper extremity	83	0.619	0.487-0.788	<.001
	Lower extremity	393	0.684	0.602-0.777	<.001
Subtype	Leiomyosarcoma	348	1		
	Liposarcoma	129	0.937	0.758-1.159	.548
	Other	916	1.282	1.129-1.455	<.001
Grade	Low (I/II)	181	1		
	High (III/NA)	680	1.422	1.196-1.690	<.001
Depth	Unknown (X)	532	1.556	1.303-1.857	<.001
	Deep	571	1		
	Superficial	136	0.698	0.571-0.853	<.001
Size (T-stadium)	Unknown	568	1.124	0.998-1.267	.054
	≤5cm (T1)	121	1		
	>5cm (T2)	913	1.195	0.983-1.454	.074
	Unknown (Tx/T0)	359	1.501	1.215-1.859	<.001
Nodal involvement	N0	475	1		
	N1	189	1.463	1.229-1.742	<.001
	Nx (unknown)	729	1.511	1.340-1.704	<.001
No. of metastases	1	650	1		
	2	231	1.171	1.004-1.367	.045
	≥3	83	1.481	1.172-1.872	.001
	Unknown	429	1.100	0.970-1.247	.137
Pulmonary metastases	No	796	1		
	Yes	597	0.914	0.819-1.020	.108
Treatment-related characteristics					
Treatment modalities ^b	No treatment	446	1		
	CTx	316	0.406	0.350-0.471	<.001
	RTx	159	0.526	0.438-0.632	<.001
	Surgery	206	0.387	0.326-0.460	<.001
	CTx and RTx	52	0.293	0.217-0.397	<.001
	CTx and surgery	107	0.258	0.206-0.322	<.001

Characteristics		N	HR	95% CI	p ^a
	RTx and surgery	79	0.189	0.143-0.249	<.001
	CTx, RTx and surgery	28	0.248	0.166-0.372	<.001
Hospital type of diagnosis	General/non-academic	1047	1		<.001
	Academic	346	0.786	0.692-0.892	<.001
Diagnosis at sarcoma center	No	1163	1		
	Yes	230	0.794	0.684-0.921	.002
Resection margins	R0	68	1		
	R1	22	0.763	0.432-1.348	.352
	R2	96	1.443	1.030-2.020	.033
	Rx	234	1.625	1.211-2.179	.001
Annual surgery volume	1-10	151	1		
	10-19	44	0.736	0.512-1.058	.097
	≥20	124	0.613	0.473-0.795	<.001
Hospital type of surgery	General/non-academic	175	1		
	Academic	172	0.599	0.477-0.752	<.001
	Unknown	73	1.047	0.793-1.381	.747
Surgery in sarcoma center	No	284	1		
	Yes	136	0.617	0.492-0.773	<.001
Annual volume (first-line) CTx	1-10	254	1		
	10-19	96	0.867	0.676-1.112	.260
	≥20	50	0.951	0.693-1.306	.758
Hospital type of (first-line) CTx	General	190	1		
	Academic	238	0.782	0.641-0.954	.015
	Unknown	75	1.065	0.811-1.398	.650
CTx in sarcoma center	No	273	1		
	Yes	224	0.707	0.588-0.850	<.001

^aVariables with p value <.05 are included in multivariable analysis. ^bTreatment modalities used, in any order (i.e., CTx and RTx can be first CTx followed by RTx, but also RTx first followed by CTx). Abbreviations: CI, confidence interval; CTx, chemotherapy; HR, hazard ratio; NA, not applicable; Nx, lymph nodes not assessed, unknown involvement; RTx, radiotherapy

Table 4. Results of multivariable Cox proportional hazards regression analysis for overall survival of patient-, tumor-, and treatment-related characteristics

Characteristics		N	HR	95% CI	p ^a
Patient-related characteristics					
Age		1393	1.011	1.007-1.015	<.001
Tumor-related characteristics					
Site	Trunk	712	1		
	(Retro)peritoneum	132	0.911	0.748-1.110	.356
	Head and neck	73	0.805	0.625-1.038	.095
	Upper extremity	83	0.723	0.565-0.926	.010
Subtype	Lower extremity	393	0.782	0.685-0.894	<.001
	Leiomyosarcoma	348	1		
	Liposarcoma	129	1.159	0.926-1.450	.197
Grade	Other	916	1.418	1.233-1.631	<.001
	Low (I/II)	181	1		
	High (III/NA)	680	1.561	1.291-1.887	<.001
Nodal involvement	Unknown (X)	532	1.347	1.115-1.628	.002
	N0	475	1		
	N1	189	1.315	1.101-1.572	.003
	Nx (unknown)	729	1.150	1.015-1.304	.029
Treatment-related characteristics					
Treatment modalities ^b	No treatment	446	1		
	CTx	316	0.462	0.393-0.543	<.001
	RTx	159	0.498	0.412-0.603	<.001
	Surgery	206	0.431	0.353-0.526	<.001
	CTx and RTx	52	0.299	0.218-0.410	<.001
	CTx and surgery	107	0.364	0.277-0.478	<.001
	RTx and surgery	79	0.257	0.189-0.350	<.001
	CTx, RTx and surgery	28	0.373	0.241-0.576	<.001
Hospital type of surgery	General/non-academic	175	1		
	Academic	172	0.688	0.542-0.874	.002
	Unknown	73	1.169	0.882-1.551	.277

^aVariables significant at an α -level of .05. ^bTreatment modalities used, in any order (i.e., CTx and RTx can be first CTx followed by RTx, but also first RTx followed by CTx). Abbreviations: CI, confidence interval; CTx, chemotherapy; HR, hazard ratio; NA, not applicable; Nx, lymph nodes not assessed; RTx, radiotherapy.

months. Probably, this group of patients represents the subgroup of patients fit enough to undergo both these treatment modalities, but also a subgroup with minimal or oligometastatic disease, although the exact intents of surgery and radiotherapy are unknown. Furthermore, because we only focused on patients with STS with metastatic disease at initial diagnosis, and clinical trials generally do not limit their inclusion to patients with synchronous metastases, these cases may represent a different, perhaps more aggressive, subgroup of STS compared with patients who initially present with non-metastatic localized STS and experience a relapse

at a later point in time. Dossett et al. showed that after pulmonary metastasectomy patients with synchronous metastases had poorer median OS than patients with metachronous metastases, with synchronous metastases also being a negative significant prognostic factor in multivariate analyses [36]. These findings may give support to the abovementioned hypothesis of patients with synchronous metastases representing a more aggressive subgroup of patients with STS, resulting in a poorer survival.

Although the current study only focuses on patients with STS with synchronous metastases, one of its strengths is that it shows survival in a “real-life” population rather than in a strictly selected trial population. Many patients are excluded from clinical trials by strict eligibility criteria but nonetheless receive these drugs when available in routine care. This, in combination with the large number of patients included in the cohort, makes it likely that this study gives a reliable reflection and accurate estimation of the “real” survival in daily clinical practice of patients with STS with synchronous metastases.

A limitation of this study is that for the patients who received chemotherapy, it is not specified which types of drugs were administered, how many lines of treatment patients received, and whether these patients received (one of) the new agents. Second, health care in general has improved over the years, and sarcoma care in The Netherlands has been largely centralized in five designated sarcoma centers [1]. Additionally, the “Will Rogers phenomenon” might have an effect [37, 38]. As mentioned, the incidence of metastatic disease at initial diagnosis increased slightly over the years, which is likely to be explained by better imaging techniques and thereby the ability to detect smaller metastases. Subsequently, it might be possible that patients with minimal metastatic disease in former years have been categorized as having localized/non-metastatic STS, and they theoretically perform worse than “true” localized STS, but in later periods, because of advancements in imaging, they are categorized as having metastatic STS, and they theoretically do better compared with other patients with (more extensive) metastatic STS. In this way, survival improves in both groups. Therefore, the trend in improvement of survival cannot completely be attributed to the new drugs alone.

Finally, the NCR started to register all patients with cancer in 1989. In this period, GIST was not yet recognized as a distinct entity, and most of these non-epithelial gastrointestinal tract tumors were classified as leiomyosarcomas. It was not until the late 1990s, after discovery of the cell of origin [39], the presence of *c-KIT* proto-oncogene mutations [40], and effectiveness of imatinib in these tumors [41, 42], that GIST was distinguished and treated as a separate entity. Therefore, it cannot be ruled out that in the earlier years of registration some of the registered leiomyosarcomas in fact were GISTs.

Recently, in 2016 another two new agents were registered for advanced or metastatic STS, thereby expanding the limited amount of (palliative) treatment options even more. One

of these agents is eribulin, which in comparison with dacarbazine significantly improves OS in patients with advanced liposarcoma who received at least two systemic treatment regimens (including an anthracycline) [43]. The second new drug is olaratumab, a PDGFR α -inhibitor. It has been conditionally approved, pending the results of the phase III trial, and temporary access for the treatment of adults with advanced STS not amenable to curative treatment (with surgery and/or radiotherapy) has been established. It is used in combination with doxorubicin as first-line treatment, improving median OS by almost a year compared with doxorubicin alone in a randomized phase II study [44]. Although only a few trials have been conducted with these new drugs, they seem promising and hopefully they will increase the survival of patients with metastatic STS further.

Conclusion

Despite new treatment options and improved health care, overall survival of patients with STS and synchronous metastases treated in 'real' life has improved only minimally and not statically significantly over the years. Nonetheless, the relatively small increase of a few months in survival might entail a valuable difference for individual patients. Hopefully, the advent of novel treatment options, such as eribulin and olaratumab, will further improve the outcome of this patient group.

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Disclosures

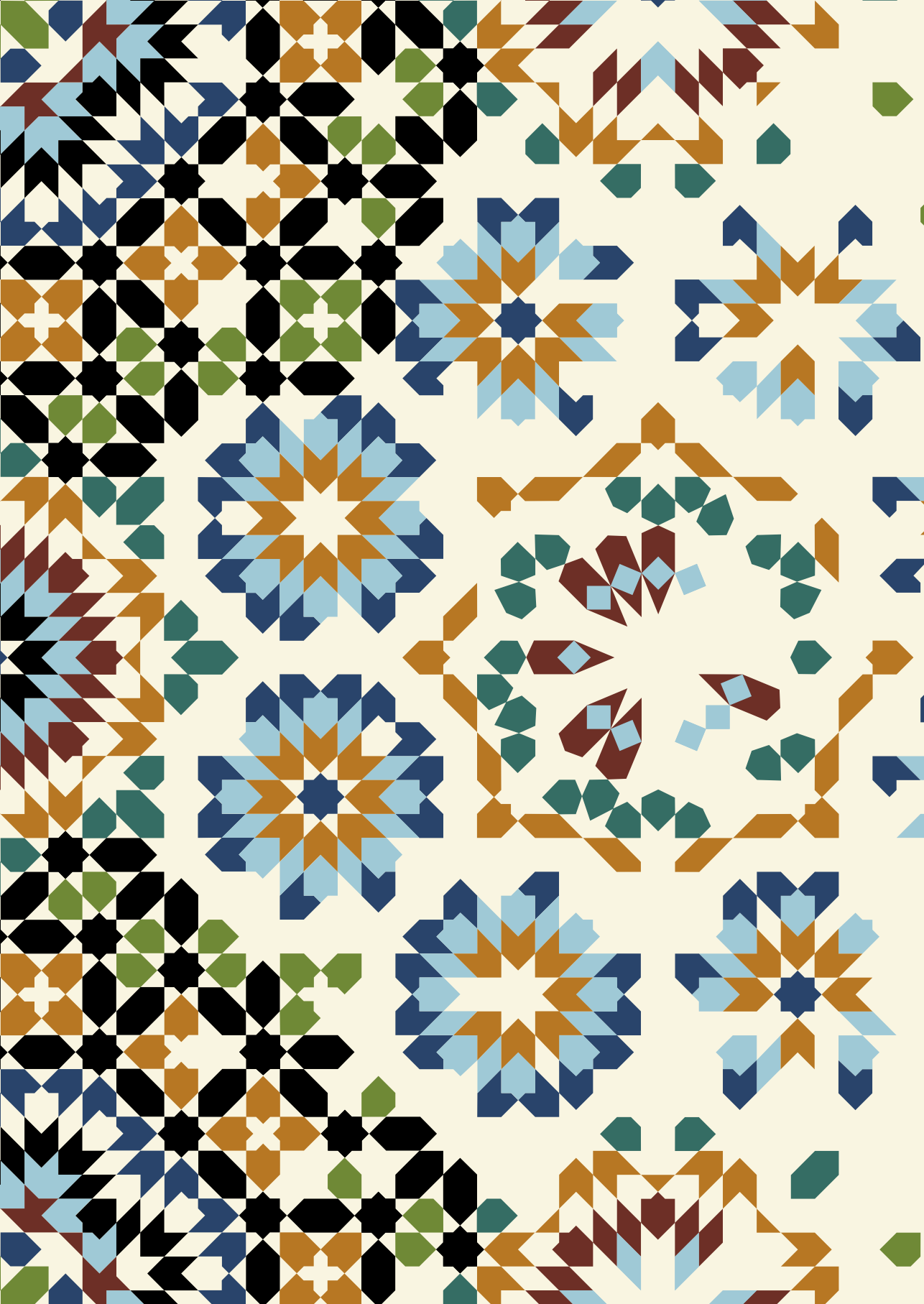
The authors indicated no financial relationships.

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CHAPTER 13

ASSOCIATION OF PAZOPANIB-INDUCED TOXICITIES WITH OUTCOME OF PATIENTS WITH ADVANCED SOFT TISSUE SARCOMA; A RETROSPECTIVE ANALYSIS BASED ON THE EUROPEAN ORGANISATION FOR RESEARCH AND TREATMENT OF CANCER (EORTC) 62043 AND 62072 CLINICAL TRIALS

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Abstract

Background: There is an unmet need for markers predicting the outcome of patients with advanced soft tissue sarcoma (STS) treated with pazopanib. Since toxicity might be related to the anti-tumor activity of the drug, the aim of this study was to determine whether pazopanib-induced proteinuria, hypothyroidism and cardiotoxicity grade 3-4 were associated with outcome.

Methods: The combined results of the EORTC 62043 and 62072 trials were retrospectively assessed and used in a landmark analysis to evaluate the effect of the toxicities on progression-free survival (PFS) and overall survival (OS), using the Kaplan-Meier method and Cox regression models.

Results: Of the 333 eligible patients, 259 patients were included in the analyses, for which a landmark time point of 60 days after randomization/registration was selected. Proteinuria occurred in 25.1%, hypothyroidism in 22.0% and cardiotoxicity grade 3-4 in 5.8% of the patients (any grade in 41.7%). There was no effect of the occurrence of proteinuria (6-months PFS 35.4% for patients with vs. 38.3% for patients without proteinuria, HR 1.01, $p=0.953$), hypothyroidism (41.2% vs. 36.5%, HR 0.82, $p=0.210$) or cardiotoxicity grade 3-4 (26.7% vs. 38.2%, HR 0.97, $p=0.897$) on PFS. Nor was there an effect of proteinuria (6-months OS 63.2% for patients with vs. 74.4% for patients without proteinuria, HR 1.22, $p=0.196$), hypothyroidism (76.2% vs. 70.5%, HR 0.75, $p=0.093$) or cardiotoxicity grade 3-4 (80.0% vs. 77.2%, HR 0.93, $p=0.801$) on OS.

Conclusion: There was no association between the occurrence of pazopanib-induced proteinuria, hypothyroidism and cardiotoxicity and outcome. Therefore, these toxicities cannot be used as predictors for pazopanib activity in patients with advanced STS.

Introduction

Soft tissue sarcomas (STS) are a heterogeneous group of tumors originating from mesenchymal tissue. These rare tumors account for approximately 1% of all adult malignancies and consist of over 50 different histological subtypes [1]. Cornerstone of the treatment of localized STS is surgery, optionally preceded or followed by neoadjuvant/ adjuvant therapy, such as radiotherapy, systemic therapy and/or isolated limb perfusion. Unfortunately, a considerable proportion of patients present with locally advanced and/or metastatic STS or will develop these stages over time. For these patients only palliative treatment remains with median overall survival times of 12–18 months [2–7]. First-line treatment usually consists of doxorubicin, sometimes in combination with ifosfamide for fit patients in need of a response [7]. Recently, the addition of olaratumab to doxorubicin as first-line treatment was conditionally approved, based on a randomized phase II study showing a significant improvement in overall survival compared to doxorubicin alone [8].

Up to the last decade, there were not many systemic treatment options for patients failing to doxorubicin-based first-line treatment, but in the past few years several other agents have become available for patients with advanced STS. One of these agents is pazopanib, an oral angiogenesis tyrosine kinase inhibitor (TKI), targeting the vascular endothelial growth factor receptor (VEGFR) and platelet derived growth factor receptor (PDGFR). Based on an improvement in progression-free survival (PFS) and an acceptable toxicity profile, pazopanib was registered as second-line treatment for patients with advanced non-adipocytic STS after failure to prior chemotherapy [9, 10].

Although pazopanib yielded an almost three-fold prolongation of PFS over placebo, the observed response rates were low (6–9%) and came at the expense of toxicities [9, 10]. On the other hand, there is also a subgroup of STS patients treated with pazopanib with a long-term response (PFS \geq 6 months, 36%) and long-term survival (OS \geq 18 months, 34%), including patients remaining progression-free for more than 2 years (3.5%) [11]. These results illustrate the need for markers predicting response and outcome at an early stage.

This unmet need for markers predicting response is underlined by the observation that many patients in daily clinical practice are worried about the effectivity of their treatment, especially in the absence of any side-effects or toxicity. The hypothesis that the occurrence of toxicity is related to the anti-tumor activity of the drug, and that toxicity therefore can be used as a biomarker of efficacy has been tested in multiple combinations of various types of drugs and different types of cancer and for different toxicities. Examples include the occurrence of sunitinib-induced hypertension in renal cell carcinoma (RCC) [12, 13] or gastrointestinal stromal tumors (GIST) [14, 15], sorafenib-induced diarrhea or hand-foot syndrome in hepatocellular cancer [16, 17], pazopanib or sunitinib-induced proteinuria in RCC [18], and

VEGF TKI-induced hypothyroidism in RCC [19]. In these studies, the occurrence of VEGF TKI-induced toxicity was associated with an improved response rate and/or survival. Recently, also the occurrence of hematological toxicity in patients with advanced STS treated with the classic chemotherapeutic agent doxorubicin was studied, but no association between the severity of hematological toxicity and response, progression-free survival or overall survival could be demonstrated [20].

For the combination of pazopanib and advanced STS, one of the most common toxicities of pazopanib, hypertension, and its association with outcome has already been studied [21]. In this study, pazopanib-induced hypertension was not associated with outcome in STS patients treated with this agent. However, what does not hold true for hypertension, might be true for other pazopanib-specific toxicities. Therefore, the aim of this study was to investigate whether the occurrence of three other common pazopanib-induced toxicities, namely proteinuria, hypothyroidism and cardiotoxicity, following treatment with pazopanib in patients with advanced STS, was associated with outcome. Additionally, this study provides more insight into the exact incidences of these pazopanib-specific toxicities in patients with advanced STS. These three toxicities were chosen, because they are pazopanib-specific, most likely not to be affected/caused by the underlying disease, and well registered during the study period.

Methods

Primary and secondary objectives

The primary objective of this study was to assess the potential association between three pazopanib-induced toxicities and progression-free survival (PFS) of STS patients treated with pazopanib: proteinuria, hypothyroidism and cardiotoxicity related adverse events of grade 3–4. Secondary objectives of this study were to assess the association between these three pazopanib-induced toxicities and overall survival (OS) of STS patients treated with pazopanib, and to describe tumor and patient characteristics of the patients having these toxicities. Additionally, the potential association between cardiotoxicity of any grade and PFS and OS was assessed.

Patient population

The potential association between these pazopanib-induced toxicities and outcome were assessed retrospectively in the combined results of two prospectively performed studies of the European Organisation for Research and Treatment of Cancer-Soft Tissue and Bone Sarcoma Group (EORTC-STBSG): the phase II EORTC 62043 trial [9] and the subsequent phase III EORTC 62072 trial [10]. Details of these studies are listed in supporting information

Table S1. All patients eligible for the 62043 trial and 62072 trial who received pazopanib were included, except for patients with liposarcoma. These patients were excluded based on results of the phase II 62043 trial, where pazopanib failed to demonstrate a sufficient beneficial effect in this STS subtype. To investigate the potential impact of treatment with pazopanib, all analyses for the three types of pazopanib-induced toxicities were also performed on the patients in the phase III 62072 trial receiving placebo.

Measurements of toxicity

Proteinuria was measured using the Urine Protein/Creatinine (UPC) ratio and was defined as an UPC ratio greater than 45 mg/mmol, which is equivalent to an albumin/creatinine ratio of greater than 30 mg/mmol. In both studies, urine creatinine and protein levels were reported at baseline, at day 8 of the first treatment period, at day 1 of each following treatment period (28 days) and 28 days after the last treatment administration. A patient was considered to have persistent proteinuria when two of the UPC ratio measurements with an interval of 1–2 weeks minimum exceeded the cutoff point of 45 mg/mmol. Because the time period between two sequential urinary samples was at least 2 weeks in both studies, patients with two consecutive positive tests were categorized as having persistent proteinuria.

To determine the presence of hypothyroidism, thyroid stimulating hormone (TSH) levels were measured and used as a biomarker. Although there is no clear cutoff point, generally an upper normal limit between 2.5 mU/L and 4.0 mU/L is used [22]. For the current study, a patient was considered as having developed hypothyroidism if at least one TSH value surpassed the threshold value of 4.0 mU/L. According to the study protocols, the TSH levels were reported at baseline and at 12 weeks in both studies. Thereafter, levels were reported every 12 weeks (62043 trial) or 16 weeks (62072 trial). Additionally, TSH levels were often measured in between, with peaks around day 8, day 28 and day 56 (coinciding with the times of UPC assessment and start of new treatment periods).

Cardiotoxicity related adverse events were defined as events which occurred after date of randomization/registration and were part of the following list: cardiac ischemia/infarction, edema, hypertension, hypotension, supraventricular arrhythmia or extrasystole, or prolonged QTc interval. Grading of the cardiotoxicity related adverse events was determined according to the National Cancer Institute-Common Terminology Criteria for Adverse Events version 3.0 (NCI-CTCAE v3.0) [23].

Statistical analyses

PFS was defined as time from date of registration/randomization to the first documentation of progression or death, whichever occurred first. If no progression or death was observed, patients were censored at the last date of follow-up. OS was defined as time between date

of registration/randomization and date of death. Patients alive at time of clinical cutoff were censored at the date of last follow-up.

To determine the appropriate landmark for further analyses, the cumulative incidence of proteinuria, hypothyroidism and cardiotoxicity related adverse events was assessed. These landmark time points were used to assess the effect of the pazopanib-induced toxicities on PFS and OS compared to patients without toxicity, using the Kaplan Meier method for univariate analyses and Cox regression models for multivariable analyses. The 6-months survival rates are calculated taking the selected landmark as starting point ($t = 0$) and are reported as percentages with their associated 95% confidence intervals (95% CI). The multivariable models were adjusted for other prognostic factors and included: performance status (0 or 1), gender (male or female), tumor grade (low, intermediate or high), age at time of randomization (≤ 50 years or > 50 years) and histological subtype (leiomyosarcoma, synovial sarcoma or other). In the multivariable model for cardiotoxicity, cardiac history (yes or no) was also included. For the multivariable analyses, an overview summarizing the hazard ratios (HRs) with corresponding 95% CIs for the three pazopanib-induced toxicities is presented. Complete results including all covariates are shown in the supplemental materials (supporting information Table S3–S10). Two-sided tested p -values $< .05$ were considered statistically significant. All statistical analyses were performed using SAS version 9.4.

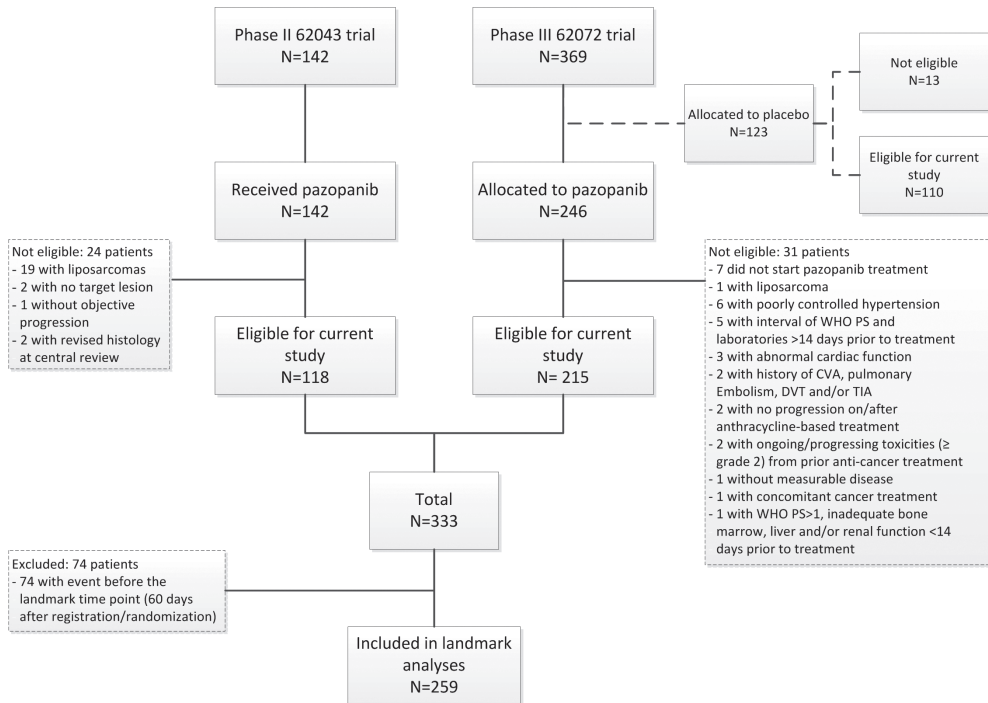
Results

Patient population

In total, 333 patients receiving pazopanib (118 out of the 62043 trial and 215 out of the 62072 trial) and 110 patients receiving placebo met the eligibility criteria and were included in the study (Figure 1). Of the 333 eligible patients receiving pazopanib, 248 patients (74%) developed at least one toxicity, of whom approximately one-third had just one toxicity, 40% a combination of two toxicities and a quarter of the patients all three toxicities. The remaining 85 patients (26%) did not experience any of the three toxicities (supporting information Table S2). Assessment of the incidence of the three toxicities showed that the majority of patients who developed proteinuria, hypothyroidism or cardiotoxicity (both of any grade and of grade 3–4) did so within 60 days after registration/randomization (supporting information Figure S1). Considering this time point as the landmark for further analyses leaves a reasonable number of patients at risk ($N = 259$).

Association between proteinuria and outcome

Overall, 65 of the 259 patients (25.1%) who received pazopanib and who were included in the landmark analyses had proteinuria at a certain point in time within the landmark period



▲ **Figure 1.** Flow chart illustrating the inclusion of patients out of the EORTC 62043 and 62072 trials in the current study.

of 60 days, of whom 22 patients had persistent proteinuria (33.8% of the patients with proteinuria, 8.5% of the total study population). These patients more often had a performance score of 1 and a high grade tumor, were more often female and >50 years of age than patients without proteinuria (Table 1).

Univariate analysis showed no difference in PFS between patients with proteinuria and patients without proteinuria (Figure 2A), nor for patients with persistent proteinuria (supporting information Figure S2A). After adjustment for other prognostic factors, multivariable analysis also showed no significant prognostic effect of proteinuria or persistent proteinuria on PFS (Table 2). The presence of proteinuria or persistent proteinuria also had no significant influence on OS (Figure 2B and Table 2; supporting information Figure S2B).

Association between hypothyroidism and outcome

Approximately one-quarter of the patients developed hypothyroidism within the landmark time point of 60 days (N = 57, 22.0%). These patients were slightly more often female and aged ≤50 years and had slightly more often a performance score of 1 than patients without hypothyroidism (Table 1).

Table 1. Baseline patient characteristics of patients with and without pazopanib-induced proteinuria, hypothyroidism or cardiotoxicity included in the landmark analysis (i.e. patients who did not experience an event, progression or death, before the selected landmark time point of 60 days).

	Proteinuria		Persistent proteinuria		Hypothyroidism		Cardiotoxicity Grade 3-4		Cardiotoxicity Any grade		Total
	No N (%)	Yes N (%)	No N (%)	Yes N (%)	No N (%)	Yes N (%)	No N (%)	Yes N (%)	No N (%)	Yes N (%)	
Performance status											
0	117 (60.3)	28 (43.1)	135 (57.0)	10 (45.5)	117 (57.9)	28 (49.1)	135 (55.3)	10 (66.7)	84 (55.6)	61 (56.5)	145 (56.0)
1	77 (39.7)	37 (56.9)	102 (43.0)	12 (54.5)	85 (42.1)	29 (50.9)	109 (44.7)	5 (33.3)	67 (44.4)	47 (43.5)	114 (44.0)
Type of regimen of advanced disease											
One line of combination therapy	42 (21.6)	7 (10.8)	48 (20.3)	1 (4.5)	40 (19.8)	9 (15.8)	48 (19.7)	1 (6.7)	29 (19.2)	20 (18.5)	49 (18.9)
Two lines of single agent therapy	23 (11.9)	3 (4.6)	25 (10.5)	1 (4.5)	25 (12.4)	1 (1.8)	24 (9.8)	2 (13.3)	12 (7.9)	14 (13.0)	26 (10.0)
Missing	129 (66.5)	55 (84.6)	164 (69.2)	20 (90.9)	137 (67.8)	47 (82.5)	172 (70.5)	12 (80.0)	110 (72.8)	74 (68.5)	184 (71.0)
Sex											
Male	79 (40.7)	24 (36.9)	95 (40.1)	8 (36.4)	84 (41.6)	19 (33.3)	98 (40.2)	5 (33.3)	59 (39.1)	44 (40.7)	103 (39.8)
Female	115 (59.3)	41 (63.1)	142 (59.9)	14 (63.6)	118 (58.4)	38 (66.7)	146 (59.8)	10 (66.7)	92 (60.9)	64 (59.3)	156 (60.2)
Tumor grade											
Low	20 (10.3)	4 (6.2)	23 (9.7)	1 (4.5)	20 (9.9)	4 (7.0)	24 (9.8)	0 (0.0)	10 (6.6)	14 (13.0)	24 (9.3)
Intermediate	67 (34.5)	20 (30.8)	81 (34.2)	6 (27.3)	66 (32.7)	21 (36.8)	80 (32.8)	7 (46.7)	51 (33.8)	36 (33.3)	87 (33.6)
High	107 (55.2)	39 (60.0)	131 (55.3)	15 (68.2)	114 (56.4)	32 (56.1)	138 (56.6)	8 (53.3)	90 (59.6)	56 (51.9)	146 (56.4)
Missing	0 (0.0)	2 (3.1)	2 (0.8)	0 (0.0)	2 (1.0)	0 (0.0)	2 (0.8)	0 (0.0)	0 (0.0)	2 (1.9)	2 (0.8)
Histological subtype											
Leiomyosarcoma	77 (39.7)	29 (44.6)	95 (40.1)	11 (50.0)	82 (40.6)	24 (42.1)	102 (41.8)	4 (26.7)	59 (39.1)	47 (43.5)	106 (40.9)
Synovial sarcoma	41 (21.1)	10 (15.4)	48 (20.3)	3 (13.6)	42 (20.8)	9 (15.8)	46 (18.9)	5 (33.3)	32 (21.2)	19 (17.6)	51 (19.7)
Other	76 (39.2)	26 (40.0)	94 (39.7)	8 (36.4)	78 (38.6)	24 (42.1)	96 (39.3)	6 (40.0)	60 (39.7)	42 (38.9)	102 (39.4)

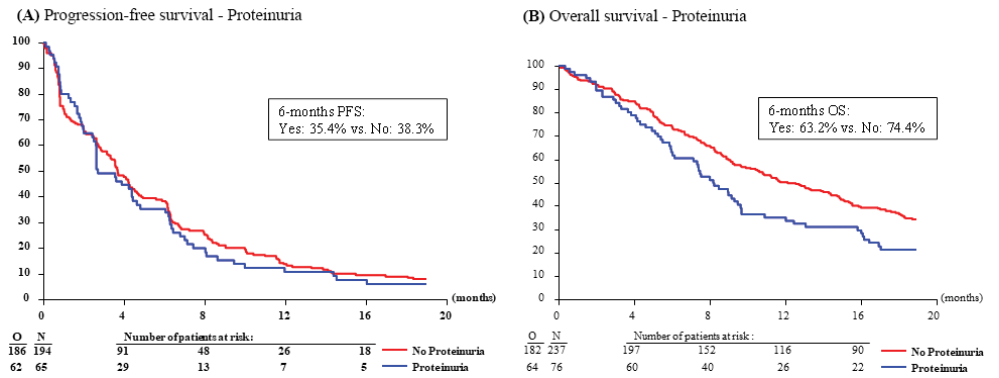
	Proteinuria	Persistent proteinuria	Hypothyroidism	Cardiotoxicity Grade 3-4	Cardiotoxicity Any grade	Total
Age at randomization						
≤50	87 (44.8)	98 (41.4)	76 (37.6)	96 (39.3)	69 (45.7)	102 (39.4)
>50	107 (55.2)	139 (58.6)	126 (62.4)	148 (60.7)	82 (54.3)	157 (60.6)
Cardiac history						
No				188 (77.0)	116 (76.8)	196 (75.7)
Yes				56 (23.0)	35 (23.2)	63 (24.3)
Type of cardiotoxicity*						
Arrhythmia				259 (100.0)	256 (98.8)	3 (1.2)
Hypertension				244 (94.2)	152 (58.7)	107 (41.3)
Ischemia				259 (100.0)	258 (99.6)	1 (0.4)

*Multiple cardiotoxicities per patient can occur, and are in that case counted multiple times for in this table.

There was no effect of the presence of hypothyroidism on PFS (Figure 3A), also not after adjustment for other prognostic factors (Table 2). Similarly, there was no significant prognostic effect of the presence of hypothyroidism on OS (Figure 3B), although an trend in favor of patients with hypothyroidism was observed (Table 2).

Association between cardiotoxicity related adverse events and outcome

In total, 108 patients (41.7%) experienced a cardiotoxicity related adverse event of any grade within the landmark period of 60 days, of whom 15 patients experienced grade 3–4 cardiotoxicity (13.9% of the patients with any grade cardiotoxicity and 5.8% of the total study population). Three patients experienced arrhythmia, one patient ischemia, but the majority of patients had hypertension (N = 107). Notably, the number of patients experiencing cardiotoxicity other than hypertension was too low to perform separate reliable analyses on. Most of the patients experiencing cardiotoxicity had a performance score of 0, were female, over 50 years old and had intermediate or high-grade tumors. Patients with cardiotoxicity grade 3–4 more often had a cardiac history (46.7%) compared to patients without toxicity (23.0%) and patients with cardiotoxicity of any grade (25.9%) (Table 1).



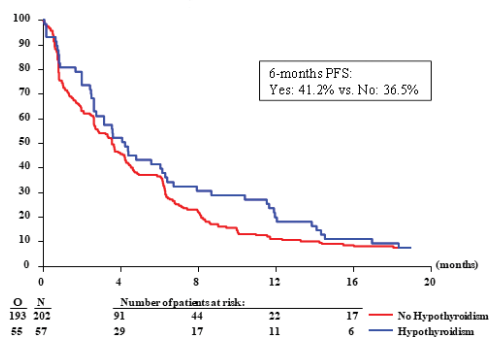
▲ **Figure 2.** Kaplan-Meier curves showing the progression-free survival (A) and overall survival (B) of patients with and without pazopanib-induced proteinuria.

Table 2. Overview of the effect of the three pazopanib-induced toxicities on PFS and OS in patients receiving pazopanib at landmark time point of 60 days, univariate (6-months PFS/OS rates) and multivariable after adjustment for other prognostic factors in multivariable Cox regression analysis. The reported p-values belong to the multivariable Cox regression model. Results of the complete Cox regression analyses are shown in supplemental tables S3-S10.

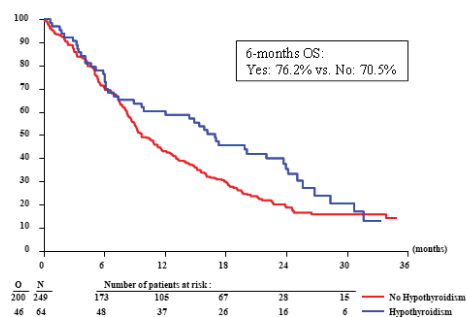
	Progression-free survival			Overall survival		
	6m PFS (95%CI)	HR (95% CI)	p-value	6m OS (95% CI)	HR (95% CI)	p-value
Proteinuria						
No	38.3% (31.4-45.1)	1	.953	74.4% (68.3-79.5)	1	.196
Yes	35.4% (24.0-46.9)	1.01 (0.75, 1.36)		63.2% (51.3-72.9)	1.22 (0.90, 1.64)	
Persistent proteinuria						
No	37.7% (31.5-43.8)	1	.937	73.3% (67.7-78.0)	1	.169
Yes	36.4% (17.4-55.7)	0.98 (0.62, 1.55)		53.9% (33.3-70.6)	1.38 (0.87, 2.19)	
Hypothyroidism						
No	36.5% (29.9-43.2)	1	.210	70.5% (64.3-75.7)	1	.093
Yes	41.2% (28.3-53.6)	0.82 (0.61, 1.12)		76.2% (63.7-84.9)	0.75 (0.54, 1.05)	
Cardiotoxicity grade 3-4						
No	38.2% (32.1-44.3)	1	.897	77.2% (71.4-82.0)	1	.801
Yes	26.7% (8.3-49.6)	0.97 (0.57, 1.64)		80.0% (50.0-93.1)	0.93 (0.52, 1.65)	
Cardiotoxicity any grade						
No	40.6% (32.7-48.3)	1	.380	75.9% (68.1-82.0)	1	.120
Yes	33.3% (24.7-42.2)	1.12 (0.87, 1.46)		79.5% (70.5-86.0)	0.81 (0.63-1.06)	

6m PFS: 6-months progression-free survival, 6m OS: 6-months overall survival, 95% CI: 95% confidence interval, HR: hazard ratio.

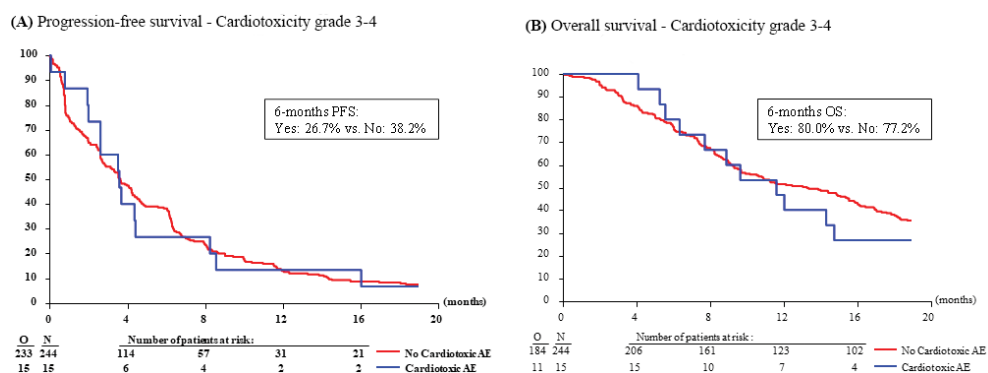
(A) Progression-free survival - Hypothyroidism



(B) Overall survival - Hypothyroidism



▲Figure 3. Kaplan-Meier curves showing the progression-free survival (A) and overall survival (B) of patients with and without pazopanib-induced hypothyroidism.



▲ **Figure 4.** Kaplan-Meier curves showing the progression-free survival (A) and overall survival (B) of patients receiving pazopanib with and without pazopanib-induced cardiotoxicity of grade 3–4.

Univariate analysis showed no difference in PFS between patients with and without cardiotoxicity related adverse events grade 3–4 (Figure 4A). Also in multivariable analysis, after adjustment for other prognostic factors, no significant effect of the occurrence of cardiotoxicity related adverse events grade 3–4 on PFS was observed (Table 2). Likewise, cardiotoxicity of any grade was not of significant influence (Table 2, supporting information Figure S3A). Additionally, there was no association between the occurrence of cardiotoxicity grade 3–4 and OS (Figure 4B), nor in multivariable analysis (Table 2). There was also no association between the occurrence of cardiotoxicity of any grade and OS (Table 2, supporting information Figure S3B).

Association between toxicity and outcome in patients receiving placebo

For all three pazopanib-induced toxicities, the analyses were also performed on the patients receiving placebo in the 62072 trial. However, no effect on PFS or OS was observed for any of the toxicities in patients receiving placebo (supporting information Tables S5, S7 and S10).

Discussion

The association between three pazopanib-induced toxicities and outcome of patients with advanced STS has been investigated in this study. We observed that pazopanib-induced proteinuria occurred in 25.1% of the patients treated with pazopanib within 60 days after start of treatment, of whom 8.5% had persistent proteinuria. Hypothyroidism was observed in 22.0% of the patients while on treatment with pazopanib. At last, cardiotoxicity (any grade) occurred in 41.7% of patients on treatment, of whom 5.8% had grade 3–4 cardiotoxicity. Additionally, we observed that cardiotoxicity other than hypertension only occurred

occasionally in 1.5% of the patients and was only of grade 1 or 2. Overall, no significant prognostic effects were observed for one of these three pazopanib-induced toxicities on PFS or OS. However, although not significant, a trend towards a better prognosis for patients developing hypothyroidism (i.e. high TSH levels) within 60 days after start of pazopanib was observed.

The incidences of the three pazopanib-induced toxicities observed in this study differ slightly from incidences observed in patients with RCC treated with pazopanib. The incidences of proteinuria (25.1%) and hypothyroidism (22.0%) observed in this study were higher than observed for RCC patients. For proteinuria, incidences of 14–18% for patients with RCC have been reported [18, 24, 25]. Importantly, the majority of these patients underwent a prior nephrectomy, which might have affected the onset of proteinuria. Hypothyroidism was observed in <10%–18% of RCC patients treated with pazopanib [24–26], but to the best of our knowledge, no clear explanation for the differences in incidence of hypothyroidism between patients with RCC and patients with STS exists. On the contrary, the incidence of any grade cardiotoxicity (including hypertension) was slightly lower than observed in RCC patients, varying from 42 to 69%, although definitions of cardiotoxicity differed among RCC studies [24, 25, 27, 28]. Comparable to STS patients treated with pazopanib, the majority of the RCC patients with cardiotoxicity had hypertension, and only a small proportion had myocardial ischemia/infarction or QTc prolongation. Inhibiting the VEGF signaling pathway itself can already induce cardiotoxicity because of its working mechanism [29], but other aspects, such as preceding cancer therapy (anthracyclines), preexisting hypertension or other cardiac history, may also play a role in inducing/worsening cardiotoxicity [30]. Even though most STS patients had received doxorubicin as first-line treatment, which is known for its cumulative cardiotoxicity, the incidence of pazopanib-induced cardiotoxicity was not higher than was described in patients with RCC.

Depending on the severity of toxicity, actions were taken according to protocols. In case of proteinuria, pazopanib was interrupted until the UPC ratio had recovered and pazopanib was restarted at a lower dose. For patients developing hypothyroidism, thyroid replacement therapy was started. At last, in case of cardiotoxicity grade 3–4, pazopanib was discontinued and the cardiotoxicity was treated, whereas for grade 1–2 cardiotoxicity, pazopanib could be continued at the current dose or restarted at a lower dose while treating the cardiotoxicity.

In line with the study on the association between pazopanib-induced hypertension and outcome [21], we did not observe a significant prognostic effect of pazopanib-induced proteinuria, hypothyroidism or cardiotoxicity in patients with advanced STS. This is in contrast to studies examining toxicities induced by anti-VEGF treatment in other types of cancer, such as RCC, hepatocellular cancer, colorectal cancer and GIST. In these studies, a significant association between sunitinib or pazopanib-induced proteinuria and OS was

observed in RCC patients [18], and bevacizumab-induced proteinuria was associated with response rate in patients with metastatic colorectal cancer [31]. Additionally, RCC patients with sunitinib-induced or sorafenib-induced hypothyroidism showed increased response rates [32], improved PFS [19, 33] and improved OS [19, 32]. Furthermore, the occurrence of other sunitinib-induced or sorafenib-induced toxicities, such as hypertension, skin toxicity and diarrhea, was significantly associated with an increase in response rate [12-14], improved PFS [12, 14, 15] and improved OS [12, 14-17, 34, 35]. To the best of our knowledge, no studies have been published regarding anti-VEGF therapy-induced cardiotoxicity and its association with outcome, except for hypertension.

The thresholds used to determine toxicity, especially for hypothyroidism, and the choice of the landmark time points might be considered as an arbitrary choice to some extent. Whereas the NCI-CTCAE and UPC ratio are well known and established methods to determine and document cardiotoxicity and proteinuria, no clear TSH cutoff point for the diagnosis of hypothyroidism has been agreed upon. Normal ranges of TSH levels may vary by individual, over the day, and even among laboratories, but generally an upper normal limit between 2.5 mU/L and 4.0 mU/L is used [22]. Hence, the TSH threshold value of 4.0 mU/L used in this study is relatively high and conservative, but allowed us to identify each case of hypothyroidism with more certainty, while still a reasonable number of patients were at risk in both groups.

Although the data included in this study were collected prospectively in the two EORTC trials, there are still some potential sources of bias and limitations related to the retrospective design of this study. There were some small differences in, for example, patient and disease characteristics between the two study populations, as well as small differences in the follow-up schedules and in the definition of PFS in the two studies, which might have influenced the outcome. Furthermore, time-to-event bias might have an impact, a type of bias where patients with a favorable response to pazopanib are more likely to continue treatment for a longer period of time, and the longer the exposure to the agent, the higher the chance of developing toxicity. To avoid this potential source of bias, a landmark analysis was used.

To investigate the potential association of the occurrence of toxicity and outcome following treatment with pazopanib, all analyses for the three types of toxicities have been performed on patients receiving the drug as well as on the patients in the phase III trial receiving placebo. As only a few of the patients receiving placebo did not experience progression/death before the landmark time point of 60 days and even a lower number of toxicities was observed in this patient group, unfortunately no real comparison could be made and no clear conclusions on a potential placebo-effect could be drawn.

The hypothesis that the occurrence of toxicity is related to efficacy of the drug and therefore outcome, prevails not only amongst physicians, but also amongst patients. Some

patients in daily clinical practice are concerned whether their treatment with pazopanib is effective if they do not experience any side-effects or toxicity. The results of this study may provide reassurance to the treating physicians as well as these patients that the absence of toxicity does not imply that there will be no benefit of treatment with pazopanib.

Conclusion

The occurrence of pazopanib-induced proteinuria, hypothyroidism or cardiotoxicity in patients with advanced STS treated with pazopanib did not have a significant predictive effect and was not associated with outcome. These toxicities can therefore not be used as predictor for pazopanib activity in these patients.

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Disclosure of interest

The authors report no conflicts of interest.

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Supporting information

Supporting Table S1. Overview of study details of the EORTC 62043 and 62072 trials.

	EORTC 62043 trial	EORTC 62072 trial
Type of trial	Phase II	Phase III
Design	Single arm	Randomized (2:1), double blind, placebo-controlled
Drug	Pazopanib 800mg daily	Pazopanib 800mg daily or placebo
Inclusion criteria	Locally advanced/metastatic STS	Metastatic non-adipocytic STS
	Ineligible to chemotherapy or failure to no more than two prior cytotoxic agents (sequentially as single agents or one combination regimen)	Failure to standard chemotherapy (including an anthracycline) in a metastatic/locally advanced setting, with a maximum of 4 previous lines or two lines of combination regimens
Primary outcome	Progression-free survival at 12 weeks	Progression-free survival
Secondary outcome	Response rate Duration of response Safety Overall survival	Overall survival Response rate Safety Quality of life
ClinicalTrial.gov number	NCT00297258	NCT00753688
Reference	Sleijfer et al., JCO, 2009	van der Graaf et al., Lancet, 2012

Supporting Table S2. Overview of the three pazopanib-induced toxicities in the 333 eligible patients.

Hypothyroidism		Proteinuria		Total (N=333)
		No	Yes	
No	Cardiotoxicity (any grade)	145	57	202
	No	85	30	115
	Yes	60	27	87
Yes	Cardiotoxicity (any grade)	77	54	131
	No	45	19	64
	Yes	32	35	67

Supporting Table S3. Complete results of the multivariable Cox regression models for PFS and OS of patients receiving pazopanib with and without proteinuria at landmark time point of 60 days.

Landmark Analysis at t=60 days	Multivariable Cox model of progression-free survival				Multivariable Cox model of overall survival				
	Covariates	Patients (N=257)	Observed Events (O=246)	Hazard Ratio (95% CI)	Wald p-value	Patients (N=311)	Observed Events (O=244)	Hazard Ratio (95% CI)	Wald p-value
Proteinuria									
No	194 (75.5)	186 (75.6)	1.00	.953	237 (76.2)	182 (74.6)	1.00	.196	
Yes	63 (24.5)	60 (24.4)	1.01 (0.75, 1.36)		74 (23.8)	62 (25.4)	1.22 (0.90, 1.64)		
Performance status									
0	145 (56.4)	139 (56.5)	1.00	.400	165 (53.1)	123 (50.4)	1.00	.001	
1	112 (43.6)	107 (43.5)	0.89 (0.69, 1.16)		146 (46.9)	121 (49.6)	1.53 (1.18, 1.99)		
Gender									
Male	101 (39.3)	98 (39.8)	1.00	.028	126 (40.5)	106 (43.4)	1.00	.114	
Female	156 (60.7)	148 (60.2)	0.74 (0.56, 0.97)		185 (59.5)	138 (56.6)	0.81 (0.62, 1.05)		
Tumor grade									
Low	24 (9.3)	19 (7.7)	1.00	.002	28 (9.0)	16 (6.6)	1.00	.017	
Intermediate	87 (33.9)	85 (34.6)	2.34 (1.43, 3.82)		105 (33.8)	85 (34.8)	2.00 (1.18, 3.37)		
High	146 (56.8)	142 (57.7)	1.79 (1.08, 2.98)		178 (57.2)	143 (58.6)	1.58 (0.92, 2.70)		
Histology subtype									
Leiomyosarcoma	106 (41.2)	104 (42.3)	1.00	.203	129 (41.5)	97 (39.8)	1.00	.011	
Synovial sarcoma	50 (19.5)	47 (19.1)	0.77 (0.58, 1.03)		60 (19.3)	50 (20.5)	1.33 (0.98, 1.79)		
Other sarcoma	101 (39.3)	95 (38.6)	0.93 (0.64, 1.34)		122 (39.2)	97 (39.8)	1.76 (1.21, 2.57)		
Age at randomization									
≤50 years	101 (39.3)	94 (38.2)	1.00	.089	122 (39.2)	93 (38.1)	1.00	.028	
>50 years	156 (60.7)	152 (61.8)	1.27 (0.96, 1.67)		189 (60.8)	151 (61.9)	1.37 (1.03, 1.82)		

Supporting Table S4. Complete results of the multivariable Cox regression models for PFS and OS of patients receiving pazopanib with and without persistent proteinuria at landmark time point of 60 days.

Landmark Analysis at t=60 days	Multivariable Cox model of progression-free survival				Multivariable Cox model of overall survival				
	Covariates	Patients (N=257)	Observed Events (O=246)	Hazard Ratio (95% CI)	Wald p-value	Patients (N=311)	Observed Events (O=244)	Hazard Ratio (95% CI)	Wald p-value
Persistent proteinuria									
No	235 (91.4)	225 (91.5)	1.00	.937	285 (91.6)	223 (91.4)	1.00	.169	
Yes	22 (8.6)	21 (8.5)	0.98 (0.62, 1.55)		26 (8.4)	21 (8.6)	1.38 (0.87, 2.19)		
Performance status									
0	145 (56.4)	139 (56.5)	1.00	.408	165 (53.1)	123 (50.4)	1.00	<.001	
1	112 (43.6)	107 (43.5)	0.90 (0.69, 1.16)		146 (46.9)	121 (49.6)	1.55 (1.20, 2.01)		
Gender									
Male	101 (39.3)	98 (39.8)	1.00	.028	126 (40.5)	106 (43.4)	1.00	.121	
Female	156 (60.7)	148 (60.2)	0.74 (0.56, 0.97)		185 (59.5)	138 (56.6)	0.81 (0.62, 1.06)		
Tumor grade									
Low	24 (9.3)	19 (7.7)	1.00	.002	28 (9.0)	16 (6.6)	1.00	.015	
Intermediate	87 (33.9)	85 (34.6)	2.34 (1.43, 3.83)		105 (33.8)	85 (34.8)	2.01 (1.20, 3.40)		
High	146 (56.8)	142 (57.7)	1.79 (1.08, 2.98)		178 (57.2)	143 (58.6)	1.58 (0.92, 2.71)		
Histology subtype									
Leiomyosarcoma	106 (41.2)	104 (42.3)	1.00	.203	129 (41.5)	97 (39.8)	1.00	.010	
Synovial sarcoma	50 (19.5)	47 (19.1)	0.77 (0.58, 1.03)		60 (19.3)	50 (20.5)	1.78 (1.22, 2.59)		
Other sarcoma	101 (39.3)	95 (38.6)	0.93 (0.64, 1.34)		122 (39.2)	97 (39.8)	1.34 (0.99, 1.80)		
Age at randomization									
≤50 years	101 (39.3)	94 (38.2)	1.00	.087	122 (39.2)	93 (38.1)	1.00	.031	
>50 years	156 (60.7)	152 (61.8)	1.27 (0.97, 1.68)		189 (60.8)	151 (61.9)	1.37 (1.03, 1.82)		

Supporting Table S5. Complete results of the multivariable Cox regression models for PFS and OS of patients receiving placebo with and without proteinuria at landmark time point of 60 days.

Landmark Analysis at t=60 days Covariates	Multivariable Cox model of progression-free survival				Multivariable Cox model of overall survival			
	Patients (N=41)	Observed Events (O=41)	Hazard Ratio (95% CI)	Wald p-value	Patients (N=101)	Observed Events (O=75)	Hazard Ratio (95% CI)	Wald p-value
Proteinuria								
No	32 (78.0)	32 (78.0)	1.00	.244	82 (81.2)	62 (82.7)	1.00	.324
Yes	9 (22.0)	9 (22.0)	1.78 (0.67, 4.69)		19 (18.8)	13 (17.3)	0.73 (0.39, 1.37)	
Performance status								
0	22 (53.7)	22 (53.7)	1.00	.128	48 (47.5)	33 (44.0)	1.00	.055
1	19 (46.3)	19 (46.3)	1.77 (0.85, 3.70)		53 (52.5)	42 (56.0)	1.58 (0.99, 2.54)	
Gender								
Male	15 (36.6)	15 (36.6)	1.00	.050	41 (40.6)	31 (41.3)	1.00	.564
Female	26 (63.4)	26 (63.4)	0.44 (0.19, 1.00)		60 (59.4)	44 (58.7)	0.86 (0.53, 1.42)	
Tumor grade								
Low	2 (4.9)	2 (4.9)	1.00	.016	3 (3.0)	2 (2.7)	1.00	.298
Intermediate	13 (31.7)	13 (31.7)	6.59 (1.10, 39.46)		25 (24.8)	17 (22.7)	2.18 (0.51, 9.35)	
High	26 (63.4)	26 (63.4)	16.82 (2.18, 129.84)		73 (72.3)	56 (74.7)	1.53 (0.32, 7.18)	
Histology subtype								
Leiomyosarcoma	19 (46.3)	19 (46.3)	1.00	.011	42 (41.6)	31 (41.3)	1.00	.841
Synovial sarcoma	3 (7.3)	3 (7.3)	0.73 (0.34, 1.60)		11 (10.9)	7 (9.3)	0.89 (0.36, 2.21)	
Other sarcoma	19 (46.3)	19 (46.3)	7.20 (1.60, 32.28)		48 (47.5)	37 (49.3)	1.11 (0.66, 1.88)	
Age at randomization								
≤50 years	19 (46.3)	19 (46.3)	1.00	.921	50 (49.5)	34 (45.3)	1.00	.123
>50 years	22 (53.7)	22 (53.7)	0.96 (0.43, 2.14)		51 (50.5)	41 (54.7)	1.52 (0.89, 2.60)	

Supporting Table S6. Complete results of the multivariable Cox regression models for PFS and OS of patients receiving pazopanib with and without hypothyroidism at landmark time point of 60 days.

Landmark Analysis at t=60days	Multivariable Cox model of progression-free survival				Multivariable Cox model of overall survival				
	Covariates	Patients (N=257)	Observed Events (O=246)	Hazard Ratio (95%CI)	Wald p-value	Patients (N=311)	Observed Events (O=244)	Hazard Ratio (95%CI)	Wald p-value
Hypothyroidism									
No	200 (77.8)	191 (77.6)	1.00	.210	247 (79.4)	198 (81.1)	1.00	.093	
Yes	57 (22.2)	55 (22.4)	0.82 (0.61, 1.12)		64 (20.6)	46 (18.9)	0.75 (0.54, 1.05)		
Performance status									
0	145 (56.4)	139 (56.5)	1.00	.457	165 (53.1)	123 (50.4)	1.00	<.001	
1	112 (43.6)	107 (43.5)	0.91 (0.70, 1.17)		146 (46.9)	121 (49.6)	1.62 (1.25, 2.10)		
Gender									
Male	101 (39.3)	98 (39.8)	1.00	.031	126 (40.5)	106 (43.4)	1.00	.157	
Female	156 (60.7)	148 (60.2)	0.74 (0.57, 0.97)		185 (59.5)	138 (56.6)	0.82 (0.63, 1.08)		
Tumor grade									
Low	24 (9.3)	19 (7.7)	1.00	.002	28 (9.0)	16 (6.6)	1.00	.016	
Intermediate	87 (33.9)	85 (34.6)	2.37 (1.45, 3.87)		105 (33.8)	85 (34.8)	2.01 (1.19, 3.39)		
High	146 (56.8)	142 (57.7)	1.82 (1.10, 3.03)		178 (57.2)	143 (58.6)	1.59 (0.93, 2.72)		
Histology subtype									
Leiomyosarcoma	106 (41.2)	104 (42.3)	1.00	.186	129 (41.5)	97 (39.8)	1.00	.017	
Synovial sarcoma	50 (19.5)	47 (19.1)	0.76 (0.57, 1.02)		60 (19.3)	50 (20.5)	1.34 (0.99, 1.80)		
Other sarcoma	101 (39.3)	95 (38.6)	0.91 (0.63, 1.31)		122 (39.2)	97 (39.8)	1.70 (1.17, 2.48)		
Age at randomization									
≤50 years	101 (39.3)	94 (38.2)	1.00	.103	122 (39.2)	93 (38.1)	1.00	.034	
>50 years	156 (60.7)	152 (61.8)	1.26 (0.95, 1.65)		189 (60.8)	151 (61.9)	1.36 (1.02, 1.80)		

Supporting Table S7. Complete results of the multivariable Cox regression models for PFS and OS of patients receiving placebo with and without hypothyroidism at landmark time point of 60 days.

Landmark Analysis at t=60days	Multivariable Cox model of progression-free survival				Multivariable Cox model of overall survival				
	Covariates	Patients (N=41)	Observed Events (O=41)	Hazard Ratio (95%CI)	Wald p-value	Patients (N=101)	Observed Events (O=75)	Hazard Ratio (95%CI)	Wald p-value
Hypothyroidism									
No	31 (75.6)	31 (75.6)	1.00	.557	85 (84.2)	64 (85.3)	1.00	.765	
Yes	10 (24.4)	10 (24.4)	1.34 (0.51, 3.55)		16 (15.8)	11 (14.7)	0.90 (0.46, 1.78)		
Performance status									
0	22 (53.7)	22 (53.7)	1.00	.149	48 (47.5)	33 (44.0)	1.00	.059	
1	19 (46.3)	19 (46.3)	1.75 (0.82, 3.76)		53 (52.5)	42 (56.0)	1.58 (0.98, 2.53)		
Gender									
Male	15 (36.6)	15 (36.6)	1.00	.057	41 (40.6)	31 (41.3)	1.00	.656	
Female	26 (63.4)	26 (63.4)	0.38 (0.14, 1.03)		60 (59.4)	44 (58.7)	0.89 (0.53, 1.49)		
Tumor grade									
Low	2 (4.9)	2 (4.9)	1.00	.015	3 (3.0)	2 (2.7)	1.00	.242	
Intermediate	13 (31.7)	13 (31.7)	5.51 (0.97, 31.47)		25 (24.8)	17 (22.7)	2.19 (0.51, 9.37)		
High	26 (63.4)	26 (63.4)	16.82 (2.11, 134.07)		73 (72.3)	56 (74.7)	1.46 (0.31, 6.81)		
Histology subtype									
Leiomyosarcoma	19 (46.3)	19 (46.3)	1.00	.015	42 (41.6)	31 (41.3)	1.00	.886	
Synovial sarcoma	3 (7.3)	3 (7.3)	0.81 (0.38, 1.73)		11 (10.9)	7 (9.3)	0.89 (0.36, 2.22)		
Other sarcoma	19 (46.3)	19 (46.3)	7.01 (1.57, 31.23)		48 (47.5)	37 (49.3)	1.08 (0.64, 1.83)		
Age at randomization									
≤50 years	19 (46.3)	19 (46.3)	1.00	.640	50 (49.5)	34 (45.3)	1.00	.187	
>50 years	22 (53.7)	22 (53.7)	1.19 (0.58, 2.44)		51 (50.5)	41 (54.7)	1.41 (0.85, 2.36)		

Supporting Table S8. Complete results of the multivariable Cox regression models for PFS and OS of patients receiving pazopanib with and without cardiotoxicity grade 3-4 at landmark time point of 60 days.

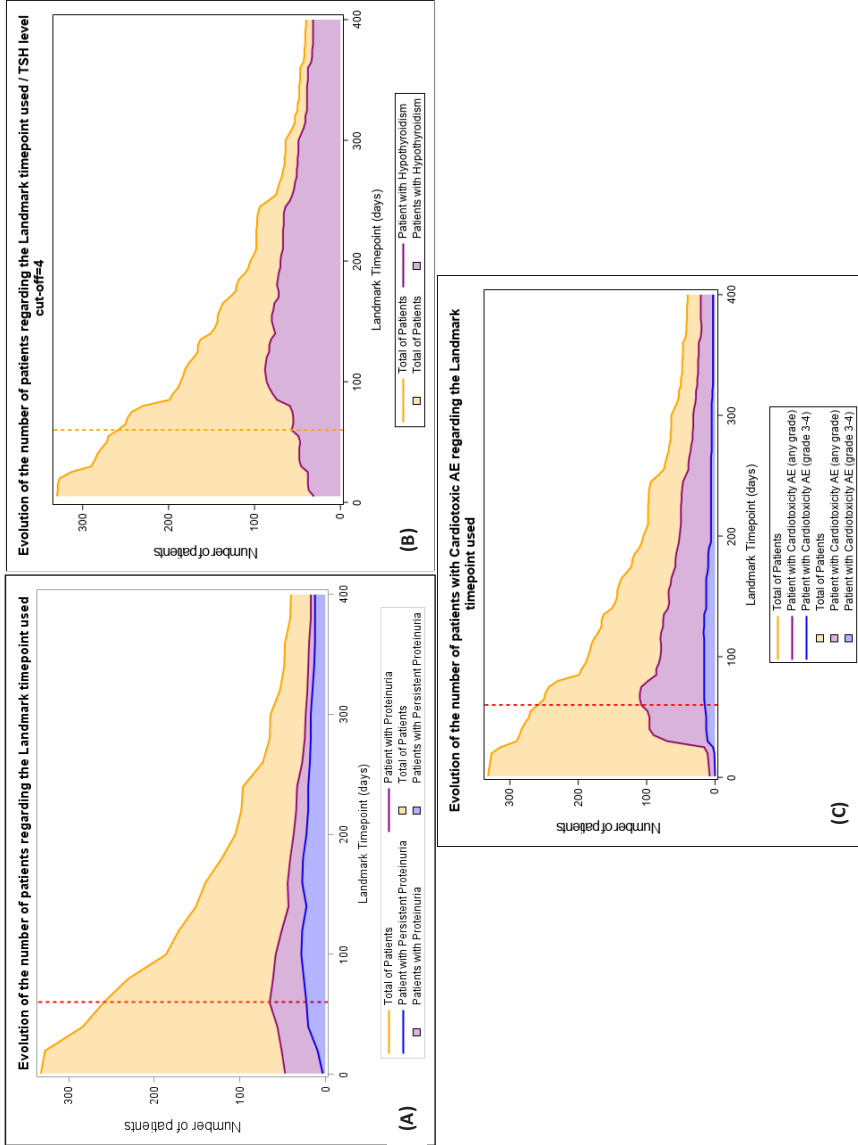
Landmark Analysis at t=60days	Multivariable Cox model of progression-free survival				Multivariable Cox model of overall survival				
	Covariates	Patients (N=257)	Observed Events (O=246)	Hazard Ratio (95%CI)	Wald p-value	Patients (N=311)	Observed Events (O=244)	Hazard Ratio (95%CI)	Wald p-value
Cardiotoxic Adverse Event (Grade 3-4)									
No	242 (94.2)	231 (93.9)	1.00	.897	294 (94.5)	231 (94.7)	1.00	.801	
Yes	15 (5.8)	15 (6.1)	0.97 (0.57, 1.64)		17 (5.5)	13 (5.3)	0.93 (0.52, 1.65)		
Performance status									
0	145 (56.4)	139 (56.5)	1.00	.403	165 (53.1)	123 (50.4)	1.00	<.001	
1	112 (43.6)	107 (43.5)	0.90 (0.69, 1.16)		146 (46.9)	121 (49.6)	1.58 (1.22, 2.04)		
Gender									
Male	101 (39.3)	98 (39.8)	1.00	.027	126 (40.5)	106 (43.4)	1.00	.129	
Female	156 (60.7)	148 (60.2)	0.74 (0.56, 0.97)		185 (59.5)	138 (56.6)	0.81 (0.62, 1.06)		
Tumor grade									
Low	24 (9.3)	19 (7.7)	1.00	.002	28 (9.0)	16 (6.6)	1.00	.013	
Intermediate	87 (33.9)	85 (34.6)	2.35 (1.43, 3.84)		105 (33.8)	85 (34.8)	2.04 (1.21, 3.43)		
High	146 (56.8)	142 (57.7)	1.79 (1.08, 2.97)		178 (57.2)	143 (58.6)	1.59 (0.93, 2.73)		
Histology subtype									
Leiomyosarcoma	106 (41.2)	104 (42.3)	1.00	.182	129 (41.5)	97 (39.8)	1.00	.009	
Synovial sarcoma	50 (19.5)	47 (19.1)	0.76 (0.57, 1.02)		60 (19.3)	50 (20.5)	1.36 (1.01, 1.84)		
Other sarcoma	101 (39.3)	95 (38.6)	0.91 (0.63, 1.31)		122 (39.2)	97 (39.8)	1.81 (1.23, 2.66)		
Age at randomization									
≤50 years	101 (39.3)	94 (38.2)	1.00	.048	122 (39.2)	93 (38.1)	1.00	.035	
>50 years	156 (60.7)	152 (61.8)	1.34 (1.00, 1.79)		189 (60.8)	151 (61.9)	1.37 (1.02, 1.83)		
Cardiac history									
No	194 (75.5)	186 (75.6)	1.00	.303	233 (74.9)	182 (74.6)	1.00	.539	
Yes	63 (24.5)	60 (24.4)	0.85 (0.61, 1.16)		78 (25.1)	62 (25.4)	1.11 (0.80, 1.52)		

Supporting Table S9. Complete results of the multivariable Cox regression models for PFS and OS of patients receiving pazopanib with and without cardiotoxicity of any grade at landmark time point of 60 days.

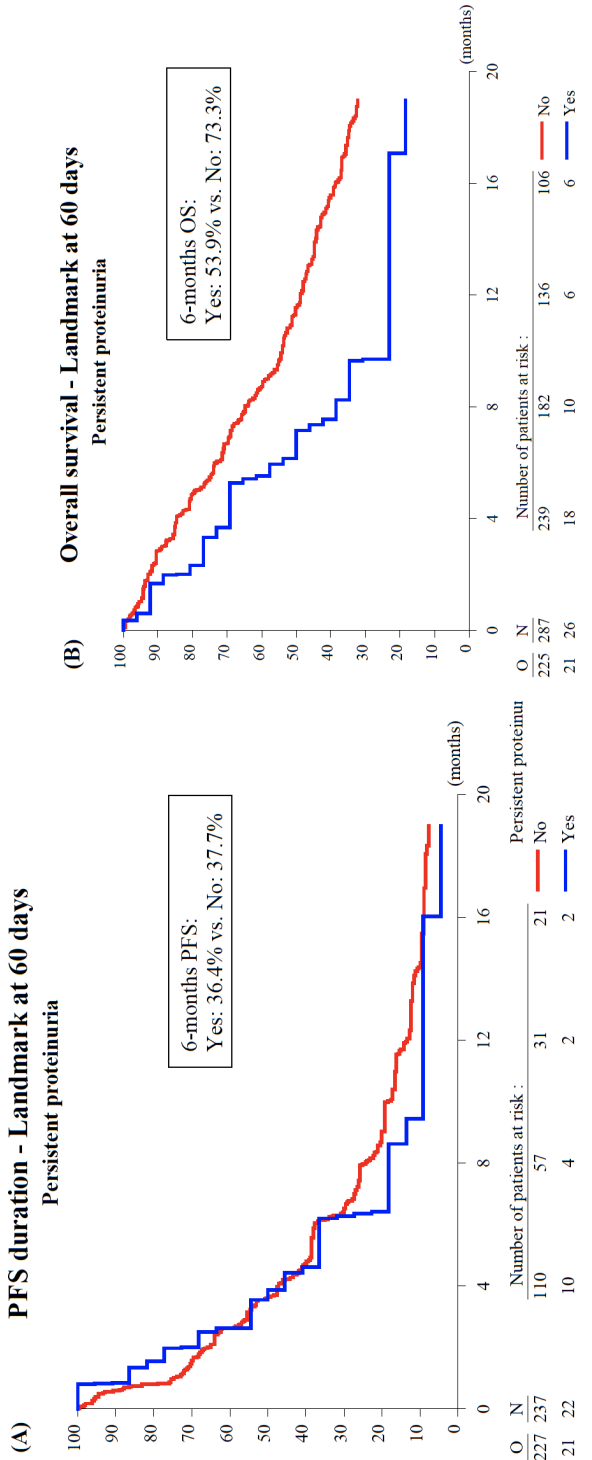
Landmark Analysis at t=60days	Multivariable Cox model of progression-free survival				Multivariable Cox model of overall survival				
	Covariates	Patients (N=257)	Observed Events (O=246)	Hazard Ratio (95%CI)	Wald p-value	Patients (N=311)	Observed Events (O=244)	Hazard Ratio (95%CI)	Wald p-value
Cardiotoxic Adverse Event (Any grade)									
No	151 (58.8)	144 (58.5)	1.00	.380	190 (61.1)	155 (63.5)	1.00	.120	
Yes	106 (41.2)	102 (41.5)	1.12 (0.87, 1.46)		121 (38.9)	89 (36.5)	0.81 (0.63, 1.06)		
Performance status									
0	145 (56.4)	139 (56.5)	1.00	.415	165 (53.1)	123 (50.4)	1.00	<.001	
1	112 (43.6)	107 (43.5)	0.90 (0.69, 1.16)		146 (46.9)	121 (49.6)	1.57 (1.21, 2.03)		
Gender									
Male	101 (39.3)	98 (39.8)	1.00	.027	126 (40.5)	106 (43.4)	1.00	.121	
Female	156 (60.7)	148 (60.2)	0.74 (0.56, 0.97)		185 (59.5)	138 (56.6)	0.81 (0.62, 1.06)		
Tumor grade									
Low	24 (9.3)	19 (7.7)	1.00	.001	28 (9.0)	16 (6.6)	1.00	.023	
Intermediate	87 (33.9)	85 (34.6)	2.40 (1.46, 3.95)		105 (33.8)	85 (34.8)	1.93 (1.14, 3.26)		
High	146 (56.8)	142 (57.7)	1.84 (1.10, 3.06)		178 (57.2)	143 (58.6)	1.51 (0.88, 2.60)		
Histology subtype									
Leiomyosarcoma	106 (41.2)	104 (42.3)	1.00	.222	129 (41.5)	97 (39.8)	1.00	.013	
Synovial sarcoma	50 (19.5)	47 (19.1)	0.77 (0.58, 1.04)		60 (19.3)	50 (20.5)	1.33 (0.98, 1.80)		
Other sarcoma	101 (39.3)	95 (38.6)	0.92 (0.63, 1.32)		122 (39.2)	97 (39.8)	1.76 (1.20, 2.58)		
Age at randomization									
≤50 years	101 (39.3)	94 (38.2)	1.00	.056	122 (39.2)	93 (38.1)	1.00	.019	
>50 years	156 (60.7)	152 (61.8)	1.33 (0.99, 1.78)		189 (60.8)	151 (61.9)	1.42 (1.06, 1.91)		
Cardiac history									
No	194 (75.5)	186 (75.6)	1.00	.273	233 (74.9)	182 (74.6)	1.00	.570	
Yes	63 (24.5)	60 (24.4)	0.84 (0.61, 1.15)		78 (25.1)	62 (25.4)	1.10 (0.80, 1.50)		

Supporting Table S10. Complete results of the multivariable Cox regression models for PFS and OS of patients receiving placebo with and without cardiotoxicity of any grade at landmark time point of 60 days.

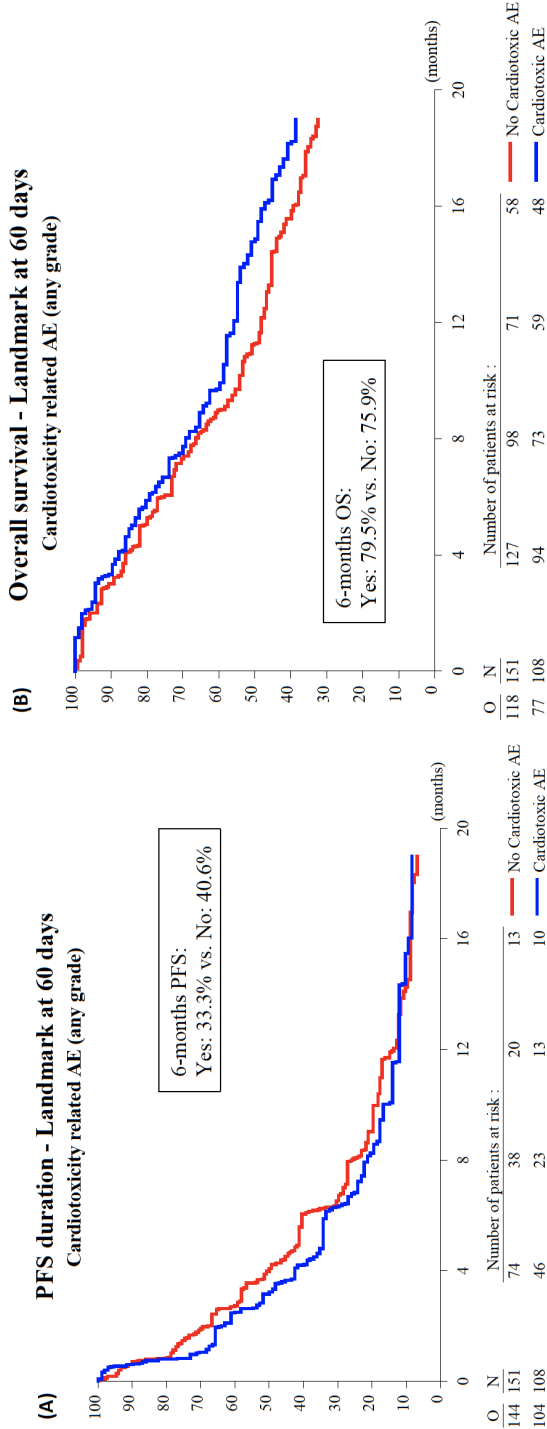
Landmark Analysis at t=60days	Multivariable Cox model of progression-free survival				Multivariable Cox model of overall survival				
	Covariates	Patients (N=41)	Observed Events (O=41)	Hazard Ratio (95%CI)	Wald p-value	Patients (N=101)	Observed Events (O=75)	Hazard Ratio (95%CI)	Wald p-value
Cardiotoxic Adverse Event (Any grade)									
No	36 (87.8)	36 (87.8)	1.00	.301	93 (92.1)	68 (90.7)	1.00	.988	
Yes	5 (12.2)	5 (12.2)	0.52 (0.15, 1.79)		8 (7.9)	7 (9.3)	1.01 (0.41, 2.46)		
Performance status									
0	22 (53.7)	22 (53.7)	1.00	.261	48 (47.5)	33 (44.0)	1.00	.034	
1	19 (46.3)	19 (46.3)	1.54 (0.73, 3.25)		53 (52.5)	42 (56.0)	1.76 (1.04, 2.96)		
Gender									
Male	15 (36.6)	15 (36.6)	1.00	.012	41 (40.6)	31 (41.3)	1.00	.801	
Female	26 (63.4)	26 (63.4)	0.29 (0.11, 0.76)		60 (59.4)	44 (58.7)	0.93 (0.55, 1.59)		
Tumor grade									
Low	2 (4.9)	2 (4.9)	1.00	.004	3 (3.0)	2 (2.7)	1.00	.218	
Intermediate	13 (31.7)	13 (31.7)	10.01 (1.42, 70.42)		25 (24.8)	17 (22.7)	2.12 (0.49, 9.17)		
High	26 (63.4)	26 (63.4)	34.89 (3.61, 337.59)		73 (72.3)	56 (74.7)	1.37 (0.29, 6.49)		
Histology subtype									
Leiomyosarcoma	19 (46.3)	19 (46.3)	1.00	.021	42 (41.6)	31 (41.3)	1.00	.964	
Synovial sarcoma	3 (7.3)	3 (7.3)	7.92 (1.72, 36.51)		11 (10.9)	7 (9.3)	0.94 (0.38, 2.35)		
Other sarcoma	19 (46.3)	19 (46.3)	1.02 (0.44, 2.34)		48 (47.5)	37 (49.3)	1.05 (0.62, 1.77)		
Age at randomization									
≤50 years	19 (46.3)	19 (46.3)	1.00	.703	50 (49.5)	34 (45.3)	1.00	.114	
>50 years	22 (53.7)	22 (53.7)	1.17 (0.53, 2.60)		51 (50.5)	41 (54.7)	1.57 (0.90, 2.76)		
Cardiac history									
No	27 (65.9)	27 (65.9)	1.00	.203	68 (67.3)	51 (68.0)	1.00	.234	
Yes	14 (34.1)	14 (34.1)	1.70 (0.75, 3.85)		33 (32.7)	24 (32.0)	0.70 (0.40, 1.25)		



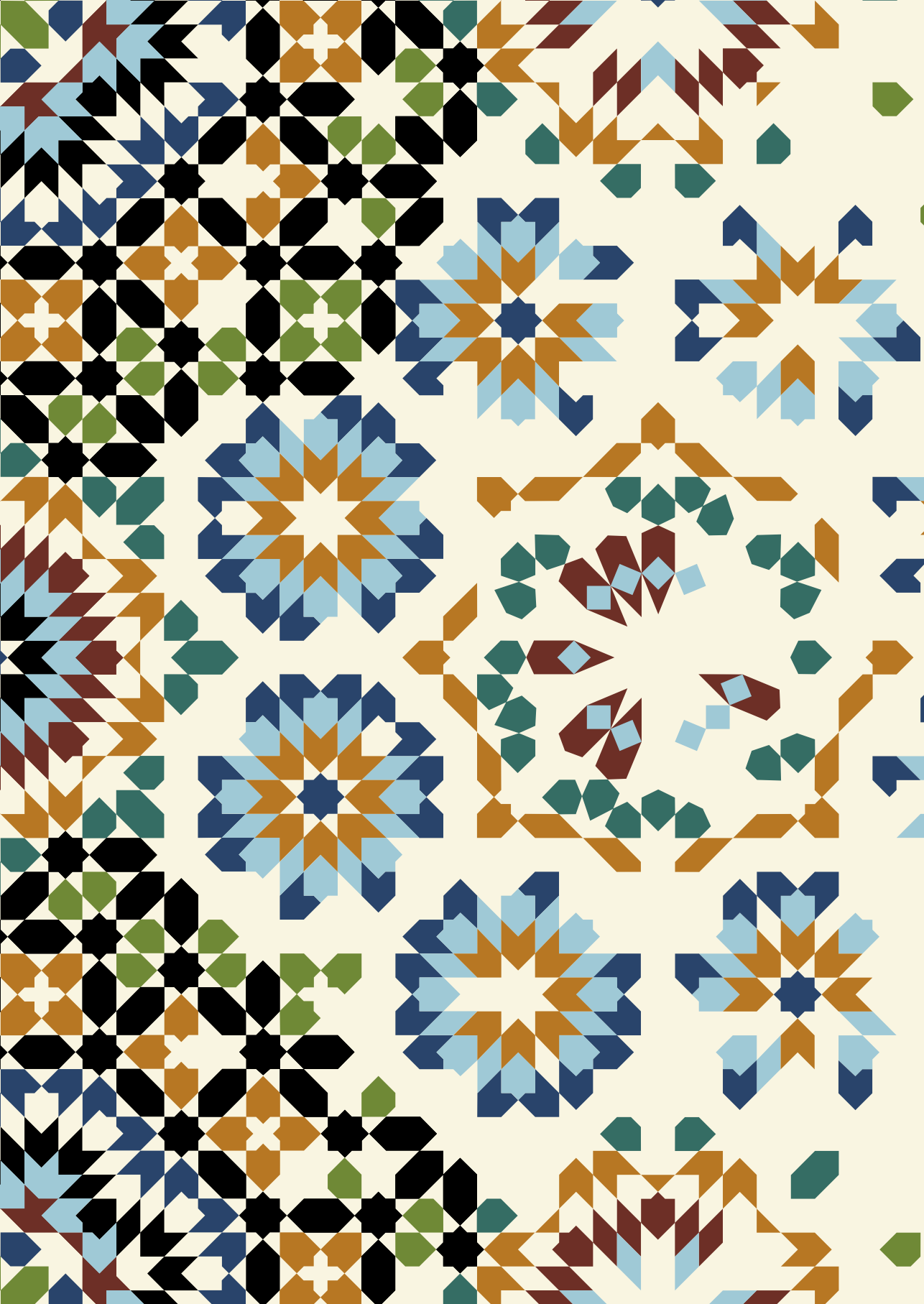
▲ **Supporting Figure S1.** Incidences and landmark choice of the three pazopanib-induced toxicities. **(A)** Number of patients with proteinuria while on treatment with pazopanib. **(B)** Number of patients with hypothyroidism (TSH cut-off point 4mU/L) while on treatment with pazopanib. **(C)** Number of patients with cardiotoxicity related adverse events while on treatment with pazopanib.

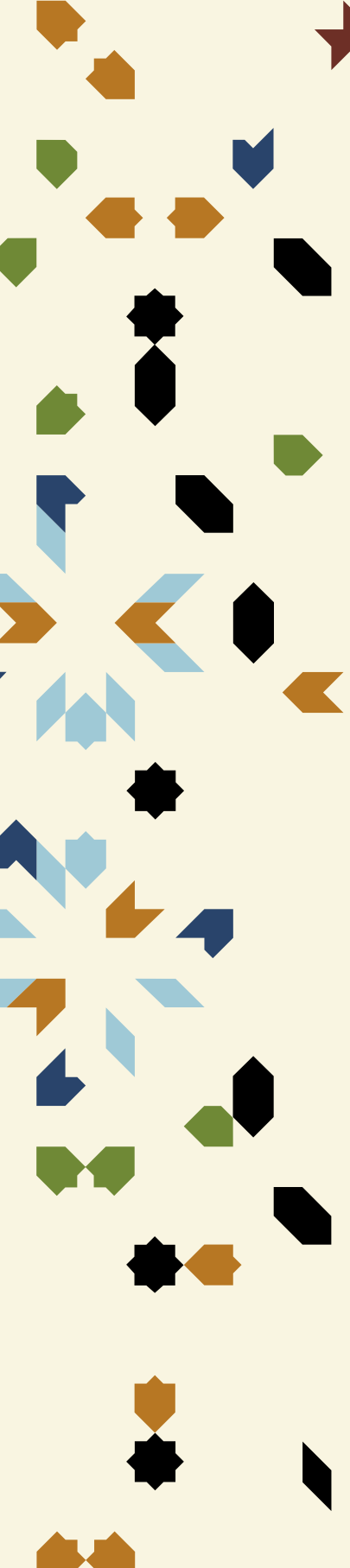


▲ **Supporting Figure S2.** Kaplan-Meier curves showing the progression-free survival (A) and overall survival (B) of patients with and without persistent proteinuria.



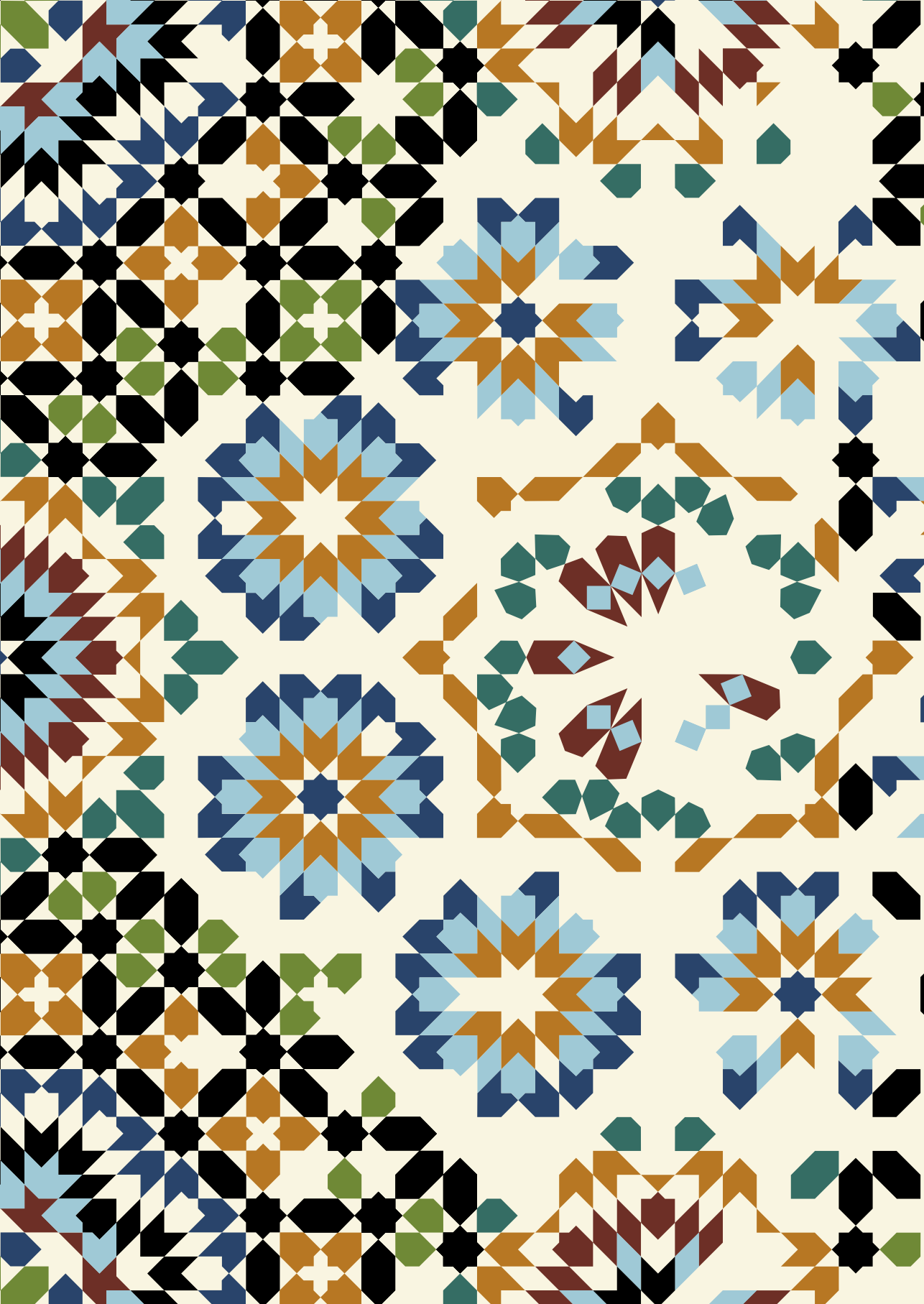
▲ **Supporting Figure S3.** Kaplan-Meier curves showing the progression-free survival **(A)** and overall survival **(B)** of patients with and without cardiotoxicity of any grade.

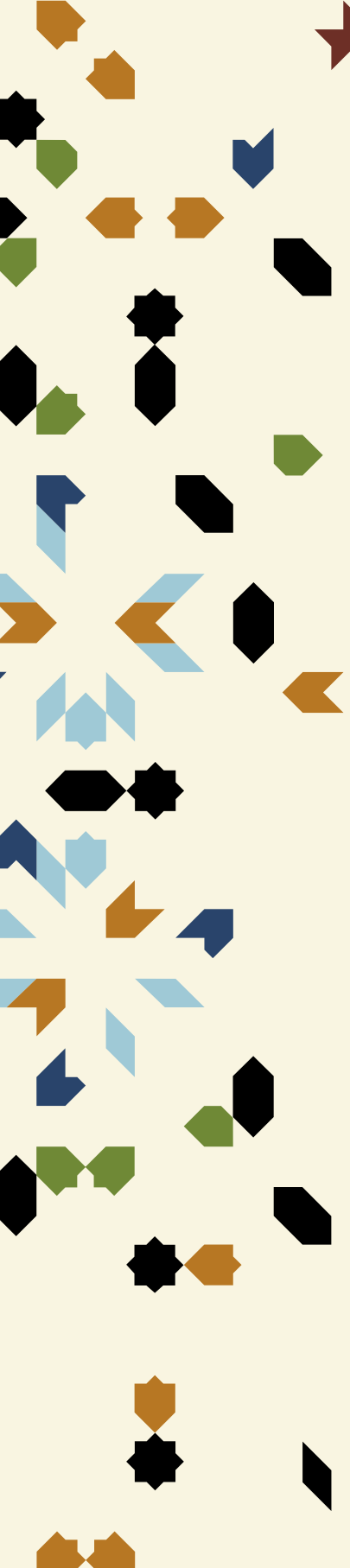




PART V

GENERAL DISCUSSION
SUMMARY
APPENDICES





CHAPTER 14

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

General discussion

Although it is already being recognized for years that soft tissue sarcoma (STS) is not one disease, but a collective term for a heterogeneous group of individual and distinct mesenchymal tumors, most STS are still treated similarly. This applies to early and localized stages as well as to the more locally advanced and metastasized setting.

Importance of soft tissue sarcoma biology

Currently, the classification of the World Health Organization is used to categorize these tumors into subtypes, primarily based on morphology [1]. As outlined in especially **chapter 2** and **chapter 4**, there still can be quite some heterogeneity on a molecular level even within one STS subtype, implying that the assessment of morphology alone might not be the most accurate diagnostic method and that other factors – such as mutational status – should also be taken into account. This intra-STs subtype heterogeneity might also partially explain the negative clinical trials, including STS subtype-specific trials [2-5] or subgroup analyses [6, 7], trials studying a histology-driven choice of treatment [8], and trials investigating targeted therapies [9, 10]. It is clear that the histology of a specific STS subtype often cannot be used to predict responsiveness to a certain drug, and it is likely that a particular genetic aberration or profile is better associated with response to that drug. For example, sensitivity to imatinib in GIST: not all GISTs are sensitive to imatinib, only those with a *KIT* exon 11 mutation [11] and to a lesser extent a *KIT* exon 9 mutation [12]. Patients with *PDGFRA*-mutated [13] or wild-type [14] GIST generally do not respond to imatinib. This hypothesis might be translated to other targeted therapies such as anti-*PDGFRA* or anti-PD1 agents.

Additionally, in an attempt to identify patients possibly sensitive for targeted therapies such as olaratumab or pembrolizumab, the *PDGFRA* or PD-L1 expression is usually determined by immunohistochemistry [15, 16], rather than identifying a specific genetic alteration predictive of response to these agents. The mere presence of a protein, as detected by immunohistochemistry, does not automatically predict response to a drug targeting that protein. Perhaps only specific alterations in the protein lead to sensitivity for a drug. Therefore, immunohistochemistry might not be the most optimal method to identify biomarkers predictive of response in STS, taking again sensitivity for imatinib in GIST as an example. All *KIT*-mutated, most *PDGFRA*-mutated and some wild-type GISTs express CD117 and/or DOG-1 [17]. These immunohistochemical biomarkers do not distinguish between the different (exon) mutations and thereby sensitivity to imatinib, and a mutation analysis is needed to make this distinction.

We advocate that treatment or eligibility for inclusion into clinical trials should not be solely based on STS subtype as classified by the WHO, but also on specific genomic alterations

of these tumors. By identifying targetable mutations or signatures in each individual STS patient, we will be able to better select patients who could benefit from personalized treatment and from inclusion in clinical trials with these specific agents. Examples include patients with homologous recombination deficient tumors who could benefit from platinum-based therapy [18], with *BRAF*-mutated tumors who could benefit from treatment with dabrafenib [19] or with an *ESR1* mutation who could benefit from treatment with fulvestrant [20].

Heterogeneity amongst liposarcomas

The results of multiple studies in this thesis and in literature show that even within one STS subtype substantial heterogeneity can exist. This confirms that STS, and even liposarcoma, is not a single entity and that we should pursue a subtype-specific and maybe even a site-specific treatment approach, illustrated by **chapter 6** and **chapter 7**.

Approximately half of all the liposarcoma patient have the well-differentiated subtype (well-differentiated liposarcoma, WDLPS). Especially in this liposarcoma subtype, tumor localization is of importance [21]. Based on prognosis, essentially two groups can be distinguished: retroperitoneal WDLPS and non-retroperitoneal WDLPS. Patients with retroperitoneal WDLPS have a poorer prognosis and ultimately die of disease because of local control issues, while patients with non-retroperitoneal WDLPS generally have an excellent prognosis and seldom die of disease. This indicates that local control is of crucial importance in retroperitoneal WDLPS and that local treatment options should be reconsidered in this specific patient group. This includes the extent of resection (resection with or without uninvolved adjacent organs) and radiotherapy. Despite improving local control, we are currently reluctant in giving radiotherapy to any WDLPS patient, because of its toxicity, varying effectivity and missing effect on survival. However, retroperitoneal WDLPS might be the exception in which improved local control could lead to improved survival. At present, this hypothesis is being evaluated in the phase III STRASS trial. The preliminary results of this trial – randomizing between neoadjuvant radiotherapy plus surgery or surgery alone [22] – showed that neoadjuvant radiotherapy improved the abdominal recurrence-free survival in the liposarcoma subgroup. At present, the final results are pending and will have to show whether the improved abdominal recurrence-free survival has led to an improvement in overall survival.

Regarding the non-retroperitoneal WDLPS, also called atypical lipomatous tumors (ALTs), we believe that we might be 'over-treating' these patients, opposite to the possible 'under-treatment' of retroperitoneal WDLPS, as outlined in **chapter 8**. We feel that an active surveillance approach might be appropriate, safe and justified in selected cases, especially in elderly patients, patients with (multiple) severe comorbidities and/or patients in whom a

radical resection cannot be achieved. Additionally, we hypothesize that the (health-related) quality of life of patients treated with active surveillance is equal or even better compared to that of surgically treated patients. However, these parameters (feasibility, safety and quality of life) need yet to be evaluated in a prospective clinical trial before implementation into daily clinical practice is possible, but these observations highlight again the need to tailor treatment based on both liposarcoma/STS subtype and tumor localization.

Besides a minimally invasive treatment approach in non-retroperitoneal WDLPS (i.e. active surveillance), we also searched for a minimally invasive method for diagnosing WDLPS in **chapter 5**, using radiomics. Radiomics is a type of artificial intelligence through which additional data is extracted from medical imaging which is not visible with the human eye, such as data regarding shape, texture, intensity, etc. Subsequently, these features are linked to the clinical data to create a diagnostic algorithm. Big data, machine learning and other forms of artificial intelligence are increasingly used in oncology and are expected to have a major impact on cancer care and research. Artificial intelligence enables the integration of different types of data, such as genomic data, imaging data and clinical data and allows for the discovery of hidden patterns and links. In general, this might lead to, for example, new drug discoveries [23] or being able to predict the response to treatment [24-26]. In non-retroperitoneal WDLPS specifically, examples of possible radiomics applications include predicting which patients will develop local recurrent disease after primary resection, who will have progressive disease (i.e. tumor growth) or in whom dedifferentiation will occur. Based on these radiomics models, treatment for non-retroperitoneal WDLPS could be tailored and individualized.

Surgical treatment of localized STS

The rarity, heterogeneity and complexity of STS highlight the need for centralization of STS care. Many studies have shown that centralization of sarcoma care has a beneficial effect on multiple outcomes, including survival [27-34]. Therefore, in an attempt to improve centralization of STS care in the Netherlands, six hospitals have been designated as centers of expertise by the Netherlands Federation of University Medical Centers (NFU), of which 5 are also member of the European Reference Network for rare adult cancer (ERN-EURACAN). Additionally, in 2012 a standardization report on the multidisciplinary cancer care, defining the conditions a hospital needs to fulfill in order to deliver good cancer care, was published in the Netherlands, which is updated annually [35].

Two nationwide studies, in the time periods 2006-2011 [36] and 2006-2015 (**chapter 9**), have shown that centralization in the Netherlands is increasing but is still in need of improvement. However, it should be realized that centralization is not only a result of the expertise centers recruiting STS patients, but mostly relies on the alertness and willingness

of physicians and patients in the general hospitals to refer/be referred to an expertise center. Creating more awareness for this rare group of diseases is therefore an important element in pursuing centralization of STS care and improving the outcomes of these patients.

Systemic treatment of metastatic STS

Despite the increase in available systemic treatment options for advanced/metastatic STS (**chapter 11**), there has been only a minimal increase in survival of patients with synchronous metastases (**chapter 12**) and most probably also for patients with metachronous metastases, given all the trials with a negative outcome reported in literature [6, 7, 10, 37-40]. These negative results and only minimal increase in survival stress that there is an unmet need for novel agents and targets. Identifying possible new targets for treatment and accompanying effective agents is obviously easier said than done, although new techniques such as whole genome sequencing – becoming more widely available and affordable – and artificial intelligence – used to discover hidden patterns and links – might be game changers in the STS field and cancer research in general.

Apart from identifying new targets and effective drugs, it is of importance to identify which patients will benefit from systemic treatment with these drugs. All kinds of predictive biomarkers have been tested and include, for example, specific genetic aberrations [41], expression levels of microRNAs [42], PD-L1 expression [43] or drug-induced toxicity (**chapter 13**) [44, 45]. However, most of these biomarkers lack reliability, validity, sensitivity and/or specificity, and have insufficient predictive power. So unfortunately, for most drugs no solid biomarkers predictive of response exist (yet).

Future perspectives

Traditionally, all STS subtypes were combined to achieve adequate patient numbers in clinical trials, which has led to a 'one size fits all' treatment approach. Based on the results in this thesis, but also other studies reported in literature, it is time to conclude that one size does not fit all. Examples include the results of **chapter 2**, **chapter 6** and **chapter 7**, in which we show that STS and even liposarcoma is not a single entity, that treatment should be tailored to a specific STS/liposarcoma subtype and may be even tailored to a specific tumor localization. Additionally, we suggest that treatment or inclusion into clinical trials should not be solely based on STS subtype as classified by the WHO, but also on the genomic aberrations present in these tumors, since with WGS a substantial number of patients were identified who could benefit from personalized targeted treatment. A switch to at least a histology-driven choice of treatment, but preferably personalized treatment, will be crucial to further improve the outcomes of STS patients in both an early and advanced stage of disease.

An example of a subtype-specific as well as a tumor localization-specific treatment for patients with localized STS is suggested in **chapter 8**. In this chapter, active surveillance is introduced for patients with non-retroperitoneal WDLPS/ALT. Recently, we have set up a prospective trial in which we aim to work towards a minimally invasive approach of ALTs (MINIMALIST trial). This trial will combine the evaluation of a minimally invasive diagnosis (prospective validation of the radiomics model developed in **chapter 5**) and the assessment of the feasibility and safety of a minimally invasive treatment approach (i.e. active surveillance as suggested in **chapter 8**). Currently, the MINIMALIST trial is open for accrual in the Erasmus MC Cancer Institute and the first patients are being included.

In the advanced/metastatic stage, ideally a clinical trial in which STS patients are randomized between treatment according to the standard lines of systemic treatment for metastatic STS (as defined in international guidelines) and treatment according to their histology plus genomic alterations should define the role of personalized treatment in this stage. As a consequence of these subtype-specific, tumor localization-specific and/or personalized trials, it will become even harder and more complicated to perform research in these rare tumors, stressing the need and importance of more intensive national and international collaborations as well as of better registration of sarcomas in (inter)national cancer registries.

Lastly, artificial intelligence will be increasingly used. Besides differentiating lipomas from WDLPS and thereby ultimately omitting a biopsy, already multiple other applications of radiomics in non-retroperitoneal WDLPS can be thought, let alone all other possible applications in other STS subtypes. The three possible radiomics applications mentioned earlier (to predict the risk of local recurrence, tumor growth and dedifferentiation) could help to further tailor and personalize treatment. In case of high risk of local recurrent disease, two different treatment approaches might be thought of: (1) more extended surgery or neoadjuvant/adjuvant therapy might be needed to lower the risk of recurrence, or (2) these patients might be candidate for treatment with active surveillance (i.e. patient selection), since the tumor will recur anyway. Radiomics could also help patient selection for active surveillance with regards to tumor growth: patients in whom no/minimal tumor growth is predicted might also be good candidates for an active surveillance approach. Lastly, in case of high risk of dedifferentiation, treatment might be intensified by extended resection or by adding radiotherapy, thereby trying to lower the risk of an unfavorable outcome.

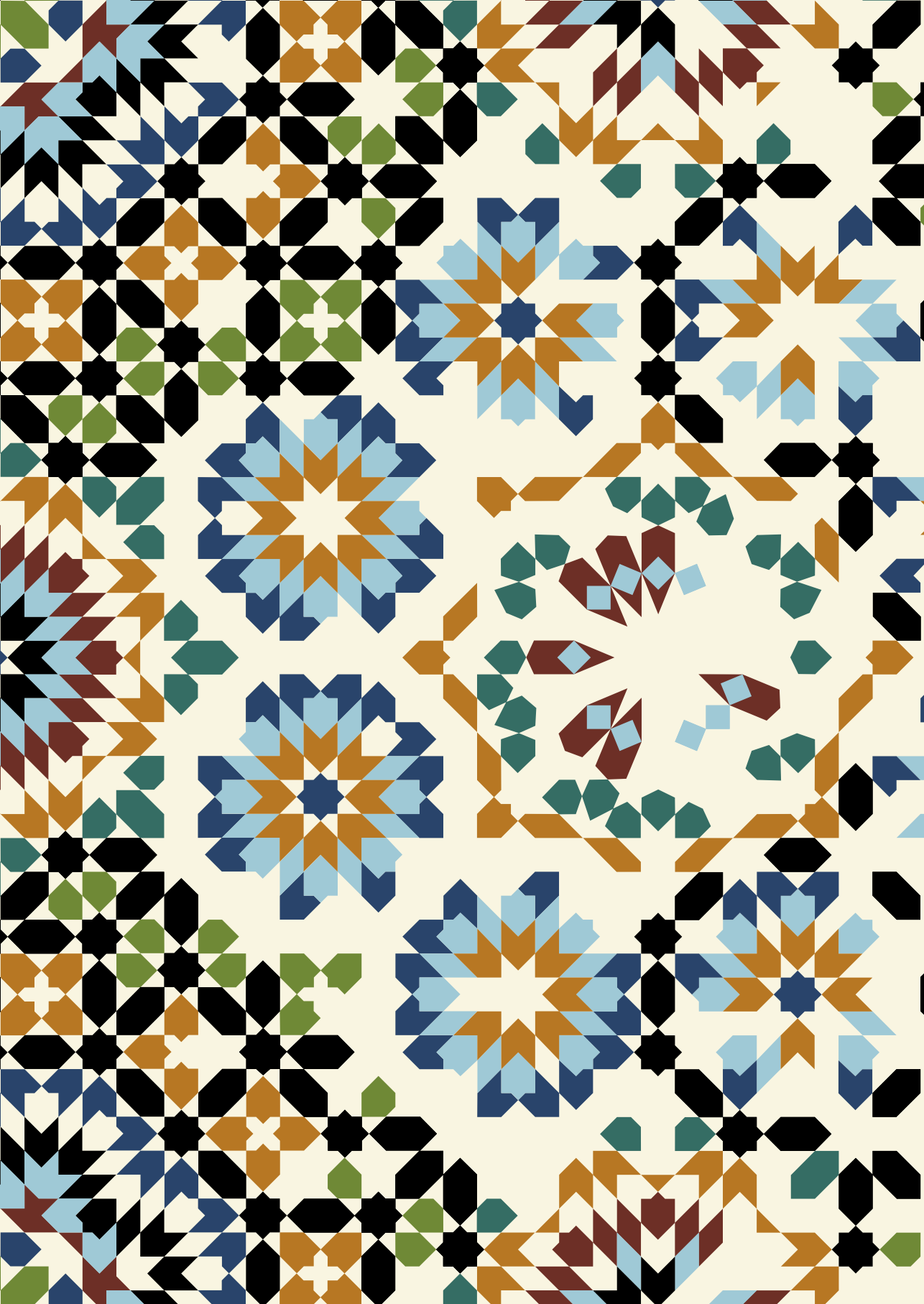
Overall, it can be concluded that there is still a lot to gain in the knowledge of soft tissue sarcomas and that the gaps of the mosaic are not all filled in.

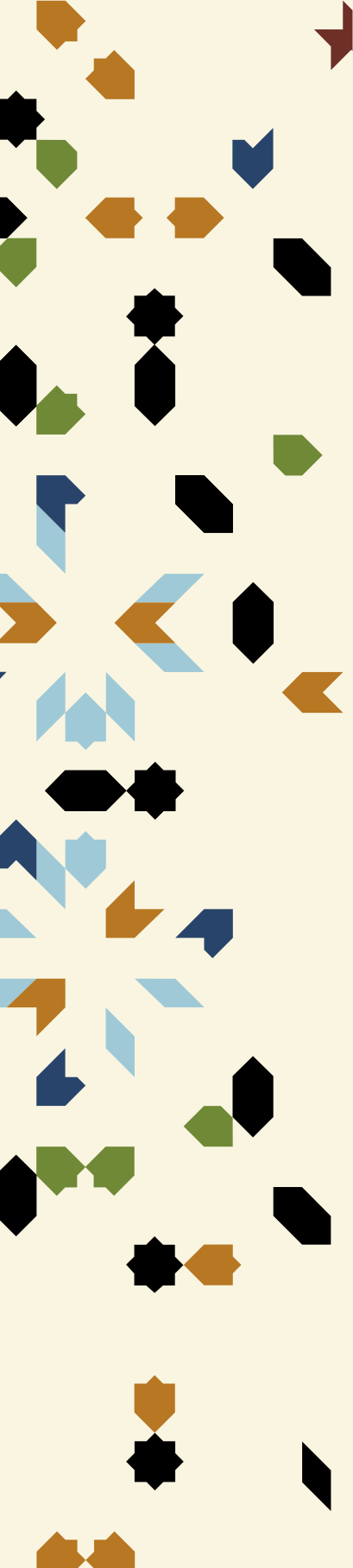
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CHAPTER 15

SUMMARY

Summary

This thesis consists of four parts. In **part I** we strived to gain more insight into the biology of soft tissue sarcomas (STS) and searched for new actionable targets for therapy. In **part II**, one of the most frequently observed STS subtypes, liposarcoma, was further examined. This includes the diagnosis and the outcomes of patients with different liposarcoma subtypes and different tumor localizations. **Part III** evaluated the surgical treatment of localized STS, on a nation-wide level as well as for a small subgroup of patients treated in the Erasmus MC Cancer Institute. Lastly, **part IV** focuses on the systemic treatment of metastatic STS. A short overview is given of the currently available therapies and therapies in the pipeline, followed by an evaluation of the survival as a result of the changes in treatment strategies.

Part I – Expanding the insight into the biology of soft tissue sarcomas

In **chapter 2**, the genomic landscape of metastatic STS (mSTS), including new targets for therapy, is described. Samples of metastatic STS were collected and analyzed by whole genome sequencing. Metastatic leiomyosarcomas on average had a higher tumor mutational burden (TMB) than the metastatic gastrointestinal stromal tumors (mGIST) or other mSTS. Kataegis was observed in 35 of the 122 samples and chromothripsis in 24 of the 122 samples. All known COSMIC mutational signatures were present and no clear differences in contributing signatures were seen between the different STS subtypes. In 86% of the samples at least one actionable target could be identified for which an FDA-approved or investigational agent is available. Examples include sorafenib for *KIT*-mutated GIST, trastuzumab for *ERBB2*-mutated leiomyosarcoma, imatinib for *KIT*-mutated angiosarcoma or fulvestrant for *ESR1*-mutated leiomyosarcoma and *ESR1*-mutated endometrial stromal cell sarcoma. Additionally, six mSTS samples had a TMB ≥ 10 and might benefit from treatment with checkpoint inhibitors. This study gives an important insight into the biology of mSTS and shows that whole genome sequencing can serve as a valuable tool to identify clinically relevant and targetable molecular aberrations. It thereby improves patient management and treatment decision making, even or especially after multiple lines of treatment.

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Chapter 3 describes the association between the overexpression of miR-26a and miR-3913, located in the 12q13-15 region, and the proliferation of well-differentiated liposarcoma (WDLPS) and dedifferentiated liposarcoma (DDLPS), characterized by amplification of the 12q13-15 region. Both microRNAs were indeed overexpressed in WDLPS and/or DDLPS. Inhibition of these microRNAs led to decreased cellular proliferation, and inhibition of miR-3913 also induced apoptosis in WDLPS cell lines. Additionally, miR-26a appeared to target the tumor suppressor *PTEN*. So, miR-26a and miR-3913 overexpression, most probably due to pathognomonic amplification of 12q13-15, seemed to promote liposarcoma development by stimulating cellular proliferation and – to a lesser extent – suppressing apoptosis, suggesting

that non-protein coding genes, like microRNAs, may also play an role in sarcomagenesis of WDLPS and DDLPS.

In **chapter 4**, the microRNA expression and DNA methylation patterns of paired primary and recurrent WDLPS were compared. The aim was to detect differences in microRNA expression levels and DNA methylation profiles, thereby identifying processes involved in recurrence. However, no distinction between primary and recurrent WDLPS could be made based on differentially expressed microRNAs or differentially methylated regions and no common drivers for recurrence could be identified. The differences, especially in DNA methylation patterns, were very heterogeneous and variable between patients.

Part II – Heterogeneity within the liposarcoma spectrum

In **chapter 5**, a new and non-invasive method to differentiate between WDLPS and lipomas was investigated. It can be difficult to distinguish between these two tumor types based on imaging, and an invasive biopsy is needed for pathological examination. Radiomics is a form of artificial intelligence which enables the extraction of imaging features from MRI scans and links them to pathological characteristics of a tumor, such as the mutational status. In this case, the *MDM2* amplification status was used to discriminate WDLPS from lipomas: *MDM2* amplification is present in WDLPS, but absent in lipomas. The radiomics model based on T1-weighted imaging features scored a mean AUC of 0.83, sensitivity of 0.68 and a specificity of 0.84. These scores were compared to the scores of three trained radiologists, who scored an AUC of 0.74/0.72/0.61, a sensitivity of 0.74/0.91/0.64, and a specificity of 0.55/0.36/0.59, respectively. From these results we concluded that radiomics is a promising, non-invasive method to differentiate between WDLPS and lipomas. However, further optimization and validation is needed before radiomics can be used in daily clinical practice.

Chapter 6 evaluated the role of primary tumor localization in the local recurrence-free survival, the distant metastasis-free survival and the disease-specific survival of patients with liposarcoma. Patients with a retroperitoneal, intrathoracic or scrotal liposarcoma developed a local recurrence more often than patients with a tumor in the extremity, trunk or head-and-neck region, but no differences in the development of metastases were observed. Patients with a retroperitoneal or intrathoracic liposarcoma had a poorer disease-specific survival, despite the observation that there were no differences in the development of metastatic disease. While most cancer patients die due to metastatic disease, these data suggest that these patients die of local disease and that for each tumor localization a different treatment approach might be preferable.

In **chapter 7**, we elaborated on liposarcomas in the extremity, the most common primary tumor localization. There were clear differences in recurrence patterns and survival between the different liposarcoma subtypes, indicating that extremity liposarcoma is not a single entity. Additionally, this study showed that an aggressive treatment approach

– including resection with wide margins and radiotherapy – led to excellent local control in extremity WDLPS, while a more conservative approach – with marginal excision and no radiotherapy – led to more local recurrences. However, this did not lead to an improved distant metastasis-free survival or disease-specific survival. Therefore, the benefits of wide excision and radiotherapy should be carefully balanced against the disadvantages and toxicity/morbidity.

Part III – Evaluation of the surgical treatment of localized soft tissue sarcoma

As a result of the observations in chapter 7, the treatment and outcomes of patients with extremity WDLPS were further examined in **chapter 8**. These patients rarely developed distant metastasis and seldom died due to their tumor. Death of disease only occurred after dedifferentiation of the tumor upon recurrence, which occurred sporadically. On the other hand, two patients died of treatment-related causes, so we wondered whether we might be ‘over-treating’ these patients. Therefore, we analyzed a small group of patients in whom deliberately an active surveillance approach was chosen. In this small cohort of patients, we concluded that active surveillance, also called wait-and-see or watchful waiting, is an appropriate and adequate treatment option in selected cases. This especially applies for elderly patients, patients with multiple or severe comorbidities and/or patients in whom a radical resection is not possible. However, the follow-up of this patient cohort is still short, and the feasibility and safety of active surveillance as treatment option for non-retroperitoneal WDLPS needs to be assessed and explored in a larger prospective clinical trial.

Chapter 9 of this thesis focuses on the centralization of soft tissue sarcoma surgery in the Netherlands. Because of the rarity of STS, it is estimated that a general practitioner only sees one patient with an STS every 20 years and a surgeon in a general hospital only once every 4 years. This, in combination with the heterogeneity amongst the STS subtypes and the fact that benign soft tissue tumors are 100x more prevalent, makes diagnosing and treating these tumors challenging and highlights the urgency for centralization. However, centralization in the Netherlands was limited until 2011 and in need of improvement. This study showed that centralization of STS surgery improved over time, although it is still highly fragmented across the country. A survival benefit was observed for patients with high-grade and deep-seated STS who were treated in a high-volume hospital (≥ 20 resections per year) compared to patients treated in low-volume hospitals (< 10 resections per year). Additionally, unplanned resection and re-resections were less often performed in high-volume hospitals than in low-volume hospitals. Therefore, we plea for further centralization of STS care.

In **chapter 10**, the unplanned resections are further analyzed using data from the Netherlands Cancer Registry. Because benign soft tissue tumors are much more common,

timely recognition of malignant STS can be challenging. It is not unusual that an STS is initially considered as benign and excised without proper diagnostic work-up and inadequate surgical margins. These unplanned resections are also called 'whoops' resections. Unplanned resection occurred in 17% of all primary STS resections. These tumors were generally smaller (≤ 5 cm), more often located superficially and in the upper extremity, and pre-operative imaging was missing more often. Most unplanned resections resulted in residual disease, and patients more often needed to undergo a re-resection or radiotherapy. After an unplanned resection, patients were often referred to/discussed with a sarcoma expertise center, especially when there was residual tumor. To prevent unplanned resections, more awareness and education is needed. However, unplanned resections of small and superficial STS are unlikely to be completely preventable, given the high incidence of benign soft tissue tumors, and are partially 'all in the game'.

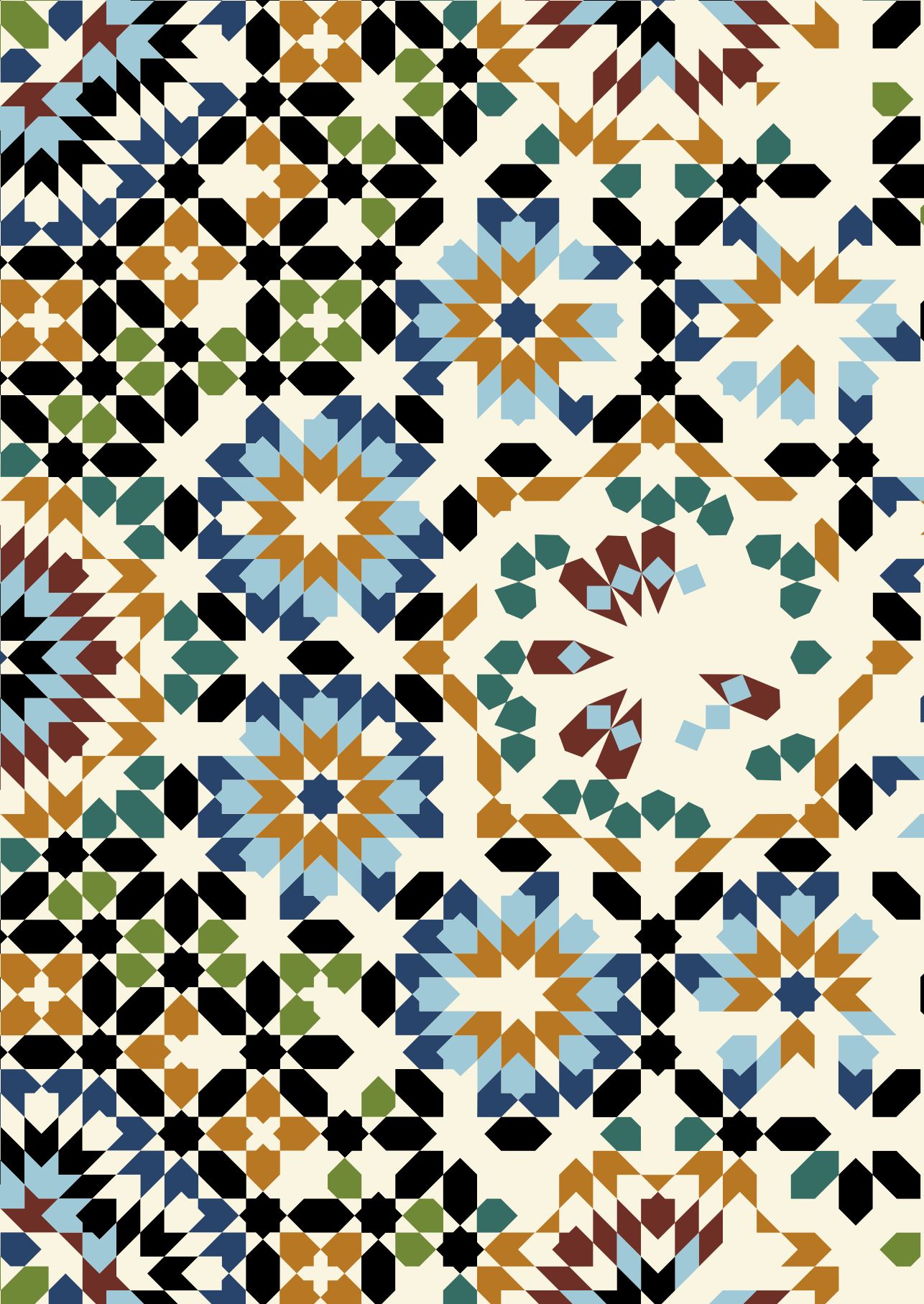
Part IV – Evaluation of the systemic treatment of metastatic soft tissue sarcoma

In **chapter 11**, a short overview of the currently available systemic therapies and the new therapies in the pipeline for metastatic STS is given. Despite the heterogeneity in terms of pathophysiology and sensitivity to chemotherapy, the first-line treatment is similar for almost all STS subtypes and consists of doxorubicin. Doxorubicin is mostly given as monotherapy, but can be combined with ifosfamide. At the time of writing this chapter, olaratumab was conditionally added to doxorubicin, based on a phase II trial showing an improvement in overall survival of almost a year. However, in the meantime, the preliminary results of the phase III trial have been presented; the benefit of the combination therapy could not be confirmed. Consequently, the conditional approval has been withdrawn. In second-line treatment and beyond, a histology-driven choice of treatment is much more common. Examples include trabectedin in (myxoid) liposarcomas and leiomyosarcomas, eribulin in liposarcomas, taxanes in angiosarcomas and gemcitabine-based regimens in leiomyosarcomas. Notwithstanding the expanding treatment options, the survival of patients with metastatic STS remains poor and there is an urgent need for new treatment strategies. Currently, all kinds of new drugs are tested, including immunotherapies, that have shown to be effective in other cancer types (such as ipilimumab, nivolumab and pembrolizumab).

Chapter 12 describes the change in overall survival of STS patients with metastatic disease at time of diagnosis (synchronous metastasis), which concerns nearly 15% of all newly diagnosed STS patients. Predilection sites of the metastases are the lungs, liver, bones and lymph nodes. These patients have a poor median overall survival, approximately 6 months, which was slightly improving over the years. The median overall survival of these patients is much poorer than the median overall survival described in the clinical trials which

compare two treatment regimens (medians of 12-24 months). Possible explanations include: 1) a considerable part of the patients in our study did not receive any treatment (due to contra-indications, unfitnes or patients' own wish); 2) the patients in this study probably were less fit than the patients included in clinical trials who need to meet strict eligibility criteria; 3) we only included patients with synchronous metastasis, while clinical trials mostly include patients who develop metastatic disease at a later point in time (metachronous metastasis). Patients with synchronous metastasis might represent a different and perhaps more aggressive subgroup of STS compared to patients with metachronous metastasis. In conclusion, the survival of STS patients with synchronous metastasis only improved minimally from 1989 until 2014, whereby the need for novel treatment strategies once again is emphasized.

Lastly, in **chapter 13** it is investigated whether there is an association between pazopanib-induced toxicity and survival of patients with metastatic STS. This study was based on the hypothesis that the occurrence of toxicity is related to the anti-tumor activity of the drug, and that toxicity therefore could serve as a biomarker of efficacy. Such a biomarker is needed, because the response rate to pazopanib is low (<10%) and most patients therefore unnecessary receive this drug, along with its side-effects and toxicity. On the other side, there is also a small subgroup with an exceptionally good and long-lasting response to pazopanib. Three toxicities were studied: proteinuria, hypothyroidism and cardiotoxicity. There was no difference in progression-free survival or overall survival between patients with or without one of the toxicities. Therefore, these three toxicities cannot be used as a biomarker for pazopanib efficacy in patients with metastatic STS.





CHAPTER 16

SAMENVATTING

Samenvatting

Dit proefschrift bestaat uit vier delen. In **deel I** wordt gepoogd meer inzicht te krijgen in de biologie van wekedelen sarcomen, waarbij ook gezocht is naar nieuwe aangrijpingspunten voor therapie. In **deel II** is één van de meest voorkomende subtypes van het wekedelen sarcoom, het liposarcoom, onder de loep genomen. Dit omvat onder andere de diagnostiek en de uitkomsten van de verschillende subtypes en lokalisaties. **Deel III** evalueert de chirurgische behandeling van het gelokaliseerde wekedelen sarcoom, zowel op nationaal niveau voor alle wekedelen sarcomen als voor een kleine subgroep van patiënten behandeld in het Erasmus MC Kanker Instituut. Ten slotte focust **deel IV** zich op de evaluatie van de systemische behandeling van het gemetastaseerd wekedelen sarcoom. Eerst wordt er een overzicht van alle huidige therapieën gegeven, gevolgd door een evaluatie van de overleving ten gevolge van de veranderingen in de systemische behandeling.

Deel I – Biologie van wekedelen sarcomen

In **hoofdstuk 2** hebben we met behulp van Whole Genome Sequencing (WGS) de genomische afwijkingen in gemetastaseerd wekedelen sarcomen in kaart gebracht. Leiomyosarcomen hadden gemiddeld een hoger aantal mutaties (tumor mutational burden, TMB) dan gastro-intestinale stromale tumoren (GIST) en andere wekedelen sarcomen. Kataegis (kleine gebieden met hypermutaties) werd gezien in 35 van de 122 samples en chromothripsis (waarbij een chromosoom (deels) verpulverd wordt in kleine stukjes) in 24 samples. Alle bekende mutatiesignaturen, zoals beschreven in de 'Catalogue Of Somatic Mutations In Cancer' (COSMIC), waren aanwezig in de sarcomen en er waren geen duidelijke verschillen in bijdragende signaturen tussen de verschillende subtypes van de wekedelen sarcomen. In 86% van de patiënten werd een 'actionale target' geïdentificeerd, een genetische afwijking waarvoor reeds geregistreerde medicatie of een middel in ontwikkeling beschikbaar is. Voorbeelden ontdekt in de data zijn: sorafenib voor GIST met een *KIT* mutatie, trastuzumab voor leiomyosarcomen met *ERBB2* mutatie, imatinib voor een angiosarcoom met een *KIT* mutatie en fulvestrant voor leiomyosarcoom en endometrium stromacel sarcoom met een *ESR1* mutatie. Verder waren er 6 patiënten met een TMB ≥ 10 die mogelijk baat kunnen hebben van behandeling met checkpoint inhibitors. Hiermee geeft deze studie een belangrijk inzicht in de biologie van het wekedelen sarcoom en laat zien dat WGS een waardevol instrument is om klinisch relevante moleculaire afwijkingen te identificeren. Daarmee kan WGS de behandeling en uitkomsten van patiënten verbeteren, zelfs of misschien juist nadat patiënten al meerdere lijnen therapie gehad hebben.

Hoofdstuk 3 beschrijft de associatie tussen de overexpressie van 2 microRNA's, miR-26a en miR-3913, en de progressie van goedgedifferentieerde en gedifferentieerde liposarcomen. MicroRNA's zijn kleine stukjes niet-coderend RNA die de expressie van andere

genen kunnen reguleren en beïnvloeden. Het goedgedifferentieerde en gedifferentieerde liposarcoom worden beiden gekenmerkt door amplificatie van chromosoom 12q13-15, waarop ook zowel miR-26a als miR-3913 gelokaliseerd zijn. Beide microRNA's bleken tot overexpressie te komen in goedgedifferentieerde en/of gedifferentieerde liposarcomen. Remming van miR-26a en miR-3913 leidde tot verminderde cellulaire proliferatie, waarbij remming van miR-3913 ook apoptose induceerde in goedgedifferentieerde liposarcoom-cellijnen. Daarbij leek miR-26a de tumor suppressor *PTEN* te reguleren. Concluderend lijkt overexpressie van miR-26a en miR-3913, meest waarschijnlijk veroorzaakt door de kenmerkende amplificatie van 12q13-15, de ontwikkeling van liposarcomen te stimuleren door proliferatie te stimuleren en – in mindere mate – apoptose te remmen. Dit suggereert dat ook niet-eiwit coderende genen, zoals microRNA's, een rol kunnen spelen in de ontwikkeling van goedgedifferentieerde en gedifferentieerde liposarcomen.

In **hoofdstuk 4** hebben we tumor samples van primaire goedgedifferentieerde liposarcomen vergeleken met samples van recidief goedgedifferentieerde liposarcomen. Dit waren gepaarde samples, wat inhoudt dat de samples van de primaire en recidief liposarcomen afkomstig van dezelfde patiënten waren. Dit hebben we gedaan op microRNA en DNA methylatie niveau, waarbij DNA methylatie een epigenetisch proces is waarmee de expressie van genen gereguleerd kan worden. Het doel was om verschillen, en daarmee processen betrokken bij recidivering, te ontdekken. Echter bleek dat er geen onderscheid gemaakt kon worden tussen primaire en recidief goedgedifferentieerde liposarcomen op basis van microRNA expressie en DNA methylatie patronen. De verschillen, met name in DNA methylatie patronen, waren erg heterogeen en variabel tussen de verschillende patiënten. Er konden geen gemeenschappelijke patronen geïdentificeerd worden, en daarmee ook geen aanwijzingen naar een specifiek proces dat betrokken zou zijn bij de recidivering van deze tumoren.

Deel II – Heterogeniteit binnen de liposarcomen

In dit deel worden de liposarcomen verder onder de loep genomen. In **hoofdstuk 5** hebben we gezocht naar een non-invasieve methode om goedgedifferentieerde liposarcomen te kunnen onderscheiden van lipomen. Deze twee tumoren kunnen op een MRI-scan er nagenoeg hetzelfde uitzien en daarom wordt er vaak een invasief biopt afgenomen. Dit biopt wordt vervolgens door de patholoog bekeken om de juiste diagnose te kunnen stellen. Radiomics is een vorm van kunstmatige intelligentie waarbij beeldkarakteristieken uit een MRI-scan gelinkt kunnen worden aan pathologische kenmerken, zoals bijvoorbeeld de mutatiestatus. In dit geval is dat amplificatie van het *MDM2*-gen: dit is aanwezig in goedgedifferentieerde liposarcomen, maar afwezig in lipomen. Het radiomics model gebaseerd op de T1-gewogen MRI-scans scoorde een gemiddelde AUC-waarde van 0.83,

een sensitiviteit van 0.68 en een specificiteit van 0.84. Deze scores zijn vergeleken met de scores van 3 radiologen. Respectievelijk scoorden zij een AUC van 0.74/0.72/0.61, sensitiviteit van 0.74/0.91/0.64 en een specificiteit van 0.55/0.36/0.59. Hieruit concludeerden we dat radiomics een veelbelovende en non-invasieve methode is om goedgedifferentieerde liposarcomen van lipomen te onderscheiden. Echter is verdere optimalisatie en validatie nodig is voordat het radiomics model bruikbaar is in de dagelijkse klinische praktijk.

Hoofdstuk 6 evalueert de rol van tumorlokalisatie in de lokaal recidief-vrije overleving, afstandsmetastase-vrije overleving en ziekte-specifieke overleving van patiënten met een liposarcoom. Hieruit bleek dat patiënten met een liposarcoom dat retroperitoneaal, scrotaal of intra-thoracaal gelokaliseerd was vaker een lokaal recidief ontwikkelden dan patiënten met een liposarcoom in één van de ledematen, de romp of het hoofd-halsgebied. Er werden geen verschillen gevonden in de ontwikkeling van afstandsmetastasen tussen de verschillende tumorlokalisaties. Patiënten met een retroperitoneaal of intra-thoracaal liposarcoom hadden een slechtere ziekte-specifieke overleving, ondanks dat deze patiënten even vaak metastasen ontwikkelden. Waar de meeste kankerpatiënten overlijden aan gemetastaseerde ziekte, suggereert deze data, in combinatie met het vaker ontwikkelen van een lokaal recidief, dat patiënten met een retroperitoneaal/intra-thoracaal liposarcoom overlijden aan lokale ziekte. Mogelijk moet de behandeling voor het liposarcoom per tumor lokalisatie aangepast worden.

In **hoofdstuk 7** wordt nader ingegaan op de liposarcomen gelokaliseerd in een ledemaat, de meest voorkomende lokalisatie van het liposarcoom. Er waren duidelijke verschillen in recidivering en overleving tussen de vier liposarcoom subtypes. Verder bleek dat bij patiënten met een goedgedifferentieerd liposarcoom een agressievere behandeling – bestaande uit ruime resectie en (neo)adjuvante radiotherapie – tot uitstekende lokale controle leidde, terwijl een meer conservatieve behandeling – met een marginale resectie zonder (neo)adjuvante therapie – resulteerde in meer lokale recidieven. Echter bleek ook dat dit uiteindelijk geen effect had op de ziekte-specifieke overleving van deze patiënten. De voordelen van ruime resectie en radiotherapie dienen daarom zorgvuldig afgewogen tegen de nadelen en toxiciteit hiervan.

Deel III – Chirurgische behandeling van het gelokaliseerde wekedelen sarcoom

Naar aanleiding van de resultaten uit hoofdstuk 7, zijn de behandeling en uitkomsten van patiënten met een goedgedifferentieerd liposarcoom in een ledemaat nader onderzocht in **hoofdstuk 8**. Het bleek dat deze patiënten maar zelden afstandsmetastasen ontwikkelden en zelden overleden aan de ziekte. Dit gebeurde alleen wanneer de patiënt een lokaal recidief met daarin dedifferentiatie had ontwikkeld, wat sporadisch voorkwam. Aan de andere kant

bleek ook dat er patiënten overleden waren aan de behandeling. Daarop vroegen we ons af of we deze patiënten niet aan het overbehandelen zijn. Met deze reden hebben we een kleine groep patiënten geanalyseerd die onder controle werden gehouden zonder actieve behandeling (active surveillance). Hieruit kon geconcludeerd worden dat active surveillance in bepaalde patiënten een goede en gerechtvaardigde behandeloptie lijkt te zijn. Dit geldt met name voor oudere patiënten, patiënten met (meerdere) comorbiditeiten en/of patiënten waarbij de tumor ongunstig gelokaliseerd ligt waardoor een radicale resectie niet haalbaar is. Echter is de follow-up van deze groep patiënten nog kort en dient de haalbaarheid en veiligheid van active surveillance als behandeling voor non-retroperitoneale goedgedifferentieerde liposarcomen nog op grotere schaal in een prospectieve studie uitgezocht te worden.

Hoofdstuk 9 van dit proefschrift richt zich op de centralisatie van wekedelen chirurgie in Nederland. Gezien de zeldzaamheid van wekedelen sarcomen, wordt er geschat dat een huisarts maar 1x in de 20 jaar een patiënt met een wekedelen sarcoom ziet en een algemeen chirurg in een perifere ziekenhuis 1x in de 4 jaar. De zeldzaamheid, in combinatie met het bestaan van vele verschillende subtypes en het feit dat goedaardige wekedelen tumoren 100x zo vaak voorkomen, maakt het diagnosticeren en behandelen van wekedelen sarcomen zeer uitdagend. Dit roept logischerwijs de noodzaak tot centralisatie op en daarom zijn in Nederland een aantal expertisecentra aangewezen. Dit onderzoek liet zien dat de centralisatie van wekedelen chirurgie verbeterd was in de afgelopen 10 jaar, alhoewel er nog steeds veel ruimte voor verbetering is. Daarnaast zagen we dat patiënten met een hooggradig en diep-gelegen sarcoom een betere overleving hadden wanneer ze geopereerd waren in een hoog-volume ziekenhuis (≥ 20 operaties per jaar) ten opzichte van patiënten die in een laag-volume ziekenhuis geopereerd waren (< 10 operaties per jaar). Verder vonden er minder vaak ongeplande resecties en re-resecties plaats in hoog-volume ziekenhuizen. Derhalve pleiten wij voor verdere centralisatie van de zorg voor wekedelen sarcomen.

In **hoofdstuk 10** worden de ongeplande resecties verder uitgelicht met data uit de Nederlandse Kankerregistratie. Gezien goedaardige wekedelen tumoren veel vaker voorkomen dan kwaadaardige wekedelen sarcomen, kan het tijdig herkennen van een sarcoom lastig zijn. Het is niet ongebruikelijk dat een wekedelen sarcoom initieel voor een goedaardige tumor wordt aangezien en verwijderd wordt zonder adequate diagnostiek voorafgaand aan de operatie en zonder de juiste chirurgische marges. Deze ongeplande sarcoomresecties worden daarom ook wel 'whoops' resecties genoemd. In 17% van alle primaire sarcoom operaties was er sprake van een ongeplande resectie. Deze wekedelen sarcomen waren over het algemeen kleiner (≤ 5 cm), oppervlakkig gelegen, gelokaliseerd in de arm en er was vaker geen beeldvorming verricht voorafgaand aan de operatie. Na de meeste ongeplande resecties was er sprake van resterende ziekte. De patiënten moesten vaker nogmaals geopereerd worden of radiotherapie ondergaan. Ook werden patiënten

na een ongeplande resecties vaker verwezen naar of besproken met een sarcoom expertisecentrum, met name als er resterende ziekte was. Om deze ongeplande resecties te voorkomen is meer onderwijs en bewustwording nodig. Echter zullen ongeplande resecties van oppervlakkige en kleine wekedelen sarcomen nooit helemaal voorkomen kunnen worden vanwege de hoge incidentie van goedaardige wekedelen tumoren en zijn deze resecties voor een deel 'all in the game'.

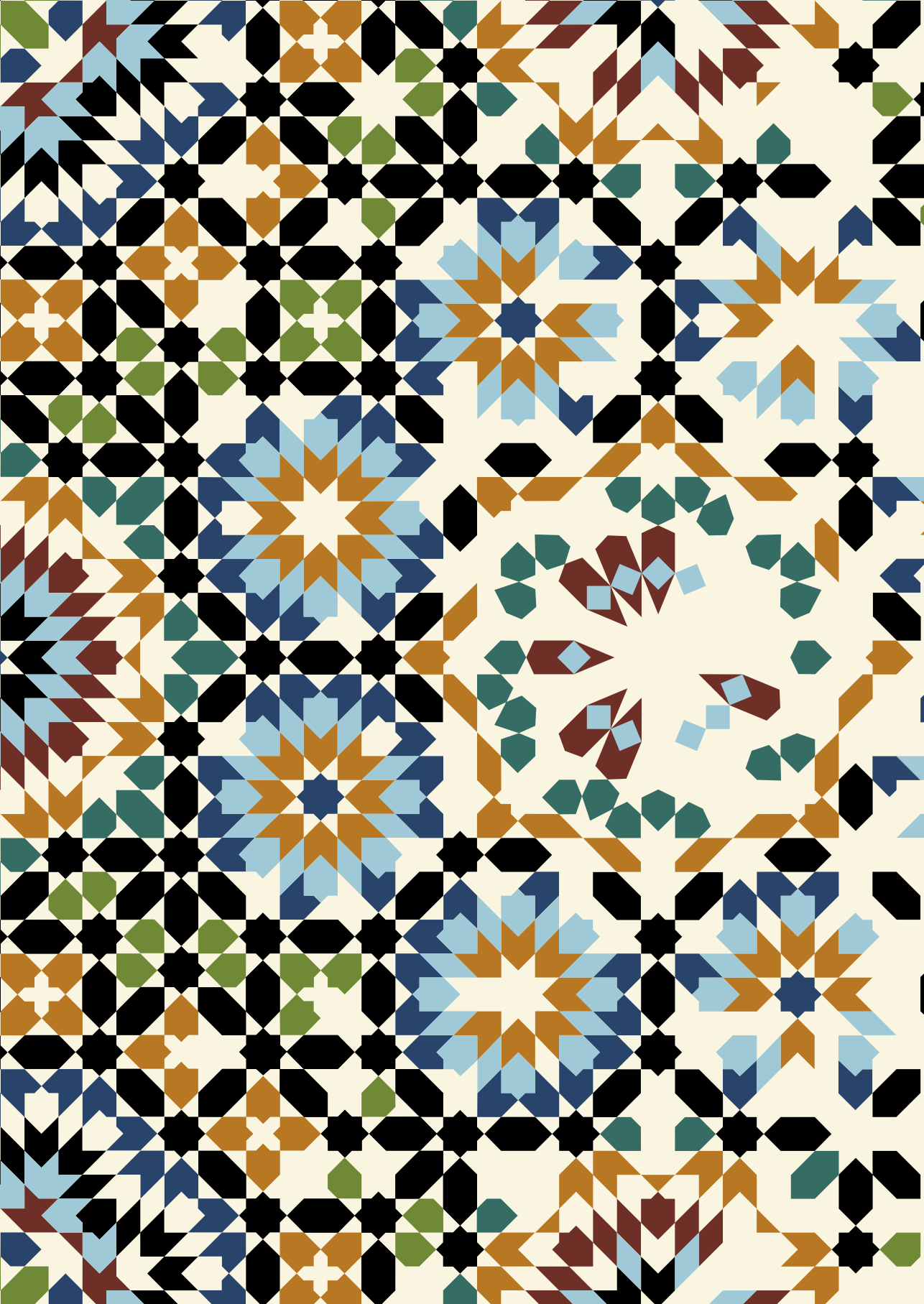
Deel IV – Systemische behandeling van het gemetastaseerde wekedelen sarcoom

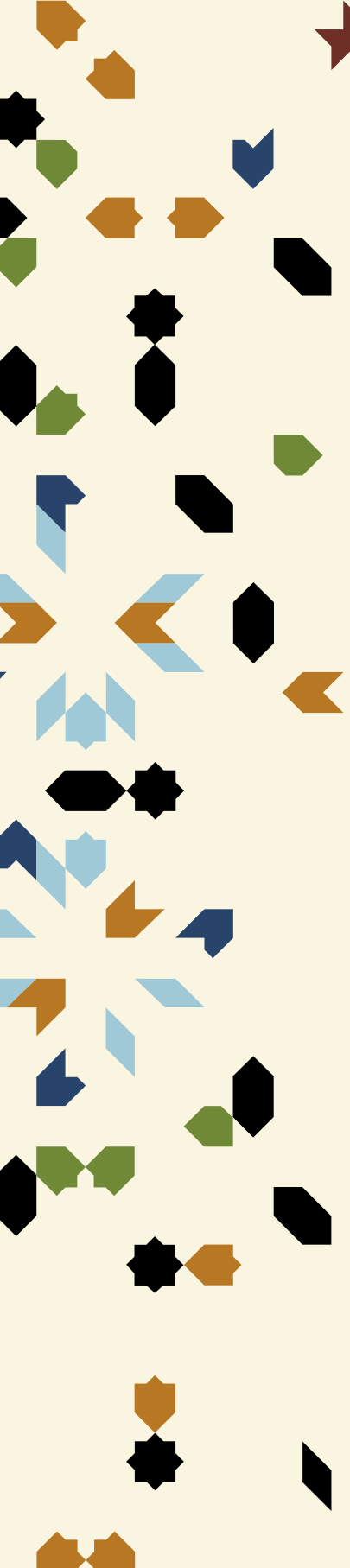
In **hoofdstuk 11** wordt een kort overzicht van zowel de huidige behandelstrategieën als nieuwe behandelingen die momenteel onderzocht worden voor het gemetastaseerd wekedelen sarcoom gegeven. Ondanks de heterogeniteit met betrekking tot de pathofysiologie en de wisselende gevoeligheid voor chemotherapie, is de eerstelijns therapie voor vrijwel alle subtypes van het wekedelen sarcoom hetzelfde. Deze bestaat uit doxorubicine, vaak als monotherapie, maar soms in combinatie met ifosfamide. Ten tijde van het schrijven van dit hoofdstuk was olaratumab (conditioneel) toegevoegd aan de eerstelijns therapie, gebaseerd op de resultaten van een fase II studie waarbij de algehele overleving bijna een jaar toenam. Echter zijn de eerste resultaten van de fase III trial inmiddels bekend; er was geen verschil in overleving tussen de groep met de combinatietherapie en de groep met doxorubicine monotherapie. Derhalve is de voorwaardelijke goedkeuring voor olaratumab inmiddels weer ingetrokken. In de tweede lijn wordt wel steeds vaker naar subtype-specifieke behandelingen gekeken. Voorbeelden zijn trabectedine in (myxoid) liposarcomen en leiomyosarcomen, eribuline in liposarcomen, taxanen in angiosarcomen en gemcitabine in leiomyosarcomen. Ondanks dat er steeds meer behandelopties bij zijn gekomen, blijft de overleving van patiënten met gemetastaseerd wekedelen sarcoom slecht en is onderzoek naar nieuwe therapieën nodig. Onder andere immunotherapieën – zoals ipilimumab, nivolumab of pembrolizumab – die reeds gebruikt worden bij andere kankersoorten, worden momenteel getest in patiënten met een wekedelen sarcoom.

Hoofdstuk 12 beschrijft de verandering in de overleving van patiënten met een wekedelen sarcoom dat bij diagnose reeds gemetastaseerd is (synchrone metastasen). Ongeveer 15% van de patiënten heeft bij diagnose al afstandsmetastasen, waarbij de voorkeurslokalisaties de longen, lever, botten en lymfeklieren waren. Deze patiënten hebben een slechte algehele overleving, mediaan ca. 6 maanden, al was er een verbeterende trend over de tijd zichtbaar. De mediane overleving van deze groep patiënten ligt een stuk lager dan de overleving die in andere klinische studies beschreven wordt (ca. 12-24 maanden), waarin verschillende chemotherapie-regimes onderzocht wordt. Hier zijn een aantal mogelijke verklaringen voor: 1) in deze studie zat ook een deel patiënten die helemaal geen

behandeling gehad hebben (niet mogelijk of eigen wens/keuze); 2) de patiënten in deze studie zijn waarschijnlijk minder fit dan de patiënten die meedoen aan een klinische studie, waarbij er vaak strenge in- en exclusiecriteria gehandhaafd worden; 3) in deze studie zitten alleen patiënten met synchrone metastasen, terwijl in de andere studies vooral patiënten zitten die pas later metastasen ontwikkeld hadden (metachrone metastasen). De patiënten die zich met synchrone metastasen presenteren, hebben mogelijk een agressievere vorm van het wekedelen sarcoom, en daardoor een slechtere prognose. Al met al is de overleving van patiënten met synchrone metastasen maar minimaal toegenomen van 1989 tot 2014, waarmee opnieuw het belang en de noodzaak van het ontwikkelen van nieuwe behandelstrategieën benadrukt wordt.

Tenslotte wordt er in **hoofdstuk 13** onderzocht of er een associatie bestaat tussen door pazopanib-geïnduceerde toxiciteit en de overleving van patiënten met gemetastaseerd wekedelen sarcoom. Deze onderzoeksvraag is gebaseerd op de hypothese dat het optreden van toxiciteit gerelateerd is aan de anti-tumor werking van het middel, en dat toxiciteit daarom gebruikt kan worden als biomarker voor de werkzaamheid van het middel. Een dergelijke biomarker is nodig, omdat het aantal patiënten dat respondeert op pazopanib heel laag ligt (<10%), en dus een groot gedeelte van de patiënten onnodig deze medicatie krijgt, inclusief bijwerkingen en toxiciteit. Echter is er ook een subgroep van patiënten die uitzonderlijk goed reageert op pazopanib en een langdurige respons heeft. In deze studie is gekeken naar 3 toxiciteiten: proteïnurie, hypothyreoïdie en cardiotoxiciteit. Er bleek geen verschil te zijn in progressie-vrije overleving of algehele overleving tussen patiënten met of zonder één van deze toxiciteiten. Deze toxiciteiten kunnen daarom niet gebruikt worden als biomarker voor de werkzaamheid van pazopanib in patiënten met gemetastaseerd wekedelen sarcoom.





APPENDICES

PHD PORTFOLIO

LIST OF PUBLICATIONS

LIST OF CONTRIBUTING

AUTHORS

ABOUT THE AUTHOR

DANKWOORD

PhD Portfolio

Name PhD student:	Melissa Vos
Department:	Medical Oncology and Surgical Oncology
Promotors:	Prof. dr. S. Sleijfer and prof. dr. C. Verhoef
Copromotors:	Dr. D.J. Grünhagen and dr. E.A.C. Wiemer
Research school:	MolMed
PhD period:	Jan 2016 – Jan 2020
Date thesis defense:	26-05-2021

Oral presentations (0.5 point each)	Year	ECTS
Medical Oncology Research Meeting	2017-2018	1.0
Chirurgendagen	2017, 2019	2.0
Annual scientific meeting dept. of Medical Oncology	2017, 2019	1.0
JNI meeting	2017	0.5
Regional IKNL meeting & symposium on sarcomas	2017	0.5
Erasmus MC Cancer Institute Research Day	2018	0.5
Symposium Experimenteel Onderzoek Heelkundige Specialisten (SEOHS)	2018	0.5
Annual Science day of the dept. of Surgery	2019	0.5
Poster presentations (0.5 point each)	Year	ECTS
Connective Tissue Oncology Society (CTOS)	2016, 2018	2.0
American Society of Clinical Oncology (ASCO)	2017	0.5
European Society for Medical Oncology (ESMO)	2017, 2019	1.5
European Society of Surgical Oncology (ESSO)	2019	0.5
Courses	Year	ECTS
Research Integrity	2016	0.3
BROK (Basiscursus Regelgeving en Organisatie voor Klinisch Onderzoekers)	2016	1.5
CPO (Course Patient Oriented research)	2016	0.3
Medical library courses in: Systematic Literature Retrieval in PubMed, Systematic Literature Retrieval in other databases and course in EndNote	2016	1.0
Annual course on Molecular Medicine	2016	0.3
Basic Introduction Course on SPSS	2016	1.0
Survival Analysis Course	2016	0.5
CC02 Biostatistical Methods I (NIHES)	2016	5.7
Basic and Translational Oncology course	2016	1.8
OpenClinica	2017	0.3
Workshop on Photoshop and Illustrator CS6	2017	0.3
Course on GraphPad Prism	2017	0.3
Course on 'R'	2017	1.8
Workshop 'Coachen van toekomstige Erasmusartsen Basis' (onderdeel Basis Kwalificatie Onderwijs) + vervoltraining 'Talentinterview'	2017	0.3
Course in English Biomedical Writing & Communication	2017-2018	3.0
Workshop on Presenting Skills	2018	1.0
Workshop on Bioconductor in 'R'	2018	0.3
Course on Epigenetic Regulation in Health and Disease	2018	0.8

Conferences (0.3 point/day)	Year	ECTS
CTOS, Lisbon, Portugal	2016	0.9
ASCO, Chicago, United States	2017	1.5
ESMO, Madrid, Spain	2017	1.5
ESSO, Budapest, Hungary	2018	0.9
CTOS, Rome, Italy	2018	0.9
ESMO, Barcelona, Spain	2019	1.5
ESSO, Rotterdam, the Netherlands	2019	0.9
Chirurgendagen, Veldhoven	2017, 2019	1.2
Daniel den Hoed day/Erasmus MC Cancer Institute Research Day	2016-2018	0.9
MolMed annual Molecular Medicine day	2016-2019	1.2
Teaching	Year	ECTS
Supervision master thesis (2x)	2016, 2018	2.0
Coaching of bachelor medical students	2017-2019	1.2
Tutor 'Kennismaking met de BeroepsPraktijk' (KBP)	2018-2019	0.6
Meetings and seminars	Year	ECTS
JNI meetings	2016-2020	1.0
JNI Oncology Lectures	2016-2020	1.0
Medical Oncology Research Meetings (MORM)	2016-2020	1.0
Surgical Journal Club of Clinical Oncology (SJOCO)	2016-2020	1.0
Sarcomen werkgroep (SWG)	2016-2020	1.0
Sarcoma Research Meetings	2016-2020	1.0
Research meetings 'Oncologische Gastro-intestinale Chirurgie' (OGC)	2018-2020	1.0
Other	Year	ECTS
Working visit Warsaw, Poland (1 week)	2016	1.5
Organizing Sarcoma Research Meetings	2016-2018	1.0
Basic Life Support examiner of medical students	2016-2019	1.0
Data monitor RISAS trial	2016-2020	1.0
Organizing monthly Medical Oncology Research Meetings	2017-2018	1.0
Prizes/grants	Year	ECTS
Travel grant of the René Vogels Stichting (€1,015)	2016	-
Grant of Stichting Coolsingel (€28,000)	2017	-
Best poster prize at ESMO 2017, Madrid, Spain	2017	-

List of publications

This thesis, in order of publication:

EJC's biennial report on metastatic soft tissue sarcoma: State of the art and future perspectives

M. Vos, S. Sleijfer.

Eur J Cancer. 2018 Jan;88:87-91.

Differences in recurrence and survival of extremity liposarcoma subtypes

M. Vos, H. Kosela-Paterczyk, P. Rutkowski, G.J.L.H. van Leenders, M. Normantowicz, A. Lecyk, S. Sleijfer, C. Verhoef, D.J. Grünhagen

Eur J Surg Oncol. 2018 Sep;44(9):1391-1397.

Increased survival of non low-grade and deep-seated soft tissue sarcoma after surgical management in high-volume hospitals: a nationwide study from the Netherlands

M. Vos, H.G.T. Blaauwgeers, V.K.Y. Ho, W.J. van Houdt, J.A. van der Hage, L.B. Been, J.J. Bonenkamp, M.H.A. Bemelmans, T. van Dalen, R.L. Haas, D.J. Grünhagen, C. Verhoef, on behalf of the Dutch Sarcoma Study Group (DSSG)

Eur J Cancer. 2019 Mar;110:98-106.

Association of pazopanib-induced toxicities with outcome of patients with advanced soft tissue sarcoma; a retrospective analysis based on the European Organisation for Research and Treatment of Cancer (EORTC) 62043 and 62072 clinical trials

M. Vos, S. Sleijfer, S. Litière, N. Touati, F. Duffaud, W.T. van der Graaf, H. Gelderblom

Acta Oncol. 2019 Jun;58(6):872-879.

Natural history of well-differentiated liposarcoma of the extremity compared to patients treated with surgery

M. Vos, D.J. Grünhagen, H. Kosela-Paterczyk, P. Rutkowski, S. Sleijfer, C. Verhoef

Surg Oncol. 2019 Jun; 29:84-89.

Minimal Increase in Survival Throughout the Years in Patients with Soft Tissue Sarcoma with Synchronous Metastases: Results of a Population-Based Study

M. Vos, V.K.Y. Ho, A.W. Oosten, C. Verhoef, S. Sleijfer

Oncologist. 2019 Jul;24(7):e526-e535.

Impact of primary tumor location on outcome of liposarcoma patients, a retrospective cohort study

M. Vos, W.C. Boeve, T.M. van Ginhoven, S. Sleijfer, C. Verhoef, D.J. Grünhagen

Eur J Surg Oncol. 2019 Dec;45(12):2437-2442.

Radiomics approach to distinguish between well differentiated liposarcomas and lipomas on MRI

M. Vos*, M.P.A. Starmans*, M.J.M. Timbergen, S.R. van der Voort, G.A. Padmos, W. Kessels, W.J. Niessen, G.J.L.H. van Leenders, D.J. Grünhagen, S. Sleijfer, C. Verhoef, S. Klein, J.J. Visser
Br J Surg. 2019 Dec;106(13):1800-1809.

MicroRNA expression and DNA methylation profiles do not distinguish between primary and recurrent well-differentiated liposarcoma

M. Vos, R. Boers, A.L. M. Vriends, J. Boers, P.F. van Kuijk, W.J. van Houdt, G.J.L.H. van Leenders, M. Wagrodzki, W.F.J. van Ijcken, J. Gribnau, D.J. Grünhagen, C. Verhoef, S. Sleijfer, E.A.C. Wiemer
PLoS One. 2020 Jan;15(1):e0228014.

Not in this thesis:

Differential diagnosis and molecular stratification of gastrointestinal stromal tumors on CT images using a radiomics approach

M.P.A. Starmans*, M.J.M. Timbergen*, **M. Vos**, M. Renckens, D.J. Grünhagen, G.J.L.H. van Leenders, R.S. Dwarkasing, W.J. Niessen, C. Verhoef, S. Sleijfer, J.J. Visser, S. Klein
Submitted.

Necrotizing enterocolitis as presenting symptom of SARS-CoV-2 infection in premature twin, a case report

M. Vos*, D.J. Timmermans*, J.H. Kreijen-Meinesz, J. W. Wieringa, L.H.P.M. Filippini
Submitted

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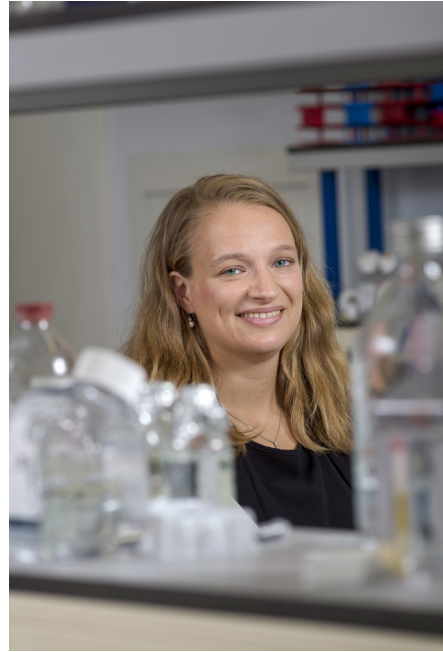
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About the author

Melissa Vos was born on September 18th 1991 in Schiedam, the Netherlands, where she also grew up. After graduating from high school (Stedelijk Gymnasium Schiedam) in 2009, she started medical school at the Erasmus University and Erasmus Medical University Center in Rotterdam. During her study, she participated in multiple committees and extra-curricular activities, and she worked in the student team of the department of Pediatric Oncology in the Erasmus MC Sophia Children's hospital. She performed her master research internship also at the department of Pediatric Oncology and was involved in basic research in the group of dr. Ronald Stam under supervision of dr. Eddy van Roon. The main focus of the project was on MLL-rearranged infant acute lymphoblastic leukemia and epigenetics. During her clinical rotations, she did an elective internship at the Hematology department of the Erasmus MC Cancer Institute and a senior internship at the Pediatrics department of the Sint Franciscus Gasthuis in Rotterdam, the Netherlands. Following her clinical rotations, she further developed interest in Pediatrics, but also in Oncology. After obtaining her MD degree in December 2015, she started her PhD at the departments of Medical Oncology and Surgical Oncology, ultimately leading to this thesis under the supervision of prof. dr. Stefan Sleijfer and prof. dr. Kees Verhoef. During her PhD, Melissa also supervised two medical students and their master theses, presented at multiple international congresses and organized several monthly research meetings. However, during her PhD, she never lost her interest in Pediatrics. She started working as a pediatrics resident (not in training) at the Haaglanden Medisch Centrum in the Hague and is currently working as a resident at the Pediatrics department in the Maasstad hospital in Rotterdam.



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De parallel met het mozaïek heeft niet alleen betrekking op de inhoud van dit proefschrift, maar ook op alle de mensen die belangrijk zijn geweest voor het tot stand komen van dit proefschrift. Zonder deze 'losse steentjes' was dit boekje er niet geweest en was mijn eigen mozaïek niet compleet geweest: "I am but a small shard in a grand mosaic." Daarom wil ik graag iedereen bedanken die op enige manier heeft bijgedragen aan mijn proefschrift, waarvan ik een aantal mensen in het bijzonder wil bedanken:

Allereerst, geachte hooggeleerde **prof. dr. S. Sleijfer** en **prof. dr. C. Verhoef**, beste Stefan en Kees, het was een voorrecht om jullie eerste gezamenlijke promovenda te zijn. Ook al was deze vorm van samenwerking nieuw, het bleek – in ieder geval in mijn ogen – al snel een gouden combinatie te zijn. Tevens was het ook een combinatie vol verrassingen waarvan ik nu soms nog steeds niet weet wat ik kan verwachten, met als toppunt vanuit het niets naar Polen 'gestuurd' worden. Ik zal het altijd stimulerende 'werkuh!' dat over de gang op A1 geroepen werd en de grappen tijdens de tumorwerkgroep missen. Noemenswaardig vind ik jullie kwaliteit om iedere onderzoeker zich op zijn/haar eigen manier te laten ontwikkelen (of in Stefans woorden: te laten 'fladderen'), zonder daarbij jullie eigen onderzoekstijl op te leggen. Ondanks de overvolle agenda's was er altijd ruimte voor overleg en had ik vaak binnen no-time respons op mijn e-mails en feedback op mijn manuscripten. Ik wil jullie beiden heel erg bedanken voor deze kans, jullie waardevolle begeleiding en fijne supervisie. Ik heb ontzettend veel van jullie geleerd.

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Geachte hooggeleerde en weledelzeergeleerde leden van de leescommissie, **prof. dr. A.J. Gelderblom**, **prof. dr. V.E.P.P. Lemmens** en **dr. A.C.H. de Vries**, hartelijk dank voor jullie expertise, tijd en het beoordelen van mijn proefschrift. Hooggeachte **overige commissieleden**, ook jullie hartelijk dank voor jullie tijd, expertise en bereidheid om in de oppositie plaats te nemen en met mij van gedachte te wisselen over de inhoud van dit proefschrift.

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