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

FILLING IN THE GAPS OF THE MOSAIC

Melissa Vos

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

FILLING IN THE GAPS OF THE MOSAIC

Melissa Vos

Printing of this thesis was financially supported by: the department of Medical Oncology of the Erasmus MC Cancer Institute, department of Surgery of the Erasmus MC University Medical Center, Erasmus University Rotterdam, Integraal Kankercentrum Nederland (IKNL), Chipsoft, ABN AMRO and Patiëntenplatform Sarcomen.

ISBN: 978-94-6416-437-4

Cover & Lay-out: Publiss | www.publiss.nl

Print: Ridderprint | www.ridderprint.nl

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

26-05-2021 om 10.30 uur door

Melissa Vos

geboren te Schiedam, Nederland

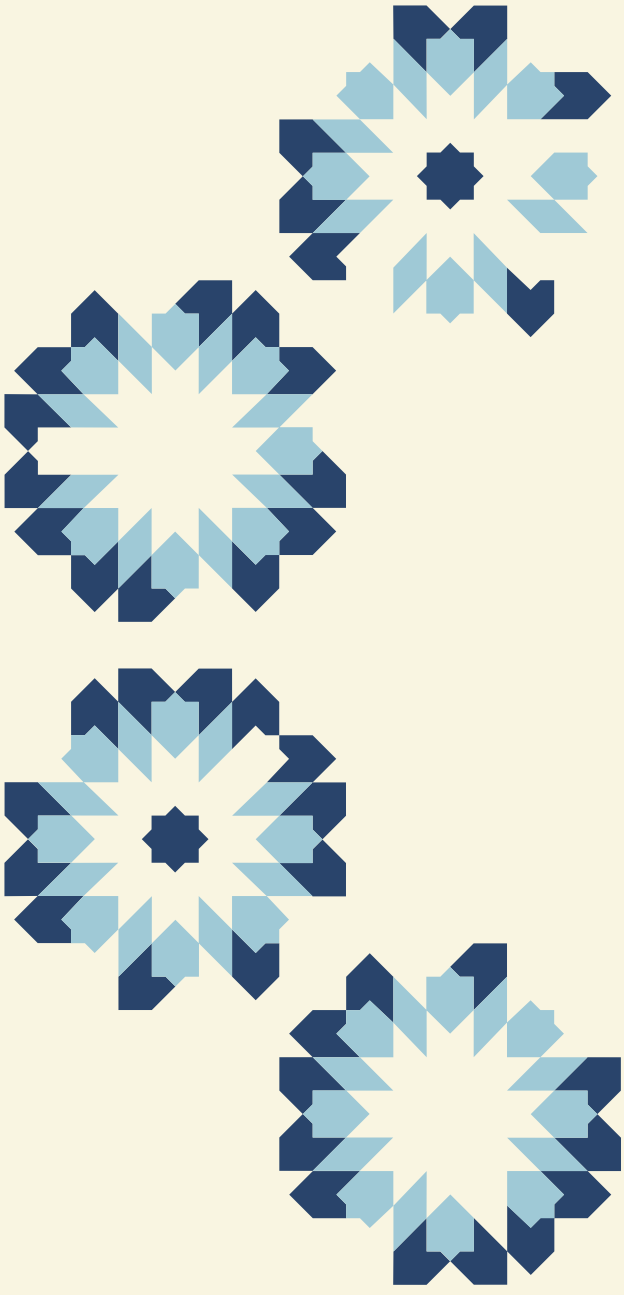
Erasmus University Rotterdam

Erasmus

Promotiecommissie

Promotoren	Prof. dr. S. Sleijfer Prof. dr. C. Verhoef
Overige leden	Prof. dr. A.J. Gelderblom Prof. dr. V.E.P.P. Lemmens Dr. A.C.H. de Vries
Copromotoren	Dr. D.J. Grünhagen Dr. E.A.C. Wiemer

Table of contents



CHAPTER 1

GENERAL INTRODUCTION AND OUTLINE OF THIS THESIS

General introduction

Clinical features

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 and DDLPS) 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.

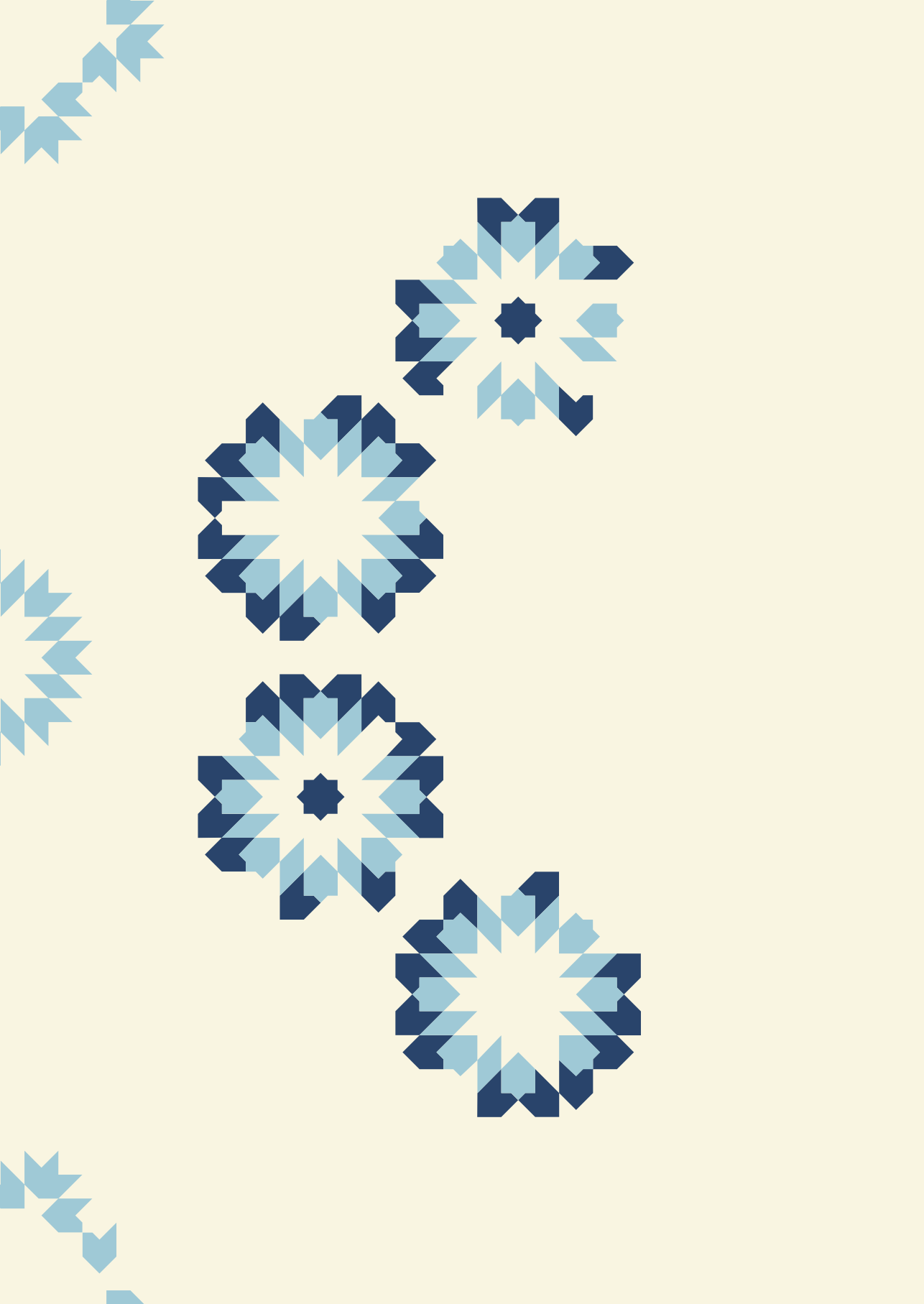
As outlined by this introduction, the mosaic theme reflects on multiple aspects of this thesis: the heterogeneity within the STS spectrum, the diversity of the subjects in this thesis and the variety of outcomes of the different chapters. Furthermore, the mosaic is still incomplete and the gaps have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

References

1. Fletcher CDM, Bridge JA, Hogendoorn PCW, Mertens F, World Health Organization, International Agency for Research on Cancer. WHO classification of tumours of soft tissue and bone. Lyon: IARC Press; 2013.
2. The Netherlands Cancer Registry. Dutch Cancer Figures (Cijfers over kanker). Nehterlands Comprehensive Cancer Organisation (IKNL); 1989-2018.
3. The Netherlands Comprehensive Cancer Registry. Bijlage D Deelrapportage voor wekedelensarcomen. The Netherlands Comprehensive Cancer Organisation (IKNL); 2014.
4. Yap J, Chuba PJ, Thomas R, Aref A, Lucas D, Severson RK, et al. Sarcoma as a second malignancy after treatment for breast cancer. Int J Radiat Oncol Biol Phys. 2002;52:1231-7.
5. Kim KS, Chang JH, Choi N, Kim H-S, Han I, Moon KC, et al. Radiation-Induced Sarcoma: A 15-Year Experience in a Single Large Tertiary Referral Center. Cancer research and treatment : official journal of Korean Cancer Association. 2016;48:650-7.
6. Mesri EA, Cesarman E, Boshoff C. Kaposi's sarcoma and its associated herpesvirus. Nature reviews Cancer. 2010;10:707-19.
7. Evans DG, Baser ME, McGaughran J, Sharif S, Howard E, Moran A. Malignant peripheral nerve sheath tumours in neurofibromatosis 1. J Med Genet. 2002;39:311-4.
8. Li FP, Fraumeni JF, Jr. Soft-tissue sarcomas, breast cancer, and other neoplasms. A familial syndrome? Ann Intern Med. 1969;71:747-52.
9. Trojani M, Contesso G, Coindre JM, Rouesse J, Bui NB, de Mascarel A, et al. Soft-tissue sarcomas of adults; study of pathological prognostic variables and definition of a histopathological grading system. Int J Cancer. 1984;33:37-42.
10. Committee EG, Euracan, Gronchi A, Frezza AM, Casali PG, De Álava E, et al. Soft tissue and visceral sarcomas: ESMO–EURACAN Clinical Practice Guidelines for diagnosis, treatment and follow-up†. Annals of Oncology. 2018;29:iv51-iv67.
11. Nijhuis PH, Schaapveld M, Otter R, Molenaar WM, van der Graaf WT, Hoekstra HJ. Epidemiological aspects of soft tissue sarcomas (STS)- Consequences for the design of clinical STS trials. Eur J Cancer. 1999;35:1705-10.
12. Coindre JM, Terrier P, Guillou L, Le Doussal V, Collin F, Ranchere D, et al. Predictive value of grade for metastasis development in the main histologic types of adult soft tissue sarcomas: a study of 1240 patients from the French Federation of Cancer Centers Sarcoma Group. Cancer. 2001;91:1914-26.
13. Chudgar NP, Brennan MF, Munhoz RR, Bucciarelli PR, Tan KS, D'Angelo SP, et al. Pulmonary metastasectomy with therapeutic intent for soft-tissue sarcoma. J Thorac Cardiovasc Surg. 2017;154:319-30 e1.
14. Billingsley KG, Burt ME, Jara E, Ginsberg RJ, Woodruff JM, Leung DH, et al. Pulmonary metastases from soft tissue sarcoma: analysis of patterns of diseases and postmetastasis survival. Ann Surg. 1999;229:602-10; discussion 10-2.
15. van Geel AN, Pastorino U, Jauch KW, Judson IR, van Coevorden F, Buesa JM, et al. Surgical treatment of lung metastases: The European Organization for Research and Treatment of Cancer-Soft Tissue and Bone Sarcoma Group study of 255 patients. Cancer. 1996;77:675-82.
16. Choong PF, Pritchard DJ, Rock MG, Sim FH, Frassica FJ. Survival after pulmonary metastasectomy in soft tissue sarcoma. Prognostic factors in 214 patients. Acta Orthop Scand. 1995;66:561-8.
17. Judson I, Verweij J, Gelderblom H, Hartmann JT, Schoffski P, Blay JY, et al. Doxorubicin alone versus intensified doxorubicin plus ifosfamide for first-line treatment of advanced or metastatic soft-tissue sarcoma: a randomised controlled phase 3 trial. Lancet Oncol. 2014;15:415-23.

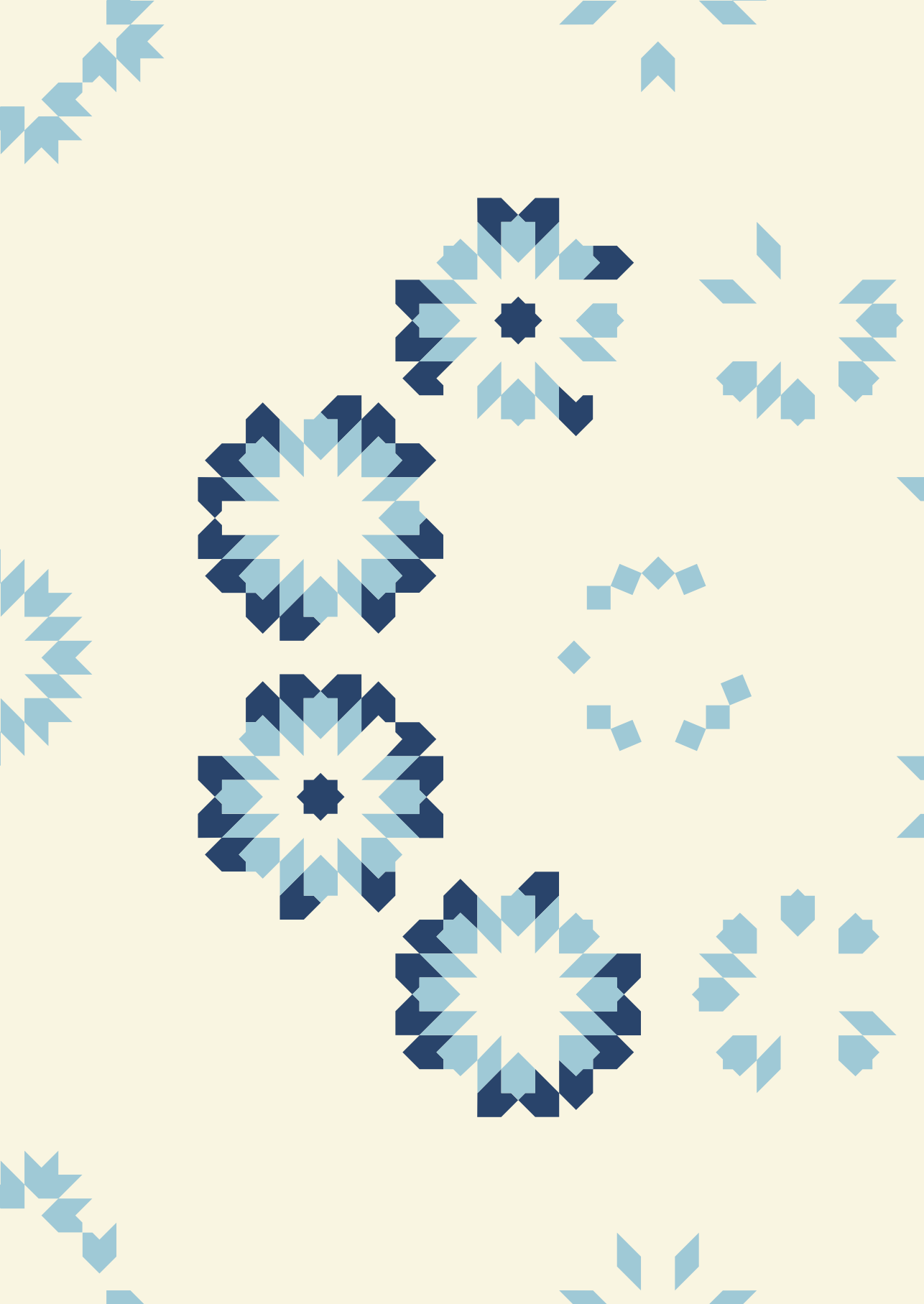
18. Tap WD, Jones RL, Van Tine BA, Chmielowski B, Elias AD, Adkins D, et al. Olaratumab and doxorubicin versus doxorubicin alone for treatment of soft-tissue sarcoma: an open-label phase 1b and randomised phase 2 trial. Lancet. 2016;388:488-97.
19. Seddon B, Strauss SJ, Whelan J, Leahy M, Woll PJ, Cowie F, et al. Gemcitabine and docetaxel versus doxorubicin as first-line treatment in previously untreated advanced unresectable or metastatic soft-tissue sarcomas (GeDDiS): a randomised controlled phase 3 trial. Lancet Oncol. 2017;18:1397-410.
20. Ryan CW, Merimsky O, Agulnik M, Blay JY, Schuetze SM, Van Tine BA, et al. PICASSO III: A Phase III, Placebo-Controlled Study of Doxorubicin With or Without Palifosfamide in Patients With Metastatic Soft Tissue Sarcoma. J Clin Oncol. 2016;34:3898-905.
21. Tap WD, Papai Z, Van Tine BA, Attia S, Ganjoo KN, Jones RL, et al. Doxorubicin plus evofosfamide versus doxorubicin alone in locally advanced, unresectable or metastatic soft-tissue sarcoma (TH CR-406/SARC021): an international, multicentre, open-label, randomised phase 3 trial. Lancet Oncol. 2017;18:1089-103.
22. Tap WD, Wagner AJ, Schöffski P, Martin-Broto J, Krarup-Hansen A, Ganjoo KN, et al. Effect of Doxorubicin Plus Olaratumab vs Doxorubicin Plus Placebo on Survival in Patients With Advanced Soft Tissue Sarcomas: The ANNOUNCE Randomized Clinical Trial. Jama. 2020;323:1266-76.
23. Demetri GD, Chawla SP, von Mehren M, Ritch P, Baker LH, Blay JY, et al. Efficacy and safety of trabectedin in patients with advanced or metastatic liposarcoma or leiomyosarcoma after failure of prior anthracyclines and ifosfamide: results of a randomized phase II study of two different schedules. J Clin Oncol. 2009;27:4188-96.
24. Demetri GD, von Mehren M, Jones RL, Hensley ML, Schuetze SM, Staddon A, et al. Efficacy and Safety of Trabectedin or Dacarbazine for Metastatic Liposarcoma or Leiomyosarcoma After Failure of Conventional Chemotherapy: Results of a Phase III Randomized Multicenter Clinical Trial. J Clin Oncol. 2016;34:786-93.
25. Grosso F, Jones RL, Demetri GD, Judson IR, Blay JY, Le Cesne A, et al. Efficacy of trabectedin (ecteinascidin-743) in advanced pretreated myxoid liposarcomas: a retrospective study. Lancet Oncol. 2007;8:595-602.
26. Schoffski P, Chawla S, Maki RG, Italiano A, Gelderblom H, Choy E, et al. Eribulin versus dacarbazine in previously treated patients with advanced liposarcoma or leiomyosarcoma: a randomised, open-label, multicentre, phase 3 trial. Lancet. 2016;387:1629-37.
27. Sleijfer S, Ray-Coquard I, Papai Z, Le Cesne A, Scurr M, Schoffski P, et al. Pazopanib, a multikinase angiogenesis inhibitor, in patients with relapsed or refractory advanced soft tissue sarcoma: a phase II study from the European organisation for research and treatment of cancer-soft tissue and bone sarcoma group (EORTC study 62043). J Clin Oncol. 2009;27:3126-32.
28. van der Graaf WT, Blay JY, Chawla SP, Kim DW, Bui-Nguyen B, Casali PG, et al. Pazopanib for metastatic soft-tissue sarcoma (PALETTE): a randomised, double-blind, placebo-controlled phase 3 trial. Lancet. 2012;379:1879-86.
29. Maki RG, Wathen JK, Patel SR, Priebat DA, Okuno SH, Samuels B, et al. Randomized phase II study of gemcitabine and docetaxel compared with gemcitabine alone in patients with metastatic soft tissue sarcomas: results of sarcoma alliance for research through collaboration study 002 [corrected]. J Clin Oncol. 2007;25:2755-63.
30. Garcia-Del-Muro X, Lopez-Pousa A, Maurel J, Martin J, Martinez-Trufero J, Casado A, et al. Randomized phase II study comparing gemcitabine plus dacarbazine versus dacarbazine alone in patients with previously treated soft tissue sarcoma: a Spanish Group for Research on Sarcomas study. J Clin Oncol. 2011;29:2528-33.

31. Penel N, Bui BN, Bay JO, Cupissol D, Ray-Coquard I, Piperno-Neumann S, et al. Phase II trial of weekly paclitaxel for unresectable angiosarcoma: the ANGIOTAX Study. J Clin Oncol. 2008;26:5269-74.
32. Mir O, Brodowicz T, Italiano A, Wallet J, Blay J-Y, Bertucci F, et al. Safety and efficacy of regorafenib in patients with advanced soft tissue sarcoma (REGOSARC): a randomised, double-blind, placebo-controlled, phase 2 trial. The Lancet Oncology. 2016;17:1732-42.
33. Brodowicz T, Mir O, Wallet J, Italiano A, Blay JY, Bertucci F, et al. Efficacy and safety of regorafenib compared to placebo and to post-cross-over regorafenib in advanced non-adipocytic soft tissue sarcoma. Eur J Cancer. 2018;99:28-36.
34. Robbins PF, Kassim SH, Tran TL, Crystal JS, Morgan RA, Feldman SA, et al. A pilot trial using lymphocytes genetically engineered with an NY-ESO-1-reactive T-cell receptor: long-term follow-up and correlates with response. Clin Cancer Res. 2015;21:1019-27.
35. Somaiah N, Chawla SP, Block MS, Morris JC, Do KT, Kim JW, et al. Immune response, safety, and survival impact from CMB305 in NY-ESO-1+ recurrent soft tissue sarcomas (STS). Journal of Clinical Oncology. 2017;35:11006-.
36. Tawbi HA, Burgess M, Bolejack V, Van Tine BA, Schuetze SM, Hu J, et al. Pembrolizumab in advanced soft-tissue sarcoma and bone sarcoma (SARC028): a multicentre, two-cohort, single-arm, open-label, phase 2 trial. Lancet Oncol. 2017.
37. D'Angelo SP, Mahoney MR, Van Tine BA, Atkins J, Milhem MM, Jahagirdar BN, et al. Nivolumab with or without ipilimumab treatment for metastatic sarcoma (Alliance A091401): two open-label, non-comparative, randomised, phase 2 trials. Lancet Oncol. 2018;19:416-26.



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.

Abstract

In total, 122 prospectively collected metastatic soft tissue sarcoma (mSTS) biopsies, including gastrointestinal stromal tumors (mGIST, N=40), were analyzed by whole genome sequencing. Our data revealed the in-depth genomic landscape, the occurrence of kataegis and chromothripsis, mutational signatures, the presence of known and novel putative driver genes and fusion genes as well as targetable genomic alterations for improvement of personalized treatment. In all of the mGIST (including *KIT*) and in 78% of the non-GIST mSTS samples, ≥1 actionable target could be identified. Six patients with a high tumor mutational burden (≥10 mutations/Mb) were found, whom could benefit from targeted therapy.

Introduction

Soft tissue sarcomas (STS) form a group of rare and heterogeneous tumors of mesenchymal origin, comprising approximately 1% of all adult malignancies. STS consists of over 50 different subtypes, with each subtype harboring distinct pathological features and clinical behavior. STS subtypes include tumors with benign, intermediary malignant (i.e. locally aggressive) and malignant characteristics with only the latter group metastasizing. The etiology, cell of origin and biological mechanisms underlying the different STS subtypes are only partly understood, with incomplete knowledge of the pathological genomic alterations that drives these cancers [1-3]. Gastrointestinal stromal tumors (GIST), leiomyosarcomas (LMS) and liposarcomas are three of the most frequently observed STS subtypes [4]. The rarity and heterogeneity of STS hampers effective clinical trials to investigate novel treatment strategies and in-depth biological studies.

Although GISTs are part of the STS spectrum, the histopathology, treatment and prognosis of GIST patients are very different from those with other STS subtypes. The majority of GISTs (80%) harbor a gain-of-function mutation in the receptor tyrosine kinase *KIT*, followed by a smaller subgroup with a gain-of function mutation in *PDGFRA* (±10%) [4-6]. The remaining GISTs are called 'wild-type' GISTs, although mutations in *BRAF*, *SDH* subunits and *NF1* have been discovered in this subgroup [7]. In case of advanced/metastatic disease, imatinib is the current standard first-line treatment [8], directly inhibiting the kinase activity of KIT, followed by sunitinib in second line [9] and regorafenib in third line [10]. Patients with *PDGFRA*-mutated GIST, especially those with an exon 18 D842V mutation, are generally insensitive to imatinib [11-13] and most probably to sunitinib[14] as well. Overall, patients with metastatic GIST (mGIST) have a relatively favorable prognosis, with an overall survival of median 51-57 months but eventually almost all patients develop resistant disease [15-18].

Leiomyosarcomas (LMS) comprise approximately 10 % of all STS and are malignant tumours of smooth muscle origin. LMS are frequently found associated with the uterine tissues, the retroperitoneum and large blood vessels. At a genomic level, LMS exhibit a complex karyotype with variable copy-number alterations involving multiple chromosomes, few point mutations and widespread TP53 and RB1 inactivation [1, 2].

In contrast to GIST, in the remaining soft tissue sarcoma subtypes, no clinically relevant targets for treatment have been revealed yet. Despite the heterogeneity amongst these other STS subtypes, almost all patients with metastatic STS (mSTS) are treated similarly with doxorubicin-based regimens as first-line treatment [19]. Although multiple lines of systemic treatment are currently available, the prognosis of mSTS remains poor, with a median overall survival of approximately 12-18 months [20-24]. Multiple trials exploring different regimens of 'classic' chemotherapy have been conducted in the last decades, but did not result in an improved outcome [21-24]. Additionally, multiple new agents also did not or only marginally

improved outcome, including immunotherapies and targeted therapies – targeting *PDGFRA* (olaratumab) [25] and/or *VEGF* receptors (pazopanib)[26] for example.

The outcome of these trials further highlights the need to better understand the biology of these rare tumors and to identify novel therapeutic targets. Whole genome sequencing (WGS) is increasingly used to characterize tumors in order to better understand tumor biology and to find new targets and effective therapies. However, in the rare series described so far, only WGS data from predominantly primary tumors are available, while the characteristics of metastases can differ substantially from the characteristics of the primary tumors. The aim of the current study is to describe the genomic landscape by WGS of metastatic lesions from 122 metastatic STS patients and to reveal targetable genomic alterations.

Results

Study cohort and sequencing characteristics

In the context of the CPCT-02 study (NCT01855477) tumor biopsies and matched peripheral blood of 122 patients with metastatic STS (mSTS) were collected in different Dutch hospitals (Supplementary Figure 1A). The patient cohort included slightly more males (N=65, 53%) than females (N=57, 47%) with a median age at time of biopsy of 61 and 58 years, respectively (Table 1, Supplementary Figure 1E). Biopsies were of metastatic GIST (N=40), leiomyosarcoma (LMS, N=28), liposarcoma (N=10), rhabdomyosarcoma (N=8), angiosarcoma (N=7), fibrosarcoma (N=7), sarcoma NOS (N=7), myofibroblastic sarcoma (N=4), synovial sarcoma (N=4), malignant peripheral nerve sheath tumor (MPNST, N=2), clear cell sarcoma (N=1), endometrial stromal cell sarcoma (N=1), atypical fibroxanthoma (N=1), pleomorphic dermal sarcoma (N=1) and a spindle cell sarcoma (N=1) (Table 1, Supplementary Figure 1D). The biopsies were most frequently taken from the abdomen/intra-abdominal cavity (N=40), liver (N=20), trunk (N=15) and the lungs (N=12) (Table 1). The tumor biopsies had a median sequencing coverage of 103 (interquartile range [IQR] 94-111), while the matched peripheral blood samples were sequenced at a mean read coverage of 38 (IQR 34-42) (Table 1, Supplementary Figure 1C). The median estimated tumor cell contents of the biopsies was 78% (IQR 57-89) (Supplementary Figure 1B).

The genomic landscape of metastatic soft tissue sarcoma

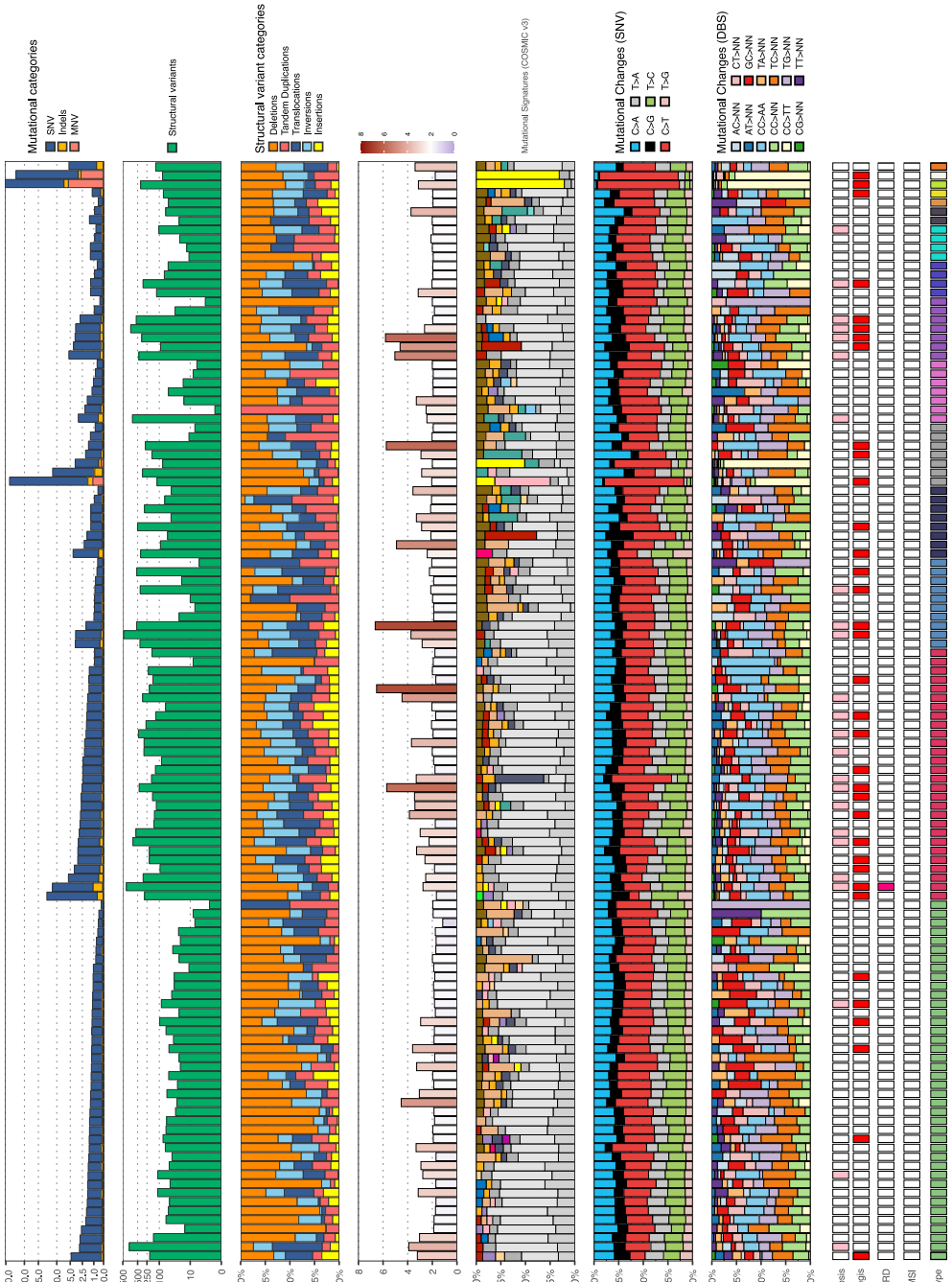
In all metastatic biopsies, a median of 3,958 single nucleotide variants (SNVs, IQR 2,686-6,960), 382 insertions and deletions (InDels, IQR 279-557), 26 multiple nucleotide variants (MNVs, IQR 16-46) and 71.5 structural variants (SVs, IQR 28-210) were observed. The median genome-wide tumor mutational burden (TMB) was 1.54 mutations per megabase (Mb)(IQR 1.06-2.63).

Table 1. Patient and tumor characteristics

		N	%
Sex	Male	65	53.3
	Female	57	46.7
Age at time of biopsy, years*		58 (49-68)	
STS subtype	GIST	40	32.8
	Leiomyosarcoma	28	23.0
	Liposarcoma	10	8.2
	Rhabdomyosarcoma	8	6.6
	Angiosarcoma	7	5.7
	Fibrosarcoma	7	5.7
	Sarcoma NOS	7	5.7
	Myofibroblastic sarcoma	4	3.3
	Synovial sarcoma	4	3.3
	MPNST	2	1.6
	Clear cell sarcoma	1	0.8
	Endometrial stromal cell sarcoma	1	0.8
	Atypical fibroxanthoma‡	1	0.8
	Pleomorphic dermal sarcoma	1	0.8
	Spindle cell sarcoma	1	0.8
Biopsy site	Abdominal	40	32.8
	Liver	20	16.4
	Trunk	15	12.3
	Lung	12	9.8
	Primary tumor	12	9.8
	Cutaneous/subcutaneous	6	4.9
	Bone	4	3.3
	Lymph node	4	3.3
	Head & neck	4	3.3
	Extremity	2	1.6
	CNS	1	0.8
	Unknown	2	1.6

*Presented as median (interquartile range). ‡Malignantly transformed into pleomorphic sarcoma NOS. GIST, gastrointestinal stromal tumor; NOS. not otherwise specified; MPNST, malignant peripheral nerve sheath tumor; CNS, central nervous system.

The overall mutational landscape distinguished mGIST (N=40) from the other mSTS (N=82) as mGIST displayed a lower TMB with a median of 1.31 somatic mutations per Mb (IQR 1.11-1.67) (Supplementary Figure 8B). For comparative reasons and taking cohort sizes into account we subdivided the remaining mSTS cohort into mLMS (N=28) and mSTS-other (N=54). The mLMS presented with a median TMB of 2.44 (IQR 1.88-3.05) and the mSTS-other with a median TMB of 1.37 (IQR 0.92-3.12)(Supplementary Figure 8B). Six of of the mSTS cases had a TMB ≥10 mutations per Mb: two LMS, two angiosarcomas, one pleomorphic


Mutational categories
SNV
Indels
MNV
Mutations per Mb
(Genome-wide; log10)
Structural variants
Structural variant categories
Deletions
Tandem Duplications
Translocations
Inversions
Insertions
Structural variants
Fill; categories
Mean ploidy
(Genome-wide)
Mutational Signatures (COSMIC v3)
Mut. Signatures
(Genome-wide)
Mutational Changes (SNV)
C>A
C>G
C>T
T>A
T>C
T>G
SNV Changes
(Relative frequency)
Mutational Changes (DBS)
AC>NN
AT>NN
CC>NN
CT>NN
TC>NN
TG>NN
TT>NN
CG>NN
DBS Changes
Chromothripsis
Kataegis
HRD
MSI
Subtype
Mutational Signatures (COSMIC v3)
Deamination of 5-methylcytosine (Sig. 1)
APOBEC activity (Sig. 2 & 13)
Defective HR; Mutated BRCA1/2 mutation (Sig. 3)
Tobacco smoking (Sig. 4)
Putative clock-like (Sig. 5)
Defective DNA MMR (Sig. 6, 15, 21, 26, 44)
Ultraviolet light exposure (Sig. 7a-d)
Polymerase epsilon activity (Sig. 9)
Alkylating agents (Sig. 11)
5-Fluorouracil (Sig. 17a-b)
Reactive oxygen species (Sig. 18)
Aristolochic acid exposure (Sig. 22)
Aflatoxin exposure (Sig. 24)
Tobacco chewing (Sig. 29)
Defective BER; MUTYH mutation (Sig. 30)
Platinum treatment (Sig. 31 & 35)
Azathioprine treatment (Sig. 32)
Defective BER; MUTYH mutation (Sig. 36)
AID activity (Sig. 84 & 85)
Possible sequencing artefact
Unknown aetiology
Filtered (<5%)
SubType
Angiosarcoma
Atypical fibroxanthoma
Clear cell sarcoma
Endometrial stromal sarcoma
Fibrosarcoma
GIST
Leiomyosarcoma
Liposarcoma
MPNST
Myofibroblastic sarcoma
NOS
Pleomorphic dermal sarcoma
Rhabdomyosarcoma
Spindle cell sarcoma
Synovial sarcoma

◂Figure 1 - Large-scale genomic alterations detected in metastatic soft tissue sarcomas.
Overview of genome-wide characteristics of the mSTS cohort ordered by subtype on decreasing median tumor mutational burden (TMB). For each mSTS sample (N=122), the following tracks are shown:

a) Number of genomic mutations per megabase over the entire genome (TMB); SNV, InDel and MNV are depicted in blue, orange and salmon respectively. Y-axis is shown in log10-scale.
b) Total number of structural variants including deletions, tandem duplications, translocations, inversions and insertions as detected by GRIDSS. Y-axis is shown in log10-scale.
c) Relative frequency of each of the structural variant categories; deletions in orange, tandem duplications in red, translocations in blue, inversions in light-blue and insertions in yellow.
d) Mean genome-wide ploidy, ranging from 0 to 8 (octaploid). Common diploid status is shown in white .
e) Relative contribution of the COSMIC single-base substitution mutational signatures (v3; N=67). Signatures with less than 5 percent overall contribution within the entire mSTS cohort were categorized under the "Filtered (<5%)" category. The proposed etiology of the signatures is denoted below.
f) Relative frequency of the pyrimidine mutations (SNV) in their six categories.
g) Relative frequency of Doublet Base Substitution (DBS) categories.
h) Presence of chromothripsis; mSTS with chromothripsis are shown in pink.
I) Presence of kataegis; mSTS with kataegis are shown in red .
J) Status of homologous recombination deficiency (HRD) as determined by CHORD, mSTS with BRCA1/2-associated HRD (p ≥0.5) are shown in red, otherwise colored white.
k) Status of microsatellite instability (MSI) was determined with MSIseqtool.
l) Soft tissue sarcoma subtypes are indicated below. Abbreviations: GIST, Gastrointestinal stromal tumor; MPNST, malignant peripheral nerve sheath tumor; NOS, not otherwise specified.

sarcoma NOS and one pleomorphic dermal sarcoma (Figure 1). The median number of SNV, InDels, MNV and SV per whole-genome for the mGIST cohort were 3328 (IQR 2,900-4,199), 372 (IQR 281-406), 21 (IQR 16-28.5) and 42 (IQR 23.8-61), respectively (Supplementary Figures 2A, 8A). In the mLMS cohort we detected 6,541 SNVs (IQR 4,903-7,988), 439 InDels (IQR 377-590), 44 MNVs (IQR 34-53) and 208 SVs (IQR 142-319) (Supplementary Figures 2A, 8A). The cohort of mSTS-other contained 3,578 SNVs (IQR 2,331-7,652), 344 InDels (IQR 260-691), 23 MNVs (IQR 12-56) and 72 SVs (IQR 20-345) (Supplementary Figures 2A, 8A). The frequency of the transitions (C>T, T>C) and transversions (C>A, C>G, T>A, T>G) and the genome-wide ratio of transition over transversion in each of the three cohorts were depicted in Supplementary Figures 2B, F. The majority of the of the somatic coding mutations for each of the subgroups were predicted to be missense mutations (mGIST: 55%, mLMS: 59%, mSTS-other: 53%) followed by synonymous mutations (mGIST: 20%, mLMS: 21%, mSTS-other: 26%)(Supplementary Figure 2E). A median of 46 (IQR 34-55), 79 (IQR 67-118) and 47 (IQR 28-123) genes with coding mutations were observed in mGIST, mLMS and mSTS-other cohorts, respectively (Supplementary Figure 8D). Most tumors had a diploid status, some mainly a polyploid status and exceptionally a haploid status (Figure 1, Supplementary Figures 2C, 8C). Three of the seven sarcoma NOS samples were polyploid. Further analyses of the structural variants in the mGIST cohort revealed a median of 3 translocations, 22 deletions, 5 tandem duplications, 2 insertions and 6 inversions (Supplementary Figure 2D). In mLMS a median of 40 translocations, 62 deletions, 30 tandem duplications, 23 insertions and 42 inversions were observed (Supplementary Figure 2D) and a median of 10 translocations, 26

deletions, 10 tandem duplications, 3 insertions and 13 inversions in the mSTS-other cohort (Supplementary Figure 2D). Supplementary Figure 3 gives and overview of the genomic sizes and absolute numbers of SV detected in the mSTS cohorts.

Clustered mutational processes: kataegis and chromothripsis

Regional hypermutation, i.e. kataegis[27, 28], was observed in 6 mGISTs (15%), in 12 mLMS (43%) and 17 of the other mSTS subtypes (32%)(Figure 1). The prevalence of the TC(A|T) → (T|G) mutational signature in kataegic foci suggested the involvement of APOBEC cytidine deaminases (Supplementary Figure 4) underpinned by a relative high expression of the APOBEC related signatures (signature 2 and 13)(Supplementary Figure 4D, F). However, we cannot exclude the involvement of a UV-signature (signature 7a-d) in three samples with kataegic events that display an overall hypermutated genome and relatively high TMB (Figure 1, Supplementary Figure 4A, D).

Chromothripsis is frequently observed in soft tissue sarcomas[29, 30] and considered the underlying event of oncogenic neochromosome formation as is observed in some liposarcoma subtypes [31]. Chromothripsis events occurred in 3 mGISTs (7.5%), 11 mLMS (39.2%) and 10 other mSTS subtypes (18.5%, Figure 1). A genomic overview is given of 3 representative mSTS samples in which chromothripsis occurred (Supplementary Figure 5). It is noted that frequently more than one chromosome is affected.

One mLMS sample, which also had a TMB≥10 and kataegis, showed signs of homologous recombination deficiency. Further inspection revealed that this sample harbored a missense mutation in *RAD21* (Supplementary Table 1), a gene which is involved in DNA damage response[32]. In none of the mSTS samples signs of microsatellite instability were detected.

Fusion genes

Fusion genes were reported in 22 mSTS cases (Supplementary Table 2). Almost all fusion genes were well-known and characteristic for specific STS subtypes, such as the fusions *FUS-DDIT3* in (myxoid) liposarcoma (N=5), *SS18-SSX1* in synovial sarcoma (N=3), *PAX3-FOXO1* in rhabdomyosarcoma (N=3), *EWSR1-ATF1* in clear cell sarcoma (N=1), *RANBP2-ALK* in myofibroblastic sarcoma (N=1), *JAZF1-SUZ12* in endometrial stromal cell sarcoma (N=1), *EWSR1-CREB3L1* in fibrosarcoma (N=1) and *NAB2-STAT6* in solitary fibrous tumors (N=2). However, the latter fusion gene was also found in one angiosarcoma sample, which has not been described before. Furthermore, a few atypical fusion genes were detected: *CIC-FOXO4* in MPNST (N=1), *HMGA2-WIF1* in liposarcoma (N=1) and *EWSR1-YY1* in spindle cell sarcoma (N=1). Some of these fusion genes have been described in other cancers, such as in salivary gland tumors (*HMGA2-WIF1*)[33] and mesothelioma (*EWSR1-YY1*)[34], but not in sarcoma. The fusion gene *CIC-FOXO4* has been described in sarcoma before, but in Ewing-like sarcoma/ undifferentiated small round cell sarcomas [35, 36] and not in MPNST.

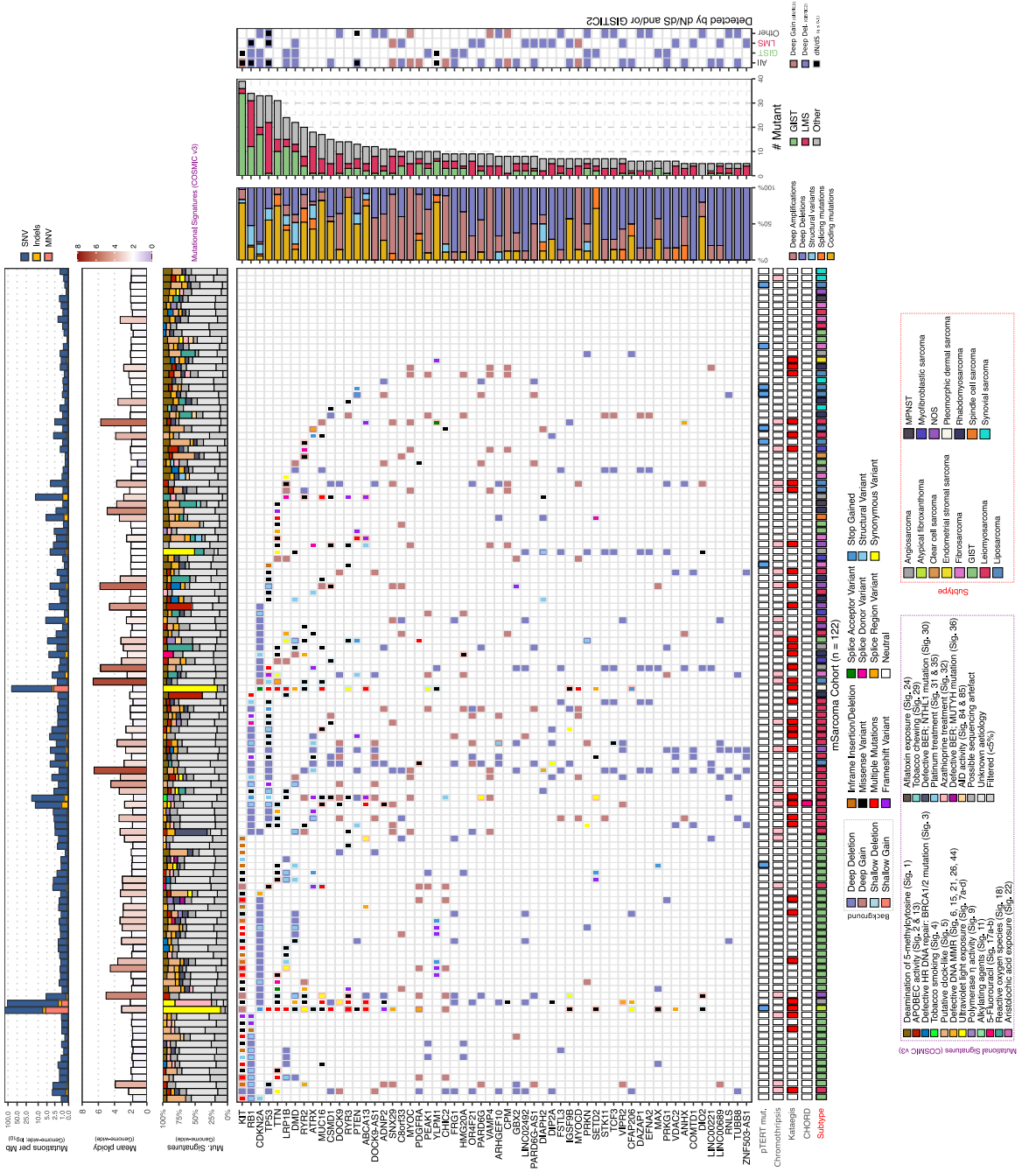
Driver genes of metastatic soft tissue sarcoma

Putative driver genes for the mSTS cohort were identified in an unbiased way and are listed in Figure 2. A total of 58 driver genes could be identified, including some of the well-known cancer drivers. The top 5 consisted of *KIT* (N=39, 32%), *RB1* (N=34, 28%), *CDKN2A* (N=33, 27%), *TP53* (N=33, 27%) and *TTN* (N=30, 26%) (Figure 2). An extended list that also includes potential driver genes in sarcomas previously reported in the literature, is presented as Supplementary Figure 10.

In mGIST, *KIT* was the top driver gene (N=34, 85%), followed by *CDKN2A* (N=17, 43%), *LRP1B* (N=12, 30%) *RB1* (N=12, 30%), and *DMD* (N=10, 25%). In 34 of the 40 GIST cases (Figure 2), a *KIT* mutation was identified. Most cases had an exon 11 mutation (N=29, 85%), alone (N=14) or in combination with a secondary mutation in exon 17 (N=10), exon 13 (N=4) or exon 14 (N=1). Four patients had a mutation in exon 9 (12%), of whom one patient also had an exon 17 mutation. The last patient had mutations in exon 13, 17 and 18. Of 26 of the 34 mGIST patients with a *KIT* mutation, data regarding pretreatment was available. Ten patients did not receive any prior systemic treatment (6 patients with exon 11 mutation, 3 patients with exon 9 mutation and 1 patient with both an exon 11 and exon 17 mutation). The remaining 16 patients were all pretreated with at least imatinib, 4 patients also with sunitinib. Eight of these pretreated patients had a secondary exon 13 or exon 17 mutation. In the 6 GIST cases without a *KIT* mutation, two patients had a mutation in *PDGFRA* (5%). No mutations in *SDH (SDHA-D)* or *BRAF* were found. For *SDH* only 6 intron SNVs in 5 patients with *KIT*-mutated GIST were found. In the remaining 4 'quadruple negative' or 'wild-type' mGIST patients different mutations were found: one patient with amplifications of *MYC* and *RAD21* and mutations in *CTNNB1* and *SMTNL2*; one patient with a mutation in *PTEN*; and two patients with a *NF1* mutation. The *CNNTB1* mutation was a S45P mutation, which is also observed in sporadic desmoid-type fibromatosis [37].

Besides these driver mutations, an oncogenic *BRAF* K601E mutation, similar as observed in melanoma, was found in one of the *KIT*-mutated mGIST samples. Additionally, in 6 mGIST samples the gene *YLPM1* was mutated, bearing frameshift or missense mutations. The function of YLPM1 is not entirely clear. It may act as repressor protein binding to the hTERT promoter reducing telomerase activity [38] or as scaffold for PP1, a phosphatase involved in the regulation of diverse cellular processes such as cell division and protein synthesis [39]. Lastly, deletion peaks were detected in chromosome 1p32, 10q and 13q14 (including *RB1*) (Supplementary Figure 7).

For mLMS *TP53* (N=21, 75%) and *RB1* (N=19, 68%) were the most commonly observed driver genes, followed by *ATRX* (N=11, 39%), *SNX29* (N=7, 25%) and *MUC16* (N=6, 21%). Recurring focal deletions were observed in 1p36, 2q37, 4q35, 10q22, 11q25, 13q14 (including *RB1*), 17p13 (including *TP53*) and 19q13, and amplifications in 15q26, 16p13 (including *SNX29*) and 17p12 (including *MYOCD*) (Supplementary Figure 7).


SNV
Indels
MNV
Mutations per Mb (Genome-wide, log10)
Mean ploidy (Genome-wide)
Mut. Signatures (Genome-wide)
Mutational Signatures (COSMIC v3)
Detected by dN/dS and/or GISTIC2
All
GIST
LMS
Other
Mutant
Deep Amplifications
Deep Deletions
Shallow Deletions
Splicing mutations
Coding mutations
KIT
NF1
CDKN2A
TP53
TTN
LRP1B
DMD
RYR2
ATRX
MUC16
CSMD1
DOCK9
PTEN
ABCA13
DOCK5-AS1
ADGRB3
SNX29
C8orf33
MYO5C
PDGFRA
PEAK1
YLPM1
CHD2
FRG1
HMG20A
ORAOV1
PARP6
VAMP4
ARHGEF10
GSK3
LINC02492
PARD6G-AS1
DAAM2
DIP2A
FSTL3
KCNF1B
MYO1D
PRKN
SETD2
STXBP1
TCF12
VPS72
CFAP206
DCAF11
EFNA2
MAX
PRAG1
VPAC2
ANKHX
COMTD1
DOCK3
LINC00221
LINC00689
TNRC18
TUBB
ZNF503-AS1
pTERT mut.
Chromothripsis
Kataegis
CHORD
Subtype
mSarcoma Cohort (n = 122)
Deep Deletion
Deep Gain
Shallow Deletion
Shallow Gain
Background
Inframe Insertion/Deletion
Missense Variant
Multiple Mutations
Frameshift Variant
Splice Acceptor Variant
Splice Donor Variant
Splice Region Variant
Neutral
Stop Gained
Structural Variant
Synonymous Variant
Subtype
Angiosarcoma
Atypical fibroxanthoma
Clear cell sarcoma
Endometrial stromal sarcoma
Fibrosarcoma
GIST
Leiomyosarcoma
Liposarcoma
MPNST
Myxofibrosarcoma
NOS
Pleomorphic dermal sarcoma
Rhabdomyosarcoma
Spindle cell sarcoma
Synovial sarcoma
Mutational Signatures (COSMIC v3)
Deamination of 5-methylcytosine (Sig. 1)
APOBEC activity (Sig. 2 & 13)
Defective HR / DNA repair- BRCA1/2 mutation (Sig. 3)
Tobacco chewing (Sig. 4)
Putative clock-like (Sig. 5)
Defective DNA MMR (Sig. 6, 15, 21, 26, 44)
Ultraviolet light exposure (Sig. 7a-d)
DNA polymerase epsilon activity (Sig. 9)
Alkylating agents (Sig. 11)
5-Fluorouracil (Sig. 17a-b)
Reactive oxygen species (Sig. 18)
Aristolochic acid exposure (Sig. 22)
Aflatoxin exposure (Sig. 24)
Tobacco chewing (Sig. 29)
Defective BER; NTHL1 mutation (Sig. 30)
Thiopurine treatment (Sig. 31)
Azathioprine treatment (Sig. 32)
Defective BER; MUTYH mutation (Sig. 36)
AID activity (Sig. 84 & 85)
Platinum chemotherapy artifact
Unknown aetiology
Filtered (<5%)

◂Figure 2 - Putative drivers and soft tissue sarcoma-associated genes within the mSTS cohort as detected by unbiased discovery (dN/dS, GISTIC2).
Overview of putative drivers harboring coding mutations within mSTS. Depicted are putative drivers as detected by dN/dS and/or GISTIC2 in the mSTS cohorts (all) or one of the mSTS subcohorts (GIST, LMS, mSTS-other). Included are also genes found mutated (non-synonymous coding variants) in at least 10 mSTS samples. The figure only includes mutated genes or genes with copy-number alterations that occur in a minimum of 5 mSTS samples. mSTS and genes are sorted based on mutual-exclusivity of the depicted putative drivers. Only GISTIC2 focal peaks with deep amplifications and deletions are shown.

a) Number of genomic mutations per megabase over the entire genome (TMB); SNV, InDel and MNV are depicted in blue, orange and salmon respectively. Y-axis is shown in log10-scale.
b) Mean genome-wide ploidy, ranging from 0 to 8 (octaploid). Diploidy is shown in white.
c) Relative contribution of the COSMIC single-base substitution mutational signatures (v3; N=67). Signatures with less than 5 percent overall contribution within the entire mSTS cohort were categorized under the "Filtered (<5%)" category. The proposed etiology of the signatures is denoted below.
d) Overview of coding mutation(s) per mSTS, background colors depict copy-number aberrations whilst the inner square depicts the type of (coding) mutation(s). The adjacent bar plots represent the relative proportions of mutational categories (coding mutations (SNV, InDels and MNV), splicing mutations, SV, deep gains (high-level amplifications resulting in many additional copies) and deep deletions (high-level losses resulting in (near) homozygous losses) per gene. The middle-outer barplot depicts the percentage of GIST (green), LMS (red) and Other (grey) mSTS which harbored a mutation. In addition, dN/dS and/or GISTIC2 support are shown on the outer-right bar plot for either the entire mSTS cohort (all) or separate subcohorts (GIST, LMS, other); GISTIC2 results are colored light purple if these genes were detected within a recurrent focal deletion and salmon if detected within a recurrent focal gain.
e) Presence of TERT promoter mutations; mSTS with TERT mutations are shown in blue.
f) Presence of chromothripsis; mSTS with chromothripsis are shown in pink.
g) Presence of kataegis; mSTS with kataegis are shown in red.
h) Status of homologous recombination deficiency (HRD) as determined by CHORD, mSTS with BRCA1/2-associated HRD (p ≥0.5) are shown in red, otherwise colored white.
i) Soft tissue sarcoma subtypes are indicated below.

Supplementary Figure 8E clearly indicates a statistically significant enrichment in mGIST for alterations within *KIT*, *CDKN2A*, *CDKN2B* and *CDKN2B-AS1*. In the mLMS group *TP53*, *RB1* and *ATRX* were found enriched as well as multiple other genes, whereas chromothripsis events were found depleted.

In the group of mSTS-other samples, the top 5 driver genes consisted of *TTN* (N=16, 30%), *CDKN2A* (N=13, 24%), *TP53* (N=11, 20%), *CSMD1* (N=10, 19%) and *MUC16* (N=10, 19%). Large recurring deletions were detected on 6q26 and 9p21 (including *CDKN2A*) and amplifications on 1q24 and 12q15 (including *MDM2*) (Supplementary Figure 7).

When the relative frequency of mSTS samples with chromosomal arm amplifications and losses was calculated in the main subgroups, we detected clear differences between mGIST, mLMS and mSTS-other (Supplementary Figure 9). In 70% of mGIST samples statistically significant losses were observed of chromosome 1p, 14p/q, 15p/q, 22p/q and in 40% of the mGIST samples losses of 9p/q, 10q and 13p/q. In addition, amplifications of 1q, 5p/q and 8q were detected in 30-50% of the mGIST samples. In about half of all mLMS samples loss of 10p/q, 13p and 16q was found. In approximately 30% of mSTS-other samples loss of 19p/q was seen with an amplification of 1q, 7p and 20q in 30% of the samples.

Mutations revealed by WGS

An oncogenic *KIT* mutation was also observed in an angiosarcoma sample which had a similar missense SNV in exon 11 of *KIT* as found in GIST. Oncogenic mutations in *ESR1* were observed in 3 cases: 1 LMS, 1 pleomorphic dermal sarcoma and 1 endometrial stromal cell sarcoma. The patients with LMS and the patient with endometrial stromal cell sarcoma had an *ESR1* Y537S mutation, which is also observed in breast cancer after treatment with aromatase inhibitors. The patient with the endometrial stromal cell sarcoma was indeed pretreated with two different aromatase inhibitors (anastrozole and exemestane), but the LMS patient did not receive any prior systemic treatment. *MDM2* was found to be amplified in 7 cases: 3 liposarcomas (suggesting dedifferentiated liposarcoma), 1 LMS, 1 rhabdomyosarcoma, 1 sarcoma NOS and 1 myofibroblastic sarcoma. Mutations in *NF1* were found in 11 cases: 3 angiosarcomas, 3 GIST, 3 LMS and one pleomorphic sarcoma NOS (which has malignantly transformed from an atypical fibroxanthoma) and a rhabdomyosarcoma. It was not detected in any of the MPNST samples, suggesting sporadic MPNST rather than neurofibromatosis type 1-associated MPNST. The gene *YLPM1* was mutated in 3 mSTS cases: 1 LMS, 1 endometrial stromal cell sarcoma and 1 pleomorphic dermal sarcoma, and was deleted in 1 angiosarcoma sample.

Mutational signatures in metastatic soft tissue Sarcoma

All of the 67 known Cosmic signatures were represented in metastatic STS (Supplementary Table 1). The most frequently observed signatures were signature 8, 39 and 40 (all of unknown etiology), and signature 1 and 5, both related to age. No clear differences in contributing signatures between GISTs, LMS and the other STS subtypes were observed.

A few cases had higher contributions of signatures 2 and 13, related to APOBEC, or signature 7 (N=3), related to UV-exposure (Figure 1, 2). The three cases with an UV-related signature were a cutaneous angiosarcoma, pleomorphic dermal sarcoma and a sarcoma NOS (which has malignantly transformed from an atypical fibroxanthoma). One angiosarcoma sample displayed a signature related to azathioprine treatment (signature 32), but this patient did not receive any systemic treatment prior to biopsy. Almost all samples partially contained signature 30, related to defective base excision repair (BER), which was predominantly present in one LMS sample. This patient was pretreated with doxorubicin and had not received radiotherapy. Although a few patients did receive pretreatment with a platinum chemotherapy, these signatures (signature 31 and 35) were not evidently present (Figure 1, 2).

To reveal potential novel mutational signatures, *de novo* single base substitutions mutational signatures were extracted from the mSTS cohort (Supplementary Figure 6D). Six signatures (Sig. A-F) were identified. The distribution of the signatures in the mSTS

cohort and their trinucleotide mutational context are shown in Supplementary Figure 6A, B. Comparison of these *de novo* mutational signatures with the 67 COSMIC signatures showed a considerable overlap with one or more of the known COSMIC signatures (Supplementary Figure 6C).

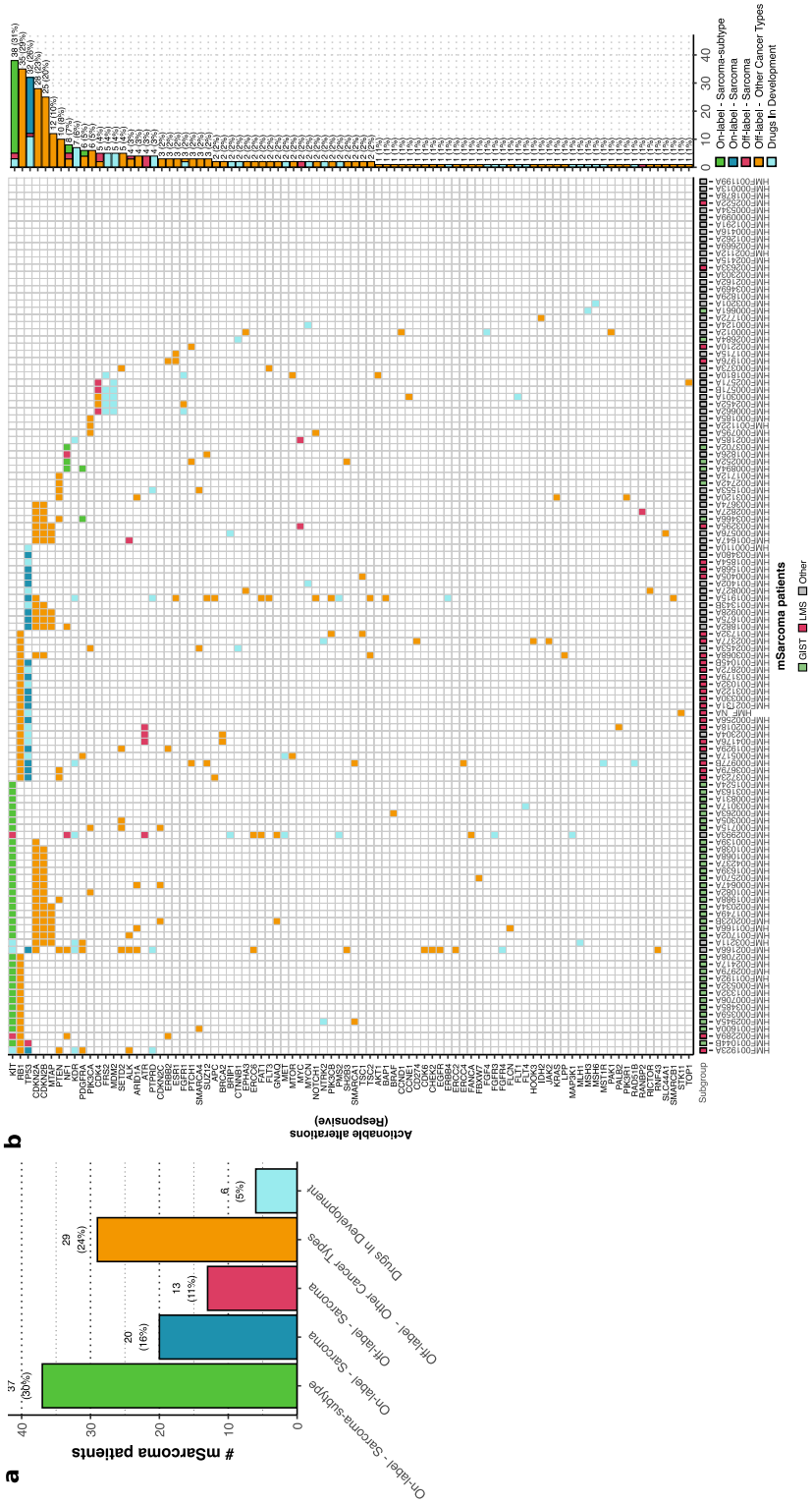
Actionable signatures, mutations and alterations with potential clinical impact

The WGS data from the mSTS cohort were also screened to reveal actionable signatures and targets. Signatures that have been linked to response to certain therapies include an MSI signature, a high TMB and a BRCAness signature. Patients with MSI or a high TMB (≥10 mutations/Mb) [40] might benefit from immunotherapy, especially anti-PD1/PD-L1 therapy. However, in none of the mSTS samples an MSI signature was observed. In 6 patients, a high TMB was identified (Figure 1) and these patients might be candidates for studies exploring checkpoint inhibitors (2 LMS, 2 angiosarcomas, 1 atypical fibroxanthoma, 1 pleomorphic dermal sarcoma). The homologous recombination deficiency observed in one of the LMS samples with a high TMB may be responsive to PARP inhibitors [41].

Lastly, we investigated which patients could potentially benefit from treatment based on specific genomic mutations and alterations found with WGS. In the whole group of mGIST and mSTS, in 105 samples (86%) at least one actionable target was identified for which an FDA-approved or investigational agent is available (Figure 3, Supplementary Table 1).

In all mGIST patients at least 1 targetable alteration was found. A mutation in *KIT* was most frequently observed, but also targetable alterations of *CDKN2A* (N=15), *CDKN2B* (N=14), *RB1* (N=12), *MTAP* (N=5), *PTEN* (N=3), *NF1* (N=3), *CDKN2C* (N=3), *PDGFRA* (N=2, D842V N=1), *PIK3CA* (N=2), *SETD2* (N=2) and *ARID1A* (N=2) were identified (Figure 3, Supplementary Table 1). Although agents against *KIT*-mutated GIST – such as imatinib, sunitinib and regorafenib – are already standard of care for GIST patients, patients with a *KIT* exon 17, V654A or T607I mutation might be candidates for trials exploring sorafenib. For the patients with a *PDGFRA* D842V mutation, although not yet listed in the different databases (CiViC; OncoKB; CGI; iClusion), there is accumulating evidence that avapritinib is effective [42, 43].

In 26 of the 28 mLMS patients (93%), at least one actionable alteration was found, including mutations in *ERBB2* (N=3), *ESR1* (N=1) and an amplification of *KIT* (N=2) who might be candidates for treatment with trastuzumab-based regimens, fulvestrant or imatinib/sunitinib/regorafenib, respectively. In addition, the tumor suppressor pathways linked to *TP53* (N=20) and *RB1* (N=20) can be targeted as well with alterations found in *CDKN2A* (N=2), *CDKN2B* (N=2), *PTEN* (N=3), *ERBB2* (N=3), *ATR* (N=2), *TSC1* (N=2), *KDR* (N=2) and *PTCH1* (N=2) (Figure 3).


a
mSarcoma patients
37 (60%)
20 (16%)
13 (11%)
29 (24%)
9 (5%)
On-label - Sarcoma-subtype
On-label - Sarcoma
Off-label - Sarcoma
Off-label - Other Cancer Types
Drugs in Development
b
Actionable alterations (Responsive)
mSarcoma patients
GIST
LMS
Other
On-label - Sarcoma-subtype
On-label - Sarcoma
Off-label - Sarcoma
Off-label - Other Cancer Types
Drugs in Development

◂Figure 3 - Clinically actionable somatic alterations observed within mSTS.

a) Overview of distinct mSTS harboring current clinically actionable alterations for on-label and off-label soft tissue sarcoma therapies. The highest sarcoma therapy option (ranked as: on-label for (specific) sarcoma subtype, on-label for (all) sarcoma, off-label for (all) sarcoma, off-label for sarcoma but used in other cancer types and drugs in development) per distinct metastatic soft tissue sarcoma is shown.

b) mSTS harboring current clinically actionable alterations per gene. The highest STS therapy option per mSTS and gene is shown. Bottom track represents the categorized sarcoma subtypes (GIST, LMS, OTH) of the mSTS cohort. The figure on the far right shows the number of samples harboring a somatic alteration in a given gene and the proposed level of therapy.

In the remaining 54 mSTS-other patients, 38 patients (70%) with at least one genomic alteration predictive for response to a drug (registered for other indication than STS) were identified (Figure 3). Examples include an angiosarcoma patient harboring the same *KIT* exon 11 mutation as observed in GIST (candidate for treatment with imatinib/sunitinib/regorafenib) and two patients (endometrial stromal cell sarcoma and pleomorphic dermal sarcoma) with an *ESR1* mutation (candidate for treatment with fulvestrant). Frequent targetable alterations were also found in *TP53* (N=12), *RB1* (N=3), *CDKN2A* (N=8), *CDKN2B* (N=6), *MTAP* (N=6), *PTEN* (N=4), *NF1* (N=4), *KDR* (N=5), *PIK3CA* (N=4), *CDK4* (N=5), *FRS2* (N=5) and *MDM2* (N=5).

Discussion

The results of this study give an important insight into the genomic aberrations found in metastatic STS and uncovered some new molecular alterations in specific STS subtypes. Important strengths of our study include the use of WGS and the use of metastatic lesions. In contrast to targeted gene panels, based on Next Generation Sequencing (NGS) or whole exome sequencing (WES), WGS provides a more reliable impression of DNA signatures and structural variants. And in patients with metastatic disease, the use of metastatic lesions better reflects the characteristics of metastatic disease than primary tumor material does; primary tumor tissue often has been obtained many years before establishing metastatic disease, while disease characteristics may have changed considerably, given the genomic instability of cancer over time and under treatment pressure.

With respect to mGIST, prior studies reported only on small series using WGS or WES, varying in size from 1 to 38 samples [44-51]. These series use either only primary (treatment-naïve) tumor samples or a mix of primary/localized and metastatic samples, or focus on a specific subgroup (for example only quadruple negative GIST [44, 47] or *PDGFRA*-mutated GIST [48, 50]), or use WES or a targeted gene panel based on NGS. The added value of this study is that it reports one of the largest series of only metastatic GIST, analyzed with WGS instead of WES/NGS. Not surprisingly, a *KIT* mutation was revealed in the majority of patients. And in line with literature, secondary KIT mutations were found in patients who were pretreated with *KIT*-targeting agents. These patients, i.e. patients with *KIT* exon 17, V654A or T607I mutations, might benefit from treatment with sorafenib [52, 53]. However, there

were also 8 imatinib-pretreated patients without any secondary *KIT* mutations. In 5% of the mGISTs (a third of the non-*KIT* mutated GISTs), a *PDGFRA* mutation was found, corresponding to the numbers observed in literature [6]. These patients, especially those with a *PDGFRA* D842V mutation, might benefit from treatment with dasatinib [54, 55] or avapritinib[42]. In the remaining 'wild-type' GISTs, different (targetable) alterations were identified, but no targetable signatures were found, such as high TMB, BRCAness signature or MSI. Although NGS previously revealed *SDH* as a potential pathogenic mutation in 'wild-type' GIST[18], in our dataset no oncogenic *SDH* mutation could be identified. A recent analysis of 29 tumor samples from 21 patients with high grade or metastatic *KIT*-mutated GIST showed an enrichment of genetic aberrations in *CDKN2A/B*, *RB1* and *TP53* amongst others, with a low mutational burden [45]. In our cohort, *RB1* was altered in 12 cases (30%) and *CDKN2A* in 16 cases (40%), but a *TP53* mutation was seen in only one case (3%). As was previously indicated, we confirmed deletion of *DMD* in 10 mGIST (25%) [56, 57]. An inactivation of *MAX* was found in 3 mGIST (7.5%) [58], and *PTEN* loss was observed in 3 mGIST (7.5%) [59]. Interestingly, we also identified *LRP1B* as putative driver gene, which appeared deleted or inactivated in 12 mGIST samples (30%). Disruption of *LRP1B* has been reported in other cancers where it may function as tumor suppressor [60-62]. Lastly, recurrent CNVs of numerous chromosomal arms in GIST were revealed using WES [63], of which we only could confirm those in 1p and 13q.

In mSTS other than GIST, several studies have tried to sketch the genomic landscape of soft tissue sarcomas, but mostly used WES or had small sample sizes, different STS subtypes and a mix of primary, local recurrent and metastatic disease. The heterogeneity in these datasets and amongst the different studies make it complicated to compare the results of our study to those reported in literature. Nonetheless, we could confirm some of the previous findings. The genes *TP53*, *ATRX* and *PTEN* were already reported to be highly mutated [2, 64-66] in tumors with generally a low mutational burden [65]. In LMS, a near-universal inactivation of *RB1* and *TP53* and widespread copy-number alterations were found [2], while in our cohort an *RB1* alteration was found in 68% of the mLMS patients, a *TP53* alteration in 75% and chromothripsis in 39% of the patients, although this was relatively high compared to the number of samples with chromothripsis in mGIST (7.5%) and the mSTS-other group (18.5%). Further, we confirmed *MYOCD* amplification in 14% of the mLMS samples [1, 67], DMD deletion in 11%[56, 57] and PTEN deletion in 14% [68]. Further analyses indicated few mutated genes but instead revealed numerous and complex chromosomal rearrangements, resulting in losses and gains of specific genes. Unbiased driver gene identification revealed novel putative oncogenes like *SNX29* (in 25% of mLMS) or *VAMP4* (14% of mLMS) or putative tumor suppressors like *CSMD1* (18% of mLMS), *ARHGEF10* (14% of mLMS) and *VIPR2* (14% of mLMS) [69]. Despite the lack of recurrent alterations, the vast majority of mLMS samples (93%) presented with at least one actionable target.

The remaining mSTS-other cohort consists of 13 different mSTS subtypes with some subtypes represented by a single patient or by limited numbers. The putative top driver gene identified is *TTN*, a gene that is frequently found mutated due to its large size [70], followed by *CDKN2A*, *TP53*, *CSMD1*, *MUC16*, *DMD*, *LRP1B*, *CPM*, *PTEN* and *ATRX*. Most putative drivers have also been highlighted in the other mSTS cohorts. *CPM*, carboxypeptidase M, may be of interest as it is found amplified in 7 samples (13%) from the mSTS-other group (3 liposarcomas, 1 sarcoma NOS, 1 angiosarcoma, 1 rhabdomyosarcoma and 1 myofibroblastic sarcoma). *CPM*, an adipocytic differentiation factor and located on chromosome 12q, has been reported to be co-amplified with *MDM2,* which is located in its vicinity, in well-differentiated liposarcomas and has been implicated to play a role in dedifferentiation from well-differentiated to dedifferentiated liposarcomas [71] and other cancers [72]. In about 70% of the mSTS-other cohort an targetable alteration was found.

Lucchesi et al. reported that by using NGS, in 41% of the soft tissue sarcoma patients an putative actionable target could be identified [66], compared to 78% in our mSTS cohort (excluding mGIST). However, there are possibly many other targetable features or leads for effectiveness of targeted therapy in certain cases that are not listed in the databases used, for example response to pazopanib in *TP53*-mutated advanced sarcoma [73]. However, the value of these actionable mutations should be investigated in clinical trials, such as the DRUP trial [74]. In this trial, patients with metastatic cancer who have no standard treatment options left receive targeted therapies that are already approved and registered for other cancer types, matched to their molecular profile. Examples include patients with *BRCA* or *ATM*-mutated tumors receiving off-label olaparib and patients with *BRAF*-mutated tumors receiving off-label dabrafenib.

As mentioned, advantages of our study over the previously published studies include the use of whole genome sequencing (instead of WES or NGS), the use of metastatic samples only and the relatively large size of this unique dataset. Additionally, it shows the clinical applicability, relevance and importance of genomics for daily clinical practice. Besides these strengths, there were also some limitations. Inherent to studying STS, there was quite some heterogeneity in the data, which has led to small subgroups, despite the relatively large size of the dataset. Second and inherent to a study in which in total of more than 6,000 patients were included in almost 50 different hospitals during daily clinical practice [75], annotation of clinical data was not always complete, especially regarding prior treatment, which made it difficult to link certain genomic alterations to resistance for a specific drug.

Conclusion

In conclusion, this study gives an important insight into the biology of mSTS. Whole genome sequencing can serve as a valuable tool to identify clinically relevant and targetable molecular aberrations in patients with mSTS and improve patient management by giving guidance on treatment choices, even or especially after multiple lines of treatment.

Methods

Patient cohort and study procedures

Patients with mSTS were recruited under the study protocol (NCT01855477) of the Center for Personalized Cancer Treatment (CPCT) and the CPCT-02 study. The CPCT-02 protocol was approved by the Medical Ethical Review Committee (METC) of the University Medical Center Utrecht. Patients were eligible for inclusion if the following criteria were met: 1) age ≥18 years; 2) locally advanced or metastatic solid tumor; 3) indication for new line of systemic treatment with registered anti-cancer agents; 4) safe biopsy option according to the intervening physician. All patients provided written informed consent before any study procedure. The study procedures consisted of the collection of image-guided percutaneous biopsy of the metastatic lesion and matched peripheral blood samples for reference DNA. For the current study, patients were included for biopsy between April 21st 2016 and January 31st 2019, resulting in a cohort of 122 distinct patients from 15 Dutch hospitals (Table 1, Supplementary Table 1, Supplementary Figure 1).

Collection, sequencing and processing of mSTS biopsies

Blood samples were collected in CellSave preservative tubes (Menarini-Silicon Biosystems, Huntington Valley, PA, USA) and shipped at room temperature to the central sequencing facility at the Hartwig Medical Foundation. Tumor samples were fresh-frozen in liquid nitrogen directly after the biopsy procedure and send to a central pathology tissue facility. Tumor cellularity was estimated by assessing a hematoxylin-eosin (HE) stained 6 µm section. Subsequently, 25 sections of 20 µm were collected for DNA isolation. DNA was isolated with an automated workflow using the Qiasymphony DSP DNA Midi kit for blood and QIAsymphony DSP DNA Mini kit for tumor samples according to the manufacturer's protocol (Qiagen Benelux B.V., Venlo, the Netherlands). DNA concentration was measured by Qubit™ fluorometric quantitation (Invitrogen, Life Technologies, Carlsbad, CA, USA). DNA libraries for Illumina sequencing were generated from 50-100ng of genomic DNA using standard protocols (Illumina, San Diego, CA, USA) and subsequently whole-genome sequenced in a HiSeq X Ten system using the paired-end sequencing protocol (2x150 bp) for both the metastatic tumor and matched blood sample.

Subsequent alignment, somatic mutation detection and *in silico* tumor cell percentage estimation were performed in a uniform manner as detailed by Priestley et al.[75]. Briefly, paired-end sequencing reads were aligned against the human reference genome (GRCh37) using BWA-mem (v0.7.5a). Duplicate reads were marked and small insertion and deletions (InDels) were realigned using GATK IndelRealigner (v3.4.46). Prior to somatic SNV and InDel variant calling, base qualities were recalibrated using GATK BQSR (v3.4.46). Somatic SNV, InDels and MNV were called by Strelka (v1.0.14) using the matched peripheral blood WGS sample for matched-normal variant calling.

Additional in-depth settings and optimizations of the HMF pipeline are described by Priestley et al.[75] and tools are available at https://github.com/hartwigmedical/.

The somatic mutations (SNV, InDels and MNV) were further annotated with Ensembl Variant Effect Predictor (VEP, version 99, cache 99_GRCh37) using GENCODE (v33) annotations in tandem with the dbNSFP plugin (version 3.5, hg19) for gnomAD population frequencies. SIFT and PolyPhen-2 scoring was applied for additional functional effect prediction.

During downstream analysis, we only retained SNV, InDels and MNV which passed all of the following heuristic filters; default Strelka filters (PASS-only), gnomAD exome (ALL) allele frequency <0.001, gnomAD genome (ALL) <0.005, not present in ≥5 samples from the Hartwig Medical Foundation germline panel-of-normals (GATK Haplotyper) and not present in ≥3 samples from the Hartwig Medical Foundation Strelka-specific somatic blacklist.

Putative protein-altering (coding) or high-impact (e.g. splicing) mutations were aggregated per sample and gene by selecting the most deleterious annotated effect (from VEP) on any known overlapping gene-wise transcript (except those transcripts flagged as retained intron and nonsense mediated decay). In addition, structural variants with a Tumor Allele Frequency (TAF) ≥0.1 that overlapped only partly with the respective coding sequences (i.e. not all exons of the respective gene), were annotated as 'Structural Variant' mutations. Multiple coding mutations and/or structural variants (SV) per gene were annotated as 'multiple mutations'.

Discovery of somatic structural variants (SV), copy-number alterations and in-frame fusions was performed using the GRIDDS, PURPLE and LINX suite. During the downstream analyses, we only retained somatic structural variants passing all default QC filters (PASS-only) and with an upstream and/or downstream TAF ≥0.1.

Mean read coverages of the reference and tumor samples were calculated using Picard Tools (v1.141; CollectWgsMetrics) based on GRCh37. Genomic and coding tumor mutational burden (TMB) was calculated as previously described by van Dessel et al.[76].

Determining purity-corrected allele frequencies for somatic alterations

To calculate the Tumor Allele Frequencies (TAF) of somatic SNV, InDels, MNV and SV, representing the tumor purity-corrected variant frequencies, we followed a previously described approach by Stephens et al.[77], implemented as: $TAF_M = f_M / p[pC_t + (1 - p)\ C_h]$ (equation 1), in which where f_M is the ratio of primary-aligned and non-duplicated reads observed for alternative allele *m* over the reference allele (VAF), *p* is the *in silico* estimated tumor purity fraction, C_t is the absolute copy-number of the segment overlapping m and C_h is the wild type (healthy) copy-number; C_h=2 for autosomes and allosomes in female samples and C_h=1 for allosomes in male samples.

Discovery of genes under evolutionary selection

We performed a dN/dS analysis on somatic mutations (SNV and InDels) using dndscv (v0.0.1.0) on respective genome sequences and transcript annotations using a custom transcript database based on ENSEMBL Genes (v99)/GENCODE (v33) annotations. We performed a dN/dS analysis over the entire mSTS cohort (N=122) and three separate dN/dS analysis on the major subgroups (mGIST; N=40, mLMS; N=28, and mSTS-other; N=54). Genes-of-interest were selected based on the statistical significance, corrected for multiple hypothesis testing (Benjamini-Hochberg), which integrated all mutation types (missense, nonsense, essential splice-site mutations and InDels; qglobal_cv≤0.1) and/or without InDels (qallsubs_cv≤0.1).

Detection and annotation of recurrent copy-number alterations

To detect recurrent copy-number alterations, we performed a GISTIC2 (v2.0.23) analysis over the entire mSTS cohort and, again, three separate GISTIC2 analyses on the major subgroups (mGIST, mLMS and other mSTS-other subtypes). The GISTIC2 was performed using the following settings:

- Genes were annotated to GISTIC2 peaks (q≤0.1) based on the following strategy;
- GISTIC2 focal peaks (all_lesions.conf_95.txt) were overlapped to genes (from verified and manually annotated loci, no pseudogenes or read-throughs and from standard chromosomes; N=36,574) from GENCODE (GRCh37; v33), taking into consideration only the genes overlapping with at least 100 base pairs within the detected GISTIC2 peak.
- If a GISTIC2 focal peak overlapped with multiple GENCODE genes, a combined database containing known drivers detected in a metastatic pan-cancer dataset (CPCT-02), COSMIC Cancer Gene Census (v85), OncoKB Cancer Gene Census (June 2019), Martincorena et al.[78] and Priestley et al.[75] were used to further pinpoint the possible target gene(s) (N=1,272), e.g. if a GISTIC2 peak overlapped both PTEN and near-adjacent non-driver gene, only PTEN would be chosen as possible target.

The list of all overlapping GENCODE (v33) genes per GISTIC2 peak can be found in Supplementary Table 1.

- If no overlapping genes were found, GISTIC2 peaks were annotated with the nearest GENCODE (v33) protein-coding gene (N=19,988).

Mutational signature analysis

Mutational signatures based on the trinucleotide contexts of SNVs was performed, mainly using the MutationalPatterns package (1.10.0) and as previously described. The 96 Single Base Substitution (SBS) mutational signatures (COSMIC v3) as established by Alexandrov et al.[79], (matrix *Sij*; *i*=96; number of trinucleotide motifs; *j*=number of signatures) were downloaded from COSMIC (as deposited on May 2019). The proposed etiology of each SBS signature was derived from Alexandrov et al.[79], Petljak et al.[80], Angus et al.[81] and Christensen et al.[82]. In addition, *de novo* mutational signature analysis by MutationalPatterns was performed based on the max. number of relevant signatures as assessed using the NMF R package (v0.21.0) with 1000 iterations (Supplementary Figure 6). By comparing the cophenetic correlation coefficient, residual sum of squares and silhouette, we opted to generate six custom *de novo* signatures. Custom signatures were correlated to existing (COSMIC v3) mutational signatures using cosine similarity.

Detection of chromothripsis

Shatterseek (v0.4) using default parameters was used to detect chromothripsis-like events. As input, we used the rounded absolute copy numbers (as derived by PURPLE) and structural variants with an TAF ≥0.1 at either end of the breakpoint. The male sex chromosome (chrY) was excluded. The criteria for a chromothripsis-like event were based on the following criteria: a) total number of intra-chromosomal structural variants involved in the event ≥25; b) max. number of oscillating CN segments (2 states) ≥7 or max. number of oscillating CN segments (3 states) ≥14; c) total size of chromothripsis event ≥20 megabase pairs (Mbp); d) satisfying the test of equal distribution of SV types ($p>0.05$); and e) satisfying the test of non-random SV distribution within the cluster region or chromosome ($p\leq0.05$).

Classification of homologous recombination deficiency genotypes

To determine Homologous Recombination Deficiency (HRD) due to possible loss-of-function of *BRCA1* and/or *BRCA2* (amongst others), we utilized the Classifier for Homologous Recombination Deficiency with default settings (CHORD; v2.0). CHORD uses a random-forest approach to classify samples into HR-deficient/HR-proficient categories.

MSI status determination

For MSI status determination we used MSIseqtool [83] and counted the number of InDels per million bases occurring in homopolymers of five or more bases or dinucleotide, trinucleotide and tetranucleotide sequences of repeat count four or more. MSIseq scores of >4 were considered MSI.

Inventory of clinically actionable somatic alterations and putative therapeutic targets

Current clinical relevance of somatic alterations in relation to putative treatment options or resistance mechanisms and trial eligibility was determined based upon the following databases; CiViC (Nov. 2018), OncoKB (Nov. 2018), CGI (Nov. 2018) and the iClusion (Dutch) clinical trial database (Sept. 2019) from iClusion (Rotterdam, the Netherlands). The databases were aggregated and harmonized using the HMF knowledgebase-importer (v1.7). This list was manually corrected for discrepancies and subsequently, we curated the linked putative treatments for current on-label and off-label mSTS and mSTS subtype treatment options, as defined within the Netherlands by the Dutch Medicines Evaluation Board (College ter Beoordeling van Geneesmiddelen; CBG).

Data availability

WGS data and corresponding clinical data have been requested from Hartwig Medical Foundation and provided under data request number DR-028. Both WGS and clinical data is freely available for academic use from the Hartwig Medical Foundation through standardized procedures and request forms can be found at https://www.hartwigmedicalfoundation.nl. No additional data were used for this study.

Code availability

Analysis and visualization have been performed using the statistical platform language R (3.6.2), all utilized custom code and scripts can be freely requested and distributed by contacting the authors.

Additional information

Supplementary information is available in the supplementary information files.

Acknowledgements

This publication and the underlying study have been made possible partly on the basis of

the data that Hartwig Medical Foundation and the Center of Personalised Cancer Treatment (CPCT) have made available.

Author contributions

M.V., H.J.G.V.D.W., S.S and E.A.C.W. wrote the manuscript, which all authors reviewed. J.V.R., H.J.G.V.D.W. and E.C. performed the bioinformatics analyses. M.V. and S.S. managed the assessment of the clinical data. N.S., M.P.J.K.L., I.M.E.D., J.J.D.H., H.G. and J.W.M.N. were the main contributors of the patient samples. E.C. coordinated the sequencing of the samples. M.P.J.K.L. and S.S. are members of the CPCT-02 study team and/or CPCT board.

Competing interest statement

The authors declare no competing interests.

References

1. The Cancer Genome Atlas Research N. Comprehensive and Integrated Genomic Characterization of Adult Soft Tissue Sarcomas. Cell. 2017;171:950-65 e28.
2. Chudasama P, Mughal SS, Sanders MA, Hubschmann D, Chung I, Deeg KI, et al. Integrative genomic and transcriptomic analysis of leiomyosarcoma. Nat Commun. 2018;9:144.
3. Behjati S, Tarpey PS, Sheldon H, Martincorena I, Van Loo P, Gundem G, et al. Recurrent PTPRB and PLCG1 mutations in angiosarcoma. Nat Genet. 2014;46:376-9.
4. Fletcher CDM, Bridge JA, Hogendoorn PCW, Mertens F, World Health Organization, International Agency for Research on Cancer. WHO classification of tumours of soft tissue and bone. Lyon: IARC Press; 2013.
5. Hirota S, Isozaki K, Moriyama Y, Hashimoto K, Nishida T, Ishiguro S, et al. Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. Science. 1998;279:577-80.
6. Szucs Z, Thway K, Fisher C, Bulusu R, Constantinidou A, Benson C, et al. Molecular subtypes of gastrointestinal stromal tumors and their prognostic and therapeutic implications. Future Oncol. 2017;13:93-107.
7. Miettinen M, Lasota J. Gastrointestinal stromal tumors. Gastroenterol Clin North Am. 2013;42:399-415.
8. Casali PG, Abecassis N, Bauer S, Biagini R, Bielack S, Bonvalot S, et al. Gastrointestinal stromal tumours: ESMO-EURACAN Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2018;29:iv68-iv78.
9. Demetri GD, van Oosterom AT, Garrett CR, Blackstein ME, Shah MH, Verweij J, et al. Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumour after failure of imatinib: a randomised controlled trial. Lancet. 2006;368:1329-38.
10. Demetri GD, Reichardt P, Kang YK, Blay JY, Rutkowski P, Gelderblom H, et al. Efficacy and safety of regorafenib for advanced gastrointestinal stromal tumours after failure of imatinib and sunitinib (GRID): an international, multicentre, randomised, placebo-controlled, phase 3 trial. Lancet. 2013;381:295-302.
11. Biron P, Cassier PA, Fumagalli E, Blesius A, Debiec-Rychter M, Adenis A, et al. Outcome of patients (pts) with PDGFRAD842V mutant gastrointestinal stromal tumor (GIST) treated with imatinib (IM) for advanced disease. Journal of Clinical Oncology. 2010;28:10051-.
12. Farag S, Somaiah N, Choi H, Heeres B, Wang WL, van Boven H, et al. Clinical characteristics and treatment outcome in a large multicentre observational cohort of PDGFRA exon 18 mutated gastrointestinal stromal tumour patients. Eur J Cancer. 2017;76:76-83.
13. Cassier PA, Fumagalli E, Rutkowski P, Schoffski P, Van Glabbeke M, Debiec-Rychter M, et al. Outcome of patients with platelet-derived growth factor receptor alpha-mutated gastrointestinal stromal tumors in the tyrosine kinase inhibitor era. Clin Cancer Res. 2012;18:4458-64.
14. Prenen H, Cools J, Mentens N, Folens C, Sciot R, Schoffski P, et al. Efficacy of the kinase inhibitor SU11248 against gastrointestinal stromal tumor mutants refractory to imatinib mesylate. Clin Cancer Res. 2006;12:2622-7.
15. Blanke CD, Rankin C, Demetri GD, Ryan CW, von Mehren M, Benjamin RS, et al. Phase III randomized, intergroup trial assessing imatinib mesylate at two dose levels in patients with unresectable or metastatic gastrointestinal stromal tumors expressing the kit receptor tyrosine kinase: S0033. J Clin Oncol. 2008;26:626-32.
16. Blanke CD, Demetri GD, von Mehren M, Heinrich MC, Eisenberg B, Fletcher JA, et al. Long-term results from a randomized phase II trial of standard- versus higher-dose imatinib mesylate for patients with unresectable or metastatic gastrointestinal stromal tumors expressing KIT. J Clin Oncol. 2008;26:620-5.

17. Patrikidou A, Domont J, Chabaud S, Ray-Coquard I, Coindre JM, Bui-Nguyen B, et al. Long-term outcome of molecular subgroups of GIST patients treated with standard-dose imatinib in the BFR14 trial of the French Sarcoma Group. Eur J Cancer. 2016;52:173-80.
18. Heinrich MC, Rankin C, Blanke CD, Demetri GD, Borden EC, Ryan CW, et al. Correlation of Long-term Results of Imatinib in Advanced Gastrointestinal Stromal Tumors With Next-Generation Sequencing Results: Analysis of Phase 3 SWOG Intergroup Trial S0033. JAMA Oncol. 2017;3:944-52.
19. Committee EG, Euracan, Gronchi A, Frezza AM, Casali PG, De Álava E, et al. Soft tissue and visceral sarcomas: ESMO–EURACAN Clinical Practice Guidelines for diagnosis, treatment and follow-up†. Annals of Oncology. 2018;29:iv51-iv67.
20. Tap WD, Jones RL, Van Tine BA, Chmielowski B, Elias AD, Adkins D, et al. Olaratumab and doxorubicin versus doxorubicin alone for treatment of soft-tissue sarcoma: an open-label phase 1b and randomised phase 2 trial. Lancet. 2016;388:488-97.
21. Judson I, Verweij J, Gelderblom H, Hartmann JT, Schoffski P, Blay JY, et al. Doxorubicin alone versus intensified doxorubicin plus ifosfamide for first-line treatment of advanced or metastatic soft-tissue sarcoma: a randomised controlled phase 3 trial. Lancet Oncol. 2014;15:415-23.
22. Tap WD, Papai Z, Van Tine BA, Attia S, Ganjoo KN, Jones RL, et al. Doxorubicin plus evofosfamide versus doxorubicin alone in locally advanced, unresectable or metastatic soft-tissue sarcoma (TH CR-406/SARC021): an international, multicentre, open-label, randomised phase 3 trial. Lancet Oncol. 2017;18:1089-103.
23. Seddon B, Strauss SJ, Whelan J, Leahy M, Woll PJ, Cowie F, et al. Gemcitabine and docetaxel versus doxorubicin as first-line treatment in previously untreated advanced unresectable or metastatic soft-tissue sarcomas (GeDDiS): a randomised controlled phase 3 trial. Lancet Oncol. 2017;18:1397-410.
24. Ryan CW, Merimsky O, Agulnik M, Blay JY, Schuetze SM, Van Tine BA, et al. PICASSO III: A Phase III, Placebo-Controlled Study of Doxorubicin With or Without Palifosfamide in Patients With Metastatic Soft Tissue Sarcoma. J Clin Oncol. 2016;34:3898-905.
25. Tap WD, Wagner AJ, Papai Z, Ganjoo KN, Yen C-C, Schoffski P, et al. ANNOUNCE: A randomized, placebo (PBO)-controlled, double-blind, phase (Ph) III trial of doxorubicin (dox) + olaratumab versus dox + PBO in patients (pts) with advanced soft tissue sarcomas (STS). Journal of Clinical Oncology. 2019;37:LBA3-LBA.
26. van der Graaf WT, Blay JY, Chawla SP, Kim DW, Bui-Nguyen B, Casali PG, et al. Pazopanib for metastatic soft-tissue sarcoma (PALETTE): a randomised, double-blind, placebo-controlled phase 3 trial. Lancet. 2012;379:1879-86.
27. Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SA, Behjati S, Biankin AV, et al. Signatures of mutational processes in human cancer. Nature. 2013;500:415-21.
28. Nik-Zainal S, Alexandrov LB, Wedge DC, Van Loo P, Greenman CD, Raine K, et al. Mutational processes molding the genomes of 21 breast cancers. Cell. 2012;149:979-93.
29. Cai H, Kumar N, Bagheri HC, von Mering C, Robinson MD, Baudis M. Chromothripsis-like patterns are recurring but heterogeneously distributed features in a survey of 22,347 cancer genome screens. BMC Genomics. 2014;15:82.
30. Cortés-Ciriano I, Lee JJ, Xi R, Jain D, Jung YL, Yang L, et al. Comprehensive analysis of chromothripsis in 2,658 human cancers using whole-genome sequencing. Nat Genet. 2020;52:331-41.
31. Garsed DW, Marshall OJ, Corbin VD, Hsu A, Di Stefano L, Schroder J, et al. The architecture and evolution of cancer neochromosomes. Cancer Cell. 2014;26:653-67.

32. Xu H, Balakrishnan K, Malaterre J, Beasley M, Yan Y, Essers J, et al. Rad21-Cohesin Haploinsufficiency Impedes DNA Repair and Enhances Gastrointestinal Radiosensitivity in Mice. PLOS ONE. 2010;5:e12112.
33. Queimado L, Lopes CS, Reis AM. WIF1, an inhibitor of the Wnt pathway, is rearranged in salivary gland tumors. Genes Chromosomes Cancer. 2007;46:215-25.
34. Panagopoulos I, Thorsen J, Gorunova L, Micci F, Haugom L, Davidson B, et al. RNA sequencing identifies fusion of the EWSR1 and YY1 genes in mesothelioma with t(14;22)(q32;q12). Genes Chromosomes Cancer. 2013;52:733-40.
35. Sugita S, Arai Y, Tonooka A, Hama N, Totoki Y, Fujii T, et al. A novel CIC-FOXO4 gene fusion in undifferentiated small round cell sarcoma: a genetically distinct variant of Ewing-like sarcoma. Am J Surg Pathol. 2014;38:1571-6.
36. Brohl AS, Solomon DA, Chang W, Wang J, Song Y, Sindiri S, et al. The genomic landscape of the Ewing Sarcoma family of tumors reveals recurrent STAG2 mutation. PLoS Genet. 2014;10:e1004475.
37. Crago AM, Chmielecki J, Rosenberg M, O'Connor R, Byrne C, Wilder FG, et al. Near universal detection of alterations in CTNNB1 and Wnt pathway regulators in desmoid-type fibromatosis by whole-exome sequencing and genomic analysis. Genes Chromosomes Cancer. 2015;54:606-15.
38. Armstrong L, Lako M, van Herpe I, Evans J, Saretzki G, Hole N. A role for nucleoprotein Zap3 in the reduction of telomerase activity during embryonic stem cell differentiation. Mech Dev. 2004;121:1509-22.
39. Ulke-Lemée A, Trinkle-Mulcahy L, Chaulk S, Bernstein NK, Morrice N, Glover M, et al. The nuclear PP1 interacting protein ZAP3 (ZAP) is a putative nucleoside kinase that complexes with SAM68, CIA, NF110/45, and HNRNP-G. Biochim Biophys Acta. 2007;1774:1339-50.
40. Ready N, Hellmann MD, Awad MM, Otterson GA, Gutierrez M, Gainor JF, et al. First-Line Nivolumab Plus Ipilimumab in Advanced Non-Small-Cell Lung Cancer (CheckMate 568): Outcomes by Programmed Death Ligand 1 and Tumor Mutational Burden as Biomarkers. J Clin Oncol. 2019;37:992-1000.
41. Seligson ND, Kautto EA, Passen EN, Stets C, Toland AE, Millis SZ, et al. BRCA1/2 Functional Loss Defines a Targetable Subset in Leiomyosarcoma. Oncologist. 2019;24:973-9.
42. Heinrich MC, mehren Mv, Jones RL, Bauer S, Kang Y, Schöffski P, et al. Avapritinib is Highly Active and Well-tolerated in Patients With Advanced GIST Driven by a Diverse Variety of Oncogenic Mutations in KIT and PDGFRA (NAVIGATOR trial). Connective Tissue Oncology Society (CTOS) Annual Meeting Rome, Italy2018.
43. Blueprint Medicines Corporation. VOYAGER: Study of Avapritinib vs Regorafenib in Patients With Locally Advanced Unresectable or Metastatic GIST (ClinicalTrials.gov Identifier: NCT03465722). NIH U.S. National Libraby of Medicine (ClinicalTrial.gov); 2018.
44. Pantaleo MA, Urbini M, Indio V, Ravegnini G, Nannini M, De Luca M, et al. Genome-Wide Analysis Identifies MEN1 and MAX Mutations and a Neuroendocrine-Like Molecular Heterogeneity in Quadruple WT GIST. Mol Cancer Res. 2017;15:553-62.
45. Heinrich MC, Patterson J, Beadling C, Wang Y, Debiec-Rychter M, Dewaele B, et al. Genomic aberrations in cell cycle genes predict progression of KIT-mutant gastrointestinal stromal tumors (GISTs). Clinical Sarcoma Research. 2019;9:3.
46. Mavroeidis L, Metaxa-Mariatou V, Papoudou-Bai A, Lampraki AM, Kostadima L, Tsinokou I, et al. Comprehensive molecular screening by next generation sequencing reveals a distinctive mutational profile of KIT/PDGFRA genes and novel genomic alterations: results from a 20-year cohort of patients with GIST from north-western Greece. ESMO Open. 2018;3:e000335.

47. Belinsky MG, Rink L, Cai KQ, Capuzzi SJ, Hoang Y, Chien J, et al. Somatic loss of function mutations in neurofibromin 1 and MYC associated factor X genes identified by exome-wide sequencing in a wild-type GIST case. BMC Cancer. 2015;15:887.
48. [48] Belinsky MG, Cai KQ, Zhou Y, Luo B, Pei J, Rink L, et al. Succinate dehydrogenase deficiency in a PDGFRA mutated GIST. BMC cancer. 2017;17:512-.
49. Klinke OK, Mizani T, Baldwin G, Bancel B, Devouassoux-Shisheboran M, Scoazec J-Y, et al. KIT Mutation and Loss of 14q May Be Sufficient for the Development of Clinically Symptomatic Very Low-Risk GIST. PloS one. 2015;10:e0130149-e.
50. Indio V, Astolfi A, Tarantino G, Urbini M, Patterson J, Nannini M, et al. Integrated Molecular Characterization of Gastrointestinal Stromal Tumors (GIST) Harboring the Rare D842V Mutation in PDGFRA Gene. Int J Mol Sci. 2018;19:732.
51. Li P, Li M, Wang K, Liu Y, Wang Y, Zhao D, et al. Genetic alterations in cell cycle regulation-associated genes may promote primary progression of gastrointestinal stromal tumors. Laboratory investigation; a journal of technical methods and pathology. 2019:10.1038/s41374-019-0322-x.
52. Park SH, Ryu MH, Ryoo BY, Im SA, Kwon HC, Lee SS, et al. Sorafenib in patients with metastatic gastrointestinal stromal tumors who failed two or more prior tyrosine kinase inhibitors: a phase II study of Korean gastrointestinal stromal tumors study group. Investigational new drugs. 2012;30:2377-83.
53. Montemurro M, Gelderblom H, Bitz U, Schütte J, Blay JY, Joensuu H, et al. Sorafenib as third- or fourth-line treatment of advanced gastrointestinal stromal tumour and pretreatment including both imatinib and sunitinib, and nilotinib: A retrospective analysis. European journal of cancer (Oxford, England : 1990). 2013;49:1027-31.
54. Trent JC, Wathen K, Von Mehren M, Samuels BL, Staddon AP, Choy E, et al. A phase II study of dasatinib for patients with imatinib-resistant gastrointestinal stromal tumor (GIST). Journal of Clinical Oncology. 2011;29:10006-.
55. Schuetze SM, Bolejack V, Thomas DG, von Mehren M, Patel S, Samuels B, et al. Association of Dasatinib With Progression-Free Survival Among Patients With Advanced Gastrointestinal Stromal Tumors Resistant to Imatinib. JAMA Oncology. 2018;4:814-20.
56. Wang Y, Marino-Enriquez A, Bennett RR, Zhu M, Shen Y, Eilers G, et al. Dystrophin is a tumor suppressor in human cancers with myogenic programs. Nat Genet. 2014;46:601-6.
57. Mauduit O, Delcroix V, Lesluyes T, Pérot G, Lagarde P, Lartigue L, et al. Recurrent DMD Deletions Highlight Specific Role of Dp71 Isoform in Soft-Tissue Sarcomas. Cancers (Basel). 2019;11.
58. Schaefer IM, Wang Y, Liang CW, Bahri N, Quattrone A, Doyle L, et al. MAX inactivation is an early event in GIST development that regulates p16 and cell proliferation. Nat Commun. 2017;8:14674.
59. Quattrone A, Wozniak A, Dewaele B, Floris G, Vanspauwen V, Van Looy T, et al. Frequent mono-allelic loss associated with deficient PTEN expression in imatinib-resistant gastrointestinal stromal tumors. Mod Pathol. 2014;27:1510-20.
60. Tabouret E, Labussière M, Alentorn A, Schmitt Y, Marie Y, Sanson M. LRP1B deletion is associated with poor outcome for glioblastoma patients. J Neurol Sci. 2015;358:440-3.
61. Takeda H, Rust AG, Ward JM, Yew CC, Jenkins NA, Copeland NG. Sleeping Beauty transposon mutagenesis identifies genes that cooperate with mutant Smad4 in gastric cancer development. Proc Natl Acad Sci U S A. 2016;113:E2057-65.
62. Liu L, Ren M, Han S, Sun L, Zhu L. Expression level and clinical significance of low-density lipoprotein receptor-related protein 1B gene in cervical squamous cell carcinoma. Int J Clin Exp Pathol. 2018;11:1701-6.

63. Schoppmann SF, Vinatzer U, Popitsch N, Mittlböck M, Liebmann-Reindl S, Jomrich G, et al. Novel Clinically Relevant Genes in Gastrointestinal Stromal Tumors Identified by Exome Sequencing. Clinical Cancer Research. 2013;19:5329.

64. Kim J, Kim JH, Kang HG, Park SY, Yu JY, Lee EY, et al. Integrated molecular characterization of adult soft tissue sarcoma for therapeutic targets. BMC Med Genet. 2018;19:216.

65. Cancer Genome Atlas Research Network. Electronic address edsc, Cancer Genome Atlas Research N. Comprehensive and Integrated Genomic Characterization of Adult Soft Tissue Sarcomas. Cell. 2017;171:950-65 e28.

66. Lucchesi C, Khalifa E, Laizet Yh, Soubeyran I, Mathoulin-Pelissier S, Chomienne C, et al. Targetable Alterations in Adult Patients With Soft-Tissue Sarcomas: Insights for Personalized TherapyTargetable Genomic Alterations in Adult Patients With Soft-Tissue SarcomasTargetable Genomic Alterations in Adult Patients With Soft-Tissue Sarcomas. JAMA Oncology. 2018;4:1398-404.

67. Pérot G, Derré J, Coindre JM, Tirode F, Lucchesi C, Mariani O, et al. Strong smooth muscle differentiation is dependent on myocardin gene amplification in most human retroperitoneal leiomyosarcomas. Cancer Res. 2009;69:2269-78.

68. Gibault L, Ferreira C, Pérot G, Audebourg A, Chibon F, Bonnin S, et al. From PTEN loss of expression to RICTOR role in smooth muscle differentiation: complex involvement of the mTOR pathway in leiomyosarcomas and pleomorphic sarcomas. Mod Pathol. 2012;25:197-211.

69. Cuppens T, Moisse M, Depreeuw J, Annibali D, Colas E, Gil-Moreno A, et al. Integrated genome analysis of uterine leiomyosarcoma to identify novel driver genes and targetable pathways. Int J Cancer. 2018;142:1230-43.

70. Oh JH, Jang SJ, Kim J, Sohn I, Lee JY, Cho EJ, et al. Spontaneous mutations in the single TTN gene represent high tumor mutation burden. NPJ Genom Med. 2020;5:33.

71. Beird HC, Wu CC, Ingram DR, Wang WL, Alimohamed A, Gumbs C, et al. Genomic profiling of dedifferentiated liposarcoma compared to matched well-differentiated liposarcoma reveals higher genomic complexity and a common origin. Cold Spring Harb Mol Case Stud. 2018;4.

72. Denis CJ, Deiteren K, Hendriks D, Proost P, Lambeir AM. Carboxypeptidase M in apoptosis, adipogenesis and cancer. Clin Chim Acta. 2013;415:306-16.

73. Koehler K, Liebner D, Chen JL. TP53 mutational status is predictive of pazopanib response in advanced sarcomas. Ann Oncol. 2016;27:539-43.

74. van der Velden DL, Hoes LR, van der Wijngaart H, van Berge Henegouwen JM, van Werkhoven E, Roepman P, et al. The Drug Rediscovery protocol facilitates the expanded use of existing anticancer drugs. Nature. 2019;574:127-31.

75. Priestley P, Baber J, Lolkema MP, Steeghs N, de Bruijn E, Shale C, et al. Pan-cancer whole-genome analyses of metastatic solid tumours. Nature. 2019;575:210-6.

76. van Dessel LF, van Riet J, Smits M, Zhu Y, Hamberg P, van der Heijden MS, et al. The genomic landscape of metastatic castration-resistant prostate cancers reveals multiple distinct genotypes with potential clinical impact. Nat Commun. 2019;10:5251.

77. Stephens PJ, Tarpey PS, Davies H, Van Loo P, Greenman C, Wedge DC, et al. The landscape of cancer genes and mutational processes in breast cancer. Nature. 2012;486:400-4.

78. Martincorena I, Raine KM, Gerstung M, Dawson KJ, Haase K, Van Loo P, et al. Universal Patterns of Selection in Cancer and Somatic Tissues. Cell. 2017;171:1029-41.e21.

79. Alexandrov LB, Kim J, Haradhvala NJ, Huang MN, Tian Ng AW, Wu Y, et al. The repertoire of mutational signatures in human cancer. Nature. 2020;578:94-101.

80. Petljak M, Alexandrov LB, Brammeld JS, Price S, Wedge DC, Grossmann S, et al. Characterizing Mutational Signatures in Human Cancer Cell Lines Reveals Episodic APOBEC Mutagenesis. Cell. 2019;176:1282-94 e20.
81. Angus L, Smid M, Wilting SM, van Riet J, Van Hoeck A, Nguyen L, et al. The genomic landscape of metastatic breast cancer highlights changes in mutation and signature frequencies. Nat Genet. 2019;51:1450-8.
82. Christensen S, Van der Roest B, Besselink N, Janssen R, Boymans S, Martens JWM, et al. 5-Fluorouracil treatment induces characteristic T>G mutations in human cancer. Nat Commun. 2019;10:4571.
83. Huang MN, McPherson JR, Cutcutache I, Teh BT, Tan P, Rozen SG. MSIseq: Software for Assessing Microsatellite Instability from Catalogs of Somatic Mutations. Sci Rep. 2015;5:13321.

Supplemental data

Supp. Table 1. Overview of included patients, data presented in figures all data presented and quantified in this manuscript. Table too large for print, will be electronically available when published.

Supplementary Table 2. Overview of the fusion genes.

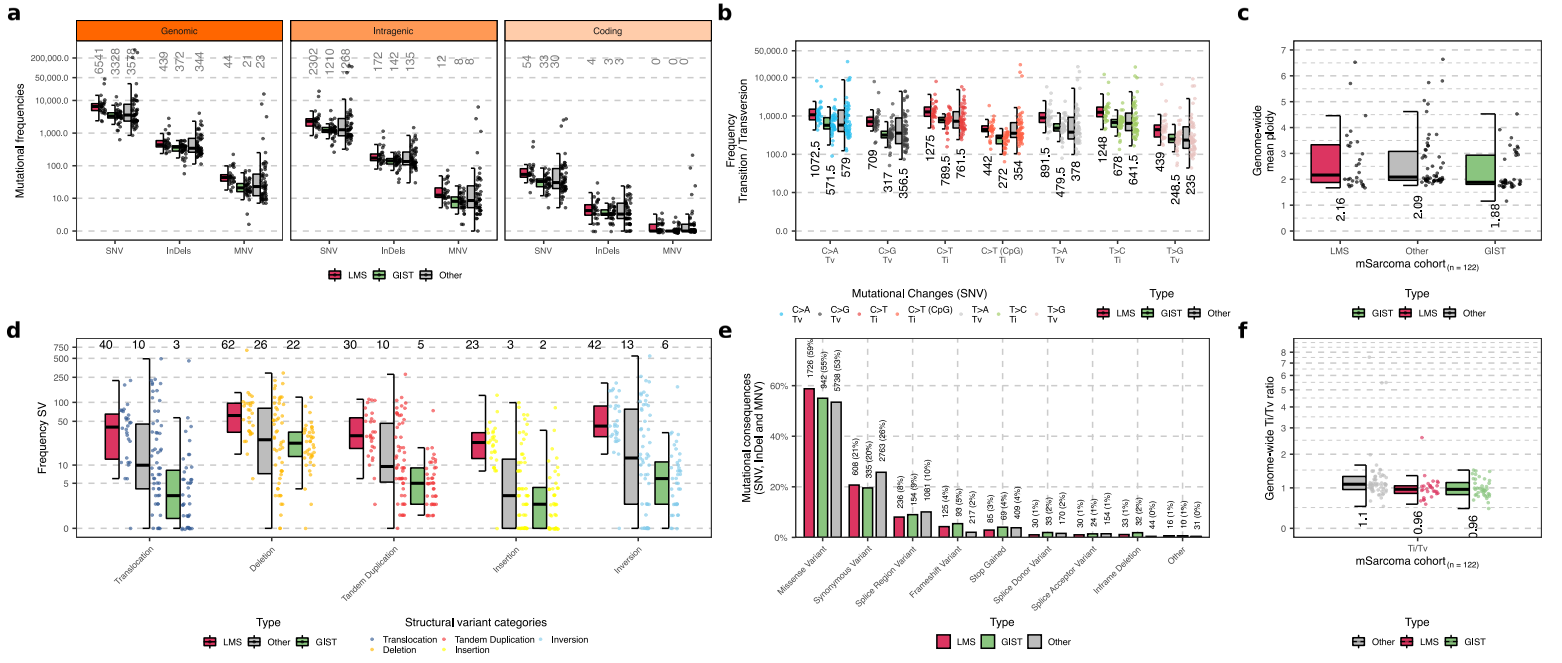
STS subtype	N	Translocation	Fusion product
Liposarcoma	5	t(12;16)(q13;p11)	*FUS-DDIT3*
Rhabdomyosarcoma	3	t(2;13)(q35;q14)	*PAX3-FOXO1*
Synovial sarcoma	3	t(X;18)(p11;q11)	*SS18-SSX1*
Clear cell sarcoma	1	t(12;22)(q13;q12)	*EWSR1-ATF1*
Myofibroblastic sarcoma	1	inv(2)(p23;q13)	*RANBP2-ALK*
Solitary fibrous tumor	2	inv(12)(q13;q13)	*NAB2-STAT6*
Fibrosarcoma	1	inv(12)(q13;q13)	*NAB2-STAT6*
Angiosarcoma	1	inv(12)(q13;q13)	*NAB2-STAT6*
Endometrial stromal sarcoma	1	t(7;17)(p15;q11)	*JAZF1-SUZ12*
MPNST	1	t(X;19)(q13;q13)	*CIC-FOXO4*
Liposarcoma	1	t(12;12)(q14;14)	*HMGA2-WIF1*
Fibrosarcoma	1	t(11;22)(p11;q12)	*EWSR1-CREB3L1*
Spindle cell sarcoma	1	t(14;22)(q32;q12)	*EWSR1-YY1*

STS: soft tissue sarcoma, GIST: gastrointestinal stromal tumor, MPNST: malignant peripheral nerve sheath tumor

▶Supp. Figure 1 - Overview of participating Dutch centers in the CPCT-02 mSTS cohort and sequencing characteristics.

a) Absolute numbers and relative frequencies of distinct patients included in the CPCT-02 mSTS cohort per participating center within the Netherlands.
b) Boxplot with individual data points of the estimated (*in silico*) tumor cell percentages based on the whole genome sequencing data with the observed median displayed.
c) Boxplot with individual data points of the mean read-coverages (WGS) of the peripheral blood reference (blue) and biopsy tissues (red) with the observed median per variable displayed.
d) Composition of the mSTS cohort with subcohorts indicated.
e) Age distribution stratified by gender of the mSTS cohort with the observed median per variable displayed in a boxplot with individual data points.

a
included patient(s)
0
10
20
30
40
NKI-AVL
Radboudumc
UMC Groningen
Erasmus MC
Maastricht UMC
UMC Utrecht
Isala
VUmc
Elisabeth-TweeSteden Ziekenhuis
Meander Medisch Centrum
Deventer Ziekenhuis
Massstad Ziekenhuis
Onze Lieve Vrouwe Gasthuis
Ziekenhuis Groep Twente
Leids Universitair Medisch Centrum
27 (22.1%)
21 (17.2%)
18 (14.8%)
15 (12.3%)
10 (8.2%)
9 (7.4%)
6 (4.9%)
5 (4.1%)
3 (2.5%)
2 (1.6%)
2 (1.6%)
1 (0.8%)
1 (0.8%)
1 (0.8%)
1 (0.8%)
b
Estimated tumor cell percentage
0%
25%
50%
75%
100%
78%
TC%
mSarcoma cohort(n = 122)
c
Mean sequencing coverage (WGS)
0x
25x
50x
75x
100x
125x
150x
175x
38
103
Peripheral blood
Tumor biopsy
d
Abs. Frequency
0
10
20
30
40
40 (32.8%)
28 (23%)
10 (8.2%)
8 (6.6%)
7 (5.7%)
7 (5.7%)
7 (5.7%)
4 (3.3%)
4 (3.3%)
2 (1.6%)
1 (0.8%)
1 (0.8%)
1 (0.8%)
1 (0.8%)
1 (0.8%)
GIST
Leiomyosarcoma
Liposarcoma
Rhabdomyosarcoma
Angiosarcoma
Fibrosarcoma
NOS
Myxofibrosarcoma
Synovial sarcoma
Atypical fibroxanthoma
MPNST
Clear cell sarcoma
Endometrial stromal sarcoma
Pleomorphic dermal sarcoma
Spindle cell sarcoma
e
Age at biopsy
10
20
30
40
50
60
70
80
90
58
61
Female (n = 57)
Male (n = 65)
mSarcoma cohort(n = 122)



▲Supp. Figure 2 - Overview of mutational landscape.

a) Number of SNVs, InDels and MNVs per whole genome sequenced sample over three resolutions; genome-wide, within intragenic regions and within coding regions with the observed median per variable displayed. Data is categorized in three mSTS cohorts (GIST, LMS, other).

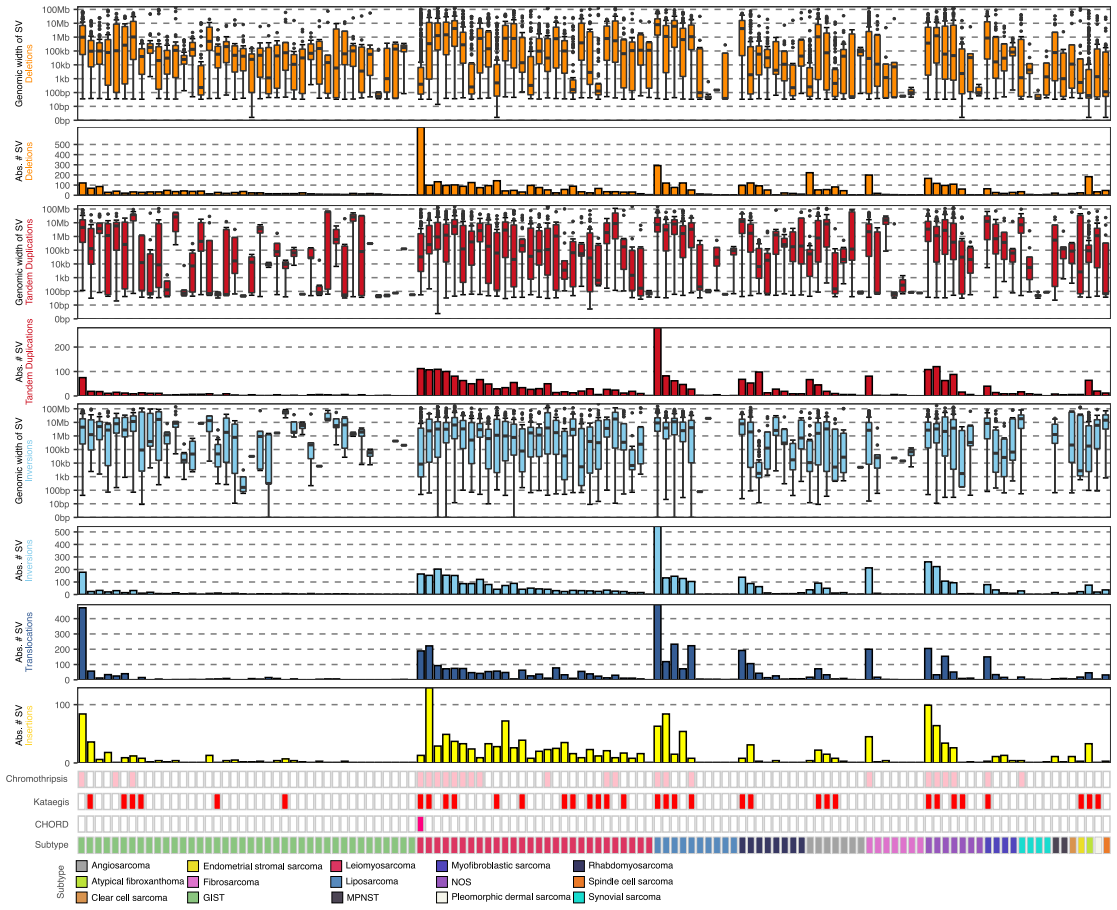
b) Type of genome-wide SNVs. Transition (Ti) and transversion (Tv), with a special attention for C to T (Ti) in CpG context, are indicated per cohort with observed median per variable displayed. Data is categorized in three mSTS cohorts (GIST, LMS, other).

c) Mean genome-wide tumor ploidy based on all autosomal chromosomes with observed median displayed. Data is categorized in three mSTS cohorts (GIST, LMS, other).

d) Frequency of inter-chromosomal translocations, deletions, tandem duplications, insertions and inversions are indicated per cohort with observed median per variable displayed. Data is categorized in three mSTS cohorts (GIST, LMS, other).

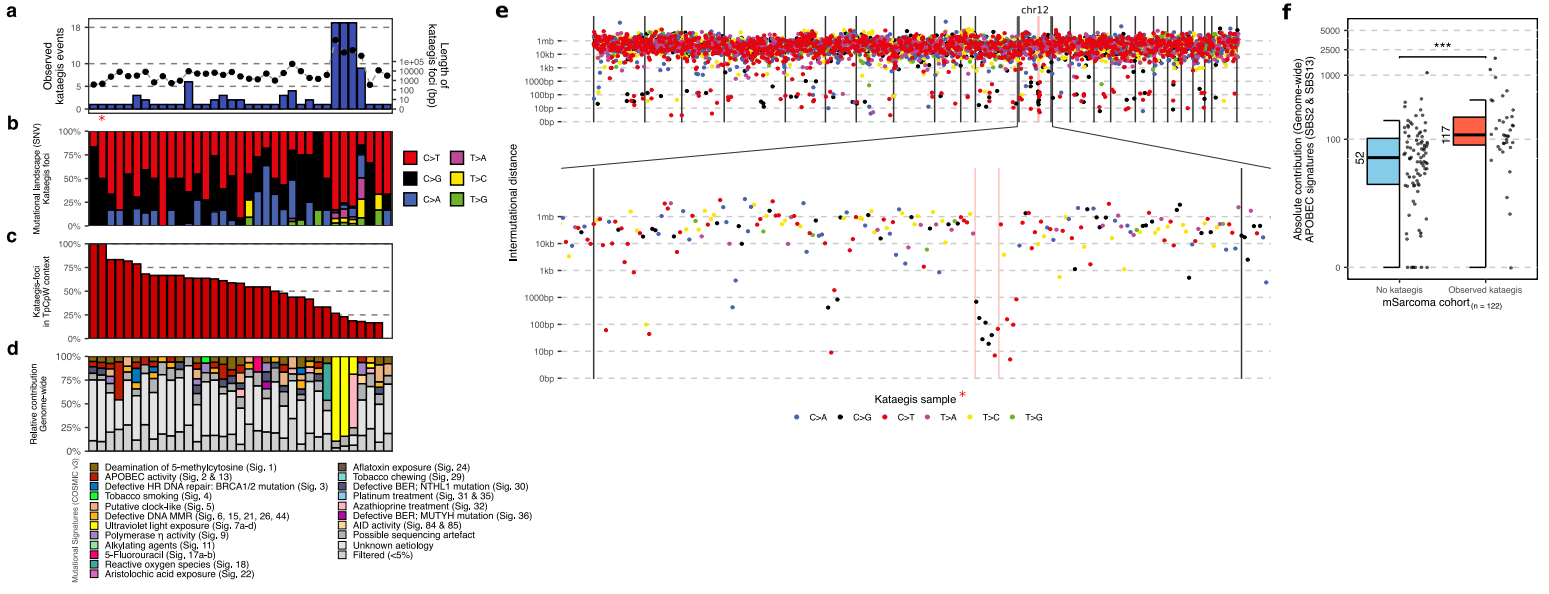
e) Mutational consequences of genomic variants overlapping genes using Ensembl Variant Effect Predictor (VEP). Data is categorized in three mSTS cohorts (GIST, LMS, other).

f) Genome-wide ratio of transitions (Ti) over transversion (Tv) with observed median displayed. Data is categorized in three mSTS cohorts (GIST, LMS, other).



▲Supp. Figure 3 - Overview of the distribution of somatically-acquired structural variants.
Overview of the genomic sizes and numbers of structural variants present in the mSTS cohort. Samples are sorted based on sarcoma subtype and decreasing number of total observed structural variants over all categories (deletions, tandem duplications, inversions, translocations and insertions).

a) Track displays boxplots representing the genomic width of deletions; Y-axis is in log10-scale. Lower track displays the total number of observed deletions.
b) Track displays boxplots representing the genomic width of tandem duplications; Y-axis is in log10-scale. Lower track displays the total number of observed tandem duplications.
c) Track displays boxplots representing the genomic width of inversions; Y-axis is in log10-scale. Lower track displays the total number of observed inversions.
d) Total number of observed translocations.
e) Total number of observed insertions.
f) Presence of chromothripsis, kataegis and/or predicted HRD (CHORD). Bottom track displays sarcoma subtypes (for color code see Figure 1).



▲Supp. Figure 4 - Observed kataegis events within the mSTS cohort.

a) Number of observed kataegis foci in the mSTS cohort (found in 35 distinct samples, blue bars) and the respective cumulative genomic width of all observed kataegis foci per sample (right y-axis; black points).

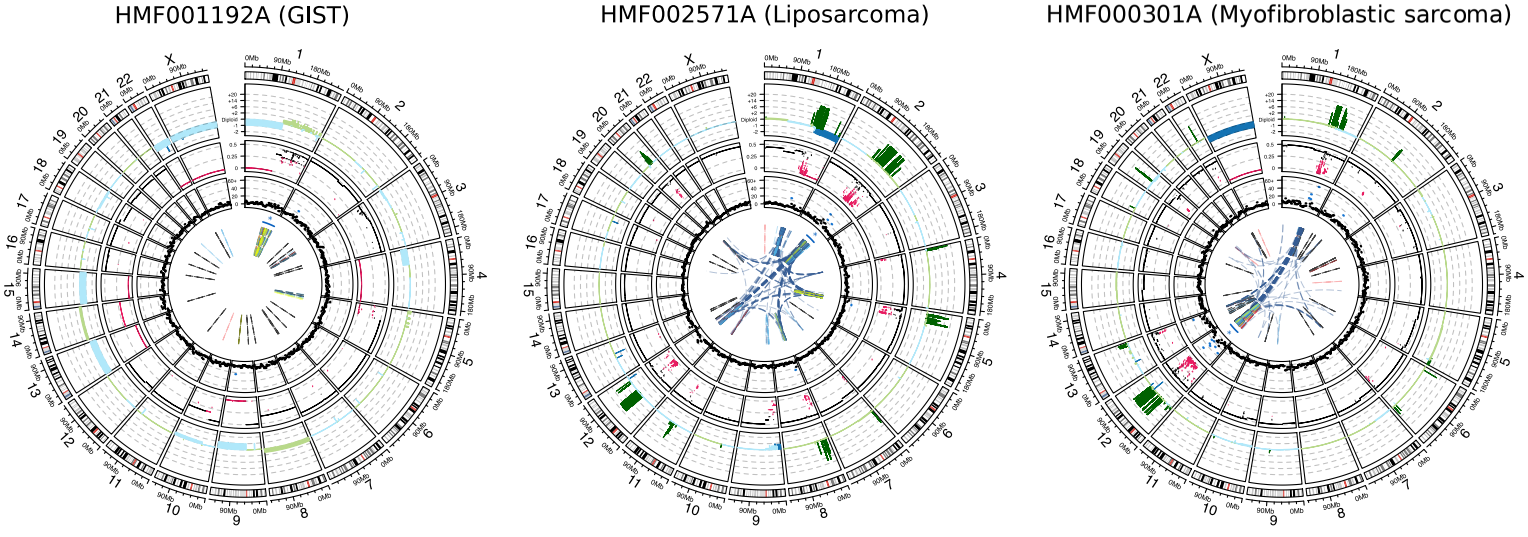
b) Relative frequency of SNV categories found in all observed kataegis foci per sample.

c) Relative frequency of SNV in observed kataegis foci with APOBEC-related TpCpW mutational context. W stands for T or A changes.

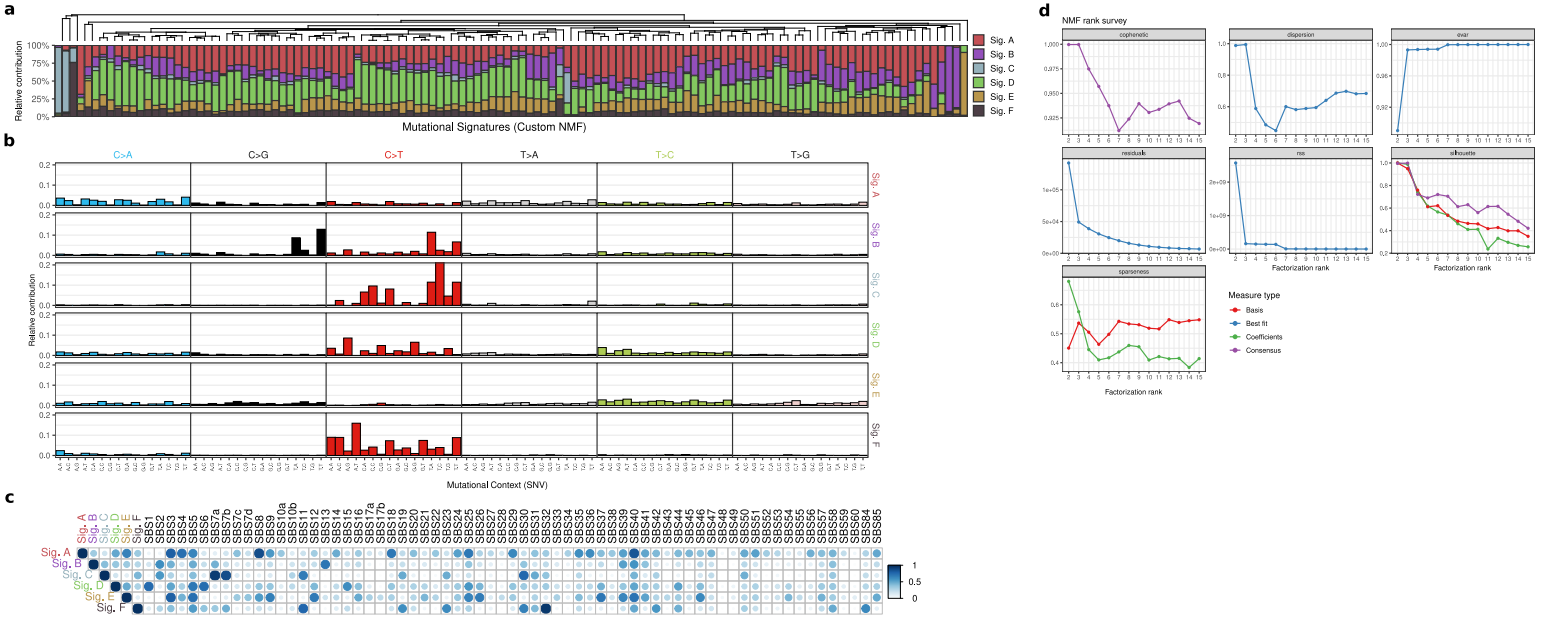
d) Genome-wide relative contribution to mutational signatures (COSMIC v3) for the respective mSTS sample.

e) Representation of a single kataegis focus on chromosome 12 within a single mSTS sample (highlighted with * in a). SNV (colored on pyrimidine mutations) are shown with relative genomic distances (in log10) to neighboring SNV. Observed kataegis focus on chromosome 12 is highlighted by a transparent red background.

f) Absolute mutational contribution of APOBEC COSMIC (v3) signatures (2 & 13) for samples without (N=87) and with observed kataegis foci (N=35). Statistical significance was tested with Wilcoxon rank-sum test and is indicated with *≤0.05, **≤0.01 and ***≤0.001.



▲Supp. Figure 5 - Genomic overview of mSTS displaying chromothripsis-like events.
Genomic representations of three chromothripsis-harboring mSTS. The outer track displays the genomic ideogram, the second-outer track displays copy-number profiles (amplification in light green; deep amplification beyond sample-specific threshold (GISTIC2) in dark green, deletions in blue; deep deletions beyond sample-specific threshold (GISTIC2) in dark blue). The third track displays TC%-corrected lower allele-frequency (LAF) values of individual copy-number segments (LAF≤0.33 in pink; LAF ≥0.33 in black). The fourth track displays the number of mutations per 5 Mbp, ranging from 0 to 60+; bins with ≥20 mutations are highlighted in blue. The fifth track highlights the regions harboring chromothripsis in a blue line. The innermost track displays the breakpoints of the structural variants; inter-chromosomal translocations in dark blue, deletions in gray, insertions in yellow, inversion in light blue and tandem duplications in red. Depicted are three representative mSTS samples: a gastrointestinal stromal tumor (GIST), a liposarcoma and a myofibroblastic sarcoma.



▲Supp. Figure 6 - *De novo* mutational signatures assessment in mSTS.

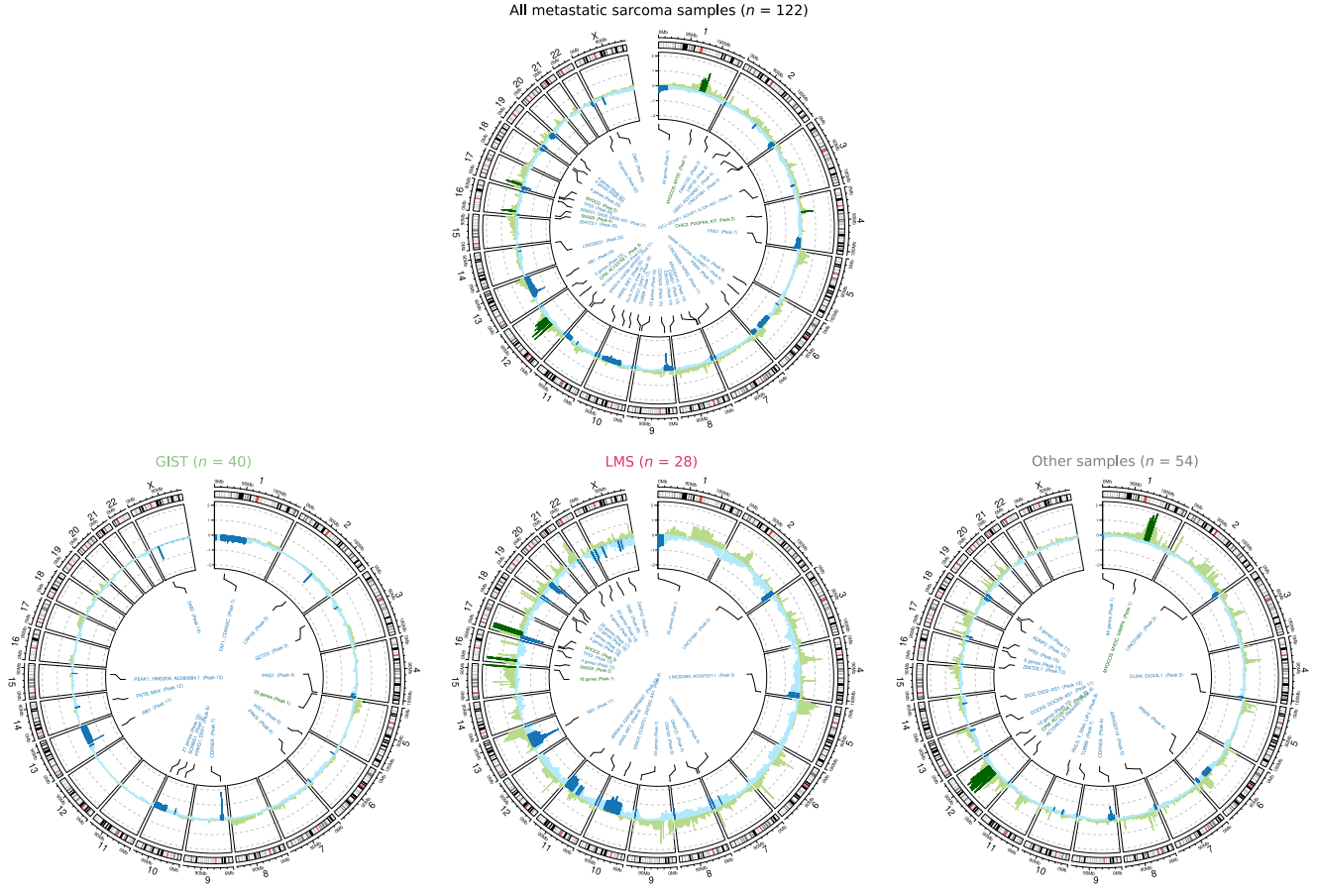
Assessment and comparison of extracted *de novo* single base substitution mutational signatures (N=6; Sig. A-F) using non-negative matrix factorization (NMF) within the mSTS cohort against the known COSMIC (v3; N=67) signatures.

a) Overview of extracted *de novo* single base substitution mutational signatures (N=6; Sig. A–F) per mSTS. mSTS are sorted based on unsupervised clustering (Ward.D; Euclidean distance; distances plotted in log10-scale) of the relative contribution of the six *de novo* mutational signatures.

b) Trinucleotide mutational contexts of the six extracted *de novo* signatures.

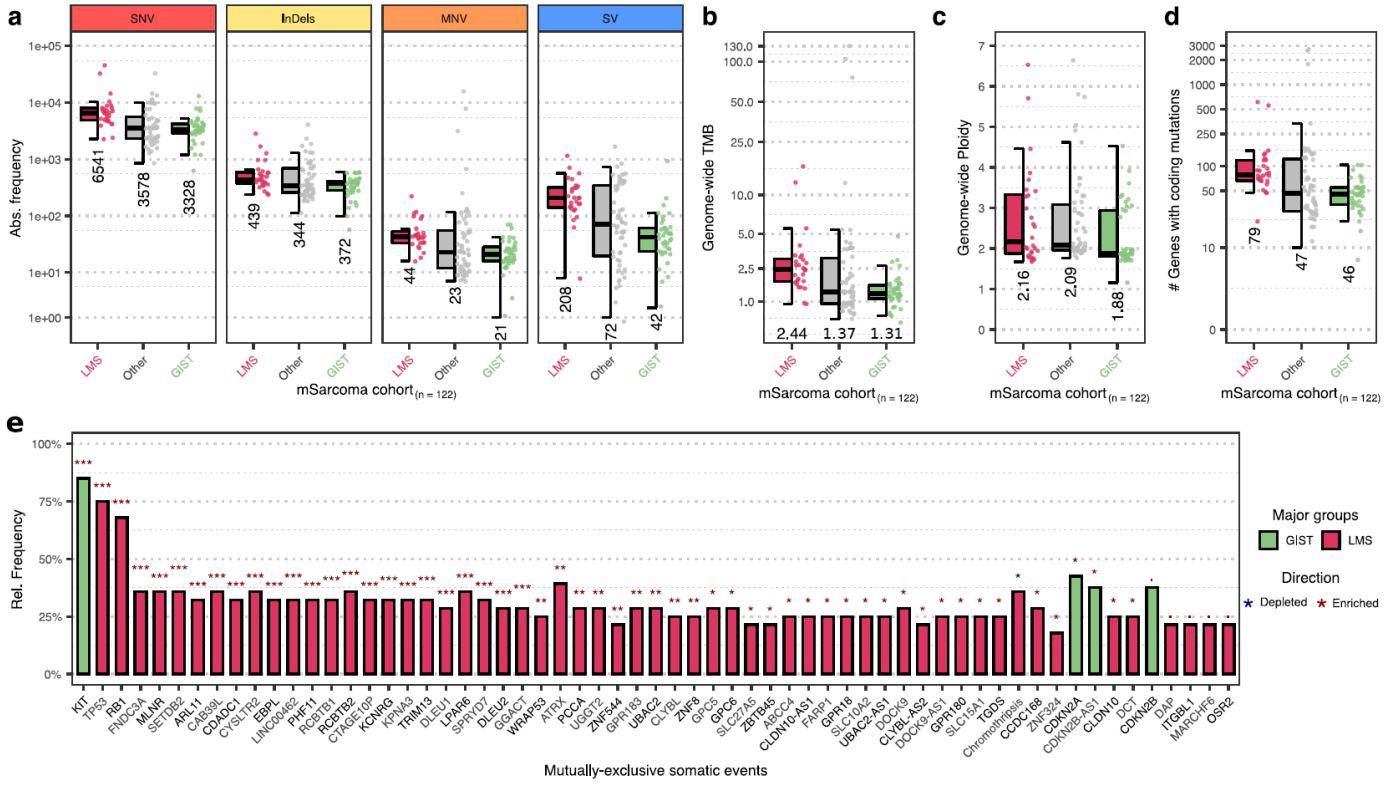
c) Cosine similarity of the *de novo* mutational signatures against the known COSMIC v3 signatures (N=67).

d) NMF quality metrics using between two to fifteen ranks over 1,000 iterations.



▲Supp. Figure 7 - Copy-number overview of mSTS cohort and subcohorts with recurring focal amplifications and deletions highlighted (GISTIC2) and unbiased driver gene analysis.

Circosplots with ideogram of recurrent copy-number aberrations as detected by GISTIC2 per sub-cohort (as shown above each circosplot). G-scores are depicted on the y-axis. Regions with amplifications (G-score >0) are depicted in green and deletions (G-score <0) in blue. Regions with significant (and recurring) copy-number aberrations (q≤0.1) are denoted with a darker shade of green or blue indicating amplification or deletion, respectively. Per region, the foci of maximal amplification or deletion (focal peaks; q≤0.1) are denoted in the inner track; the peak identifiers with associated genes are also listed and presented in Supplementary Table 1.



▲Supp. Figure 8 - Genomic characteristics per mSTS subcohort.

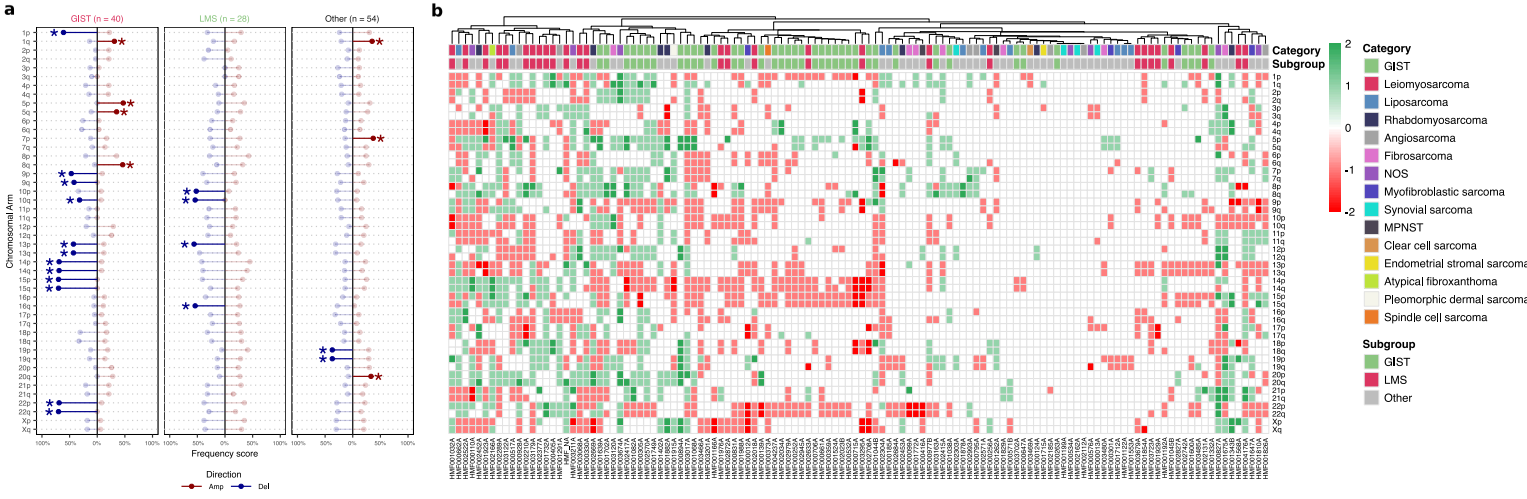
a) Number of SNVs, InDels, MNVs and SVs per whole-genome mSTS with the observed median per variable displayed. Data is categorized on mSTS subcohorts (GIST, LMS, other).

b) Tumor mutational burdens (genome-wide; log10), with the observed median per variable displayed. Data is categorized on mSTS subcohorts (GIST, LMS, other).

c) Mean genome-wide ploidy, with the observed median per variable displayed. Data is categorized on mSTS subcohorts (GIST, LMS, other).

d) Number of genes harboring somatic coding mutations, with the observed median per variable displayed. Data is categorized on mSTS subcohorts (GIST, LMS, other).

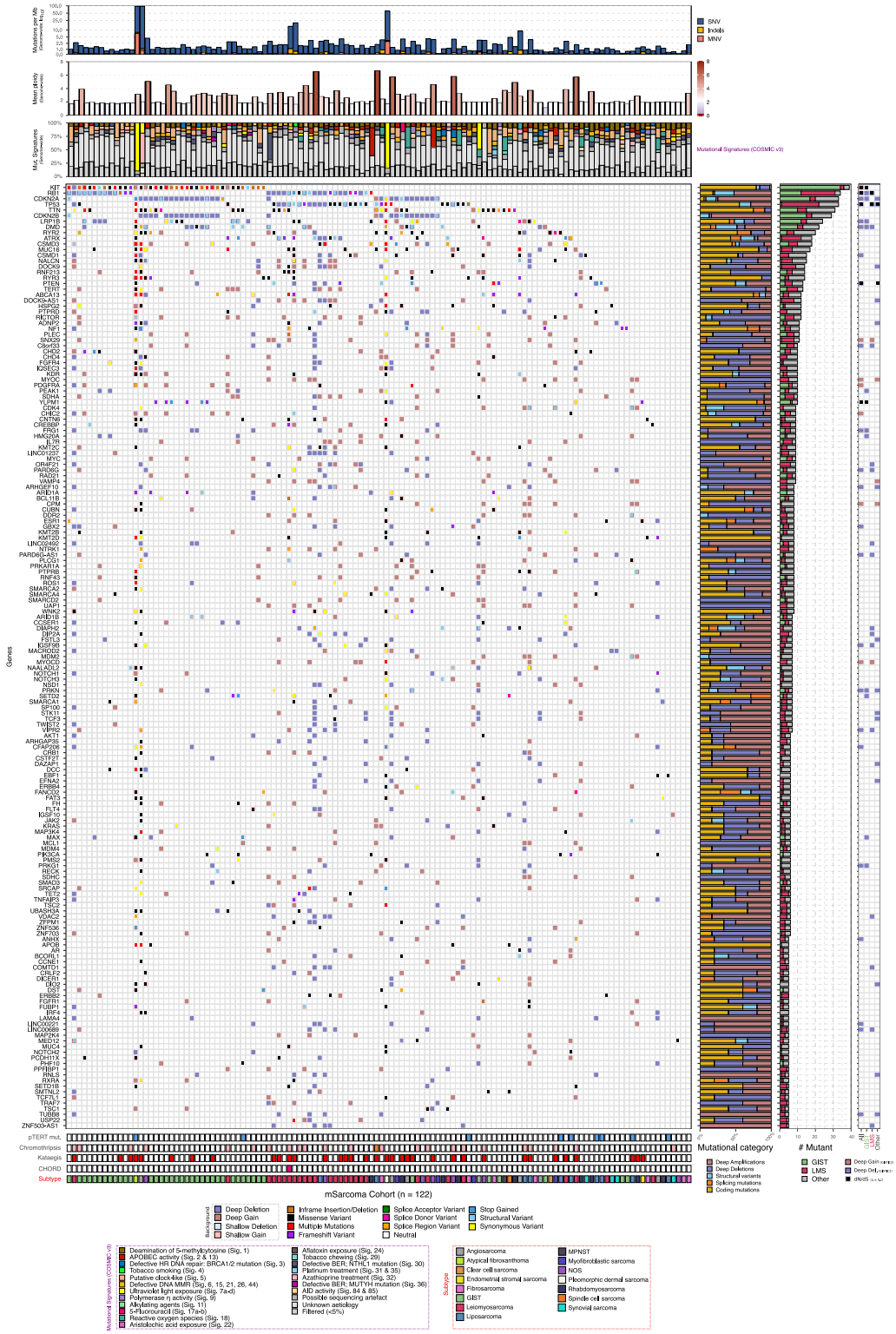
e) Mutational enrichment of mutant genes (mutations and copy-number alterations) and large-scale events (kataegis and chromothripsis) between two distinct mSTS subgroups (GIST, LMS). Statistical significance was tested using a one-sided Fisher's Exact Test with Benjamini-Hochberg correction; significance is denoted by ***(q≤0.001), **(q≤0.01), *(q≤0.05) and .(q≤0.1).



▲Supp. Figure 9 - Copy-number aberrations of chromosomal arms per mSTS subtype.

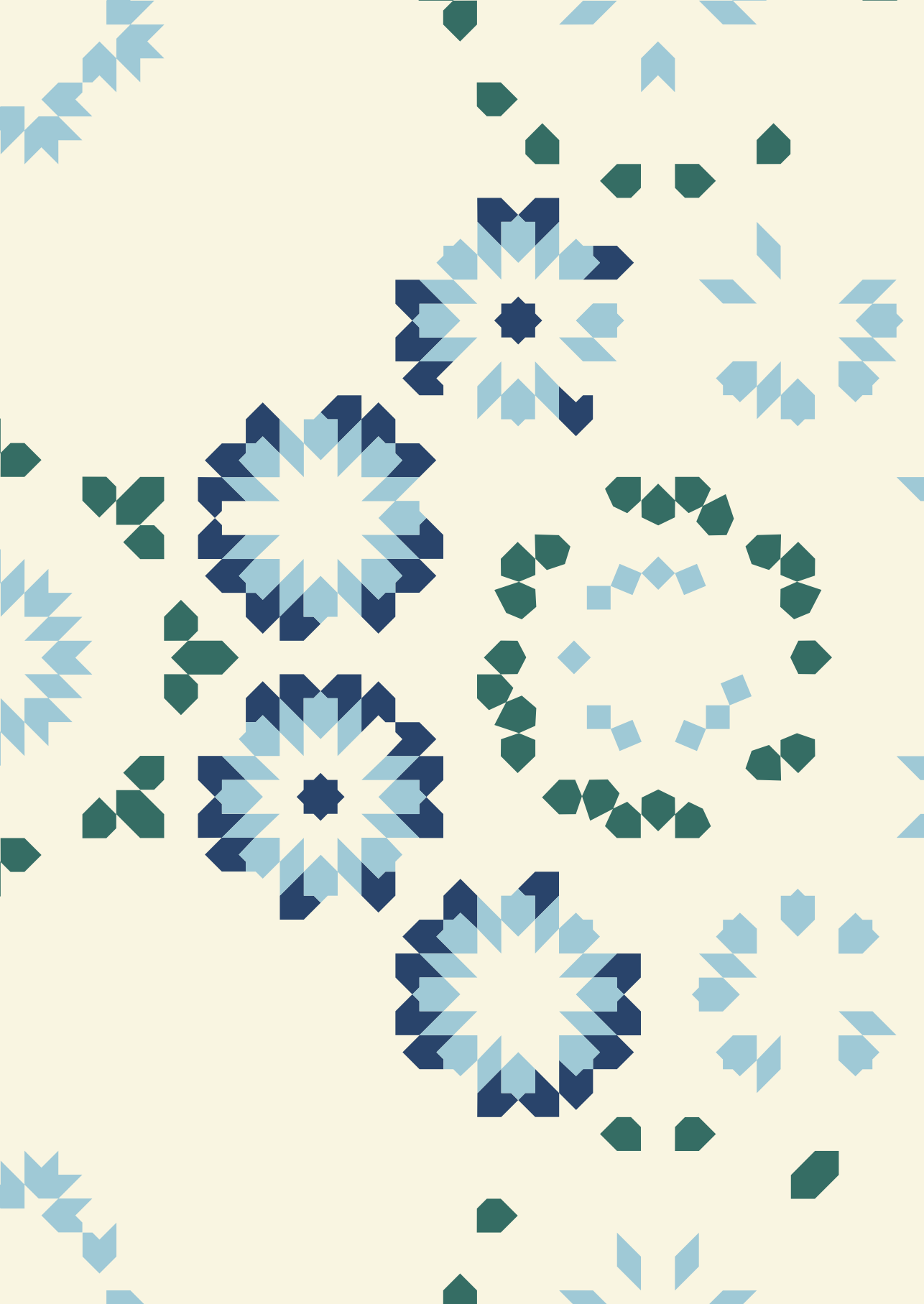
a) Overview of the relative frequency of samples with amplifications (brown) and losses (blue) per arm within the given subgroup. Statistically significant (q≤0.05) arm-level copy-number aberrations are depicted with an asterisk whilst the non-significant events are shown as transparent.

b) Unsupervised clustering (Euclidean distances, Ward.D2 method) of the mSTS samples based on the categorization of chromosomal arm copy-number aberrations, based on the GISTIC2 value per p-arm and q-arm. Top color bars depict the mSTS subtypes and mSTS sub-cohorts.


Mutations per Mb (Synonymous + Non-synonymous)
SNV
Indels
MNV
Mean ploidy (Descendant)
Mut. Signatures (COSMIC v3)
Mutational Signatures (COSMIC v3)
Genes
KIT
RB1
CDKN2A
TP53
TTN
CDKN2B
LRP1B
DMD
RYR2
ATRX
CSMD3
GRM3
OBSCN
NALCN
DOCK9
RNF213
BIRC6
PTEN
TERT
ABCA13
DOCK5-AS1
HSPG2
PTPRB
RICTOR
AZIN2
PIK3C2G
NF1
SNX29
C9orf33
CHD2
CHD4
FGFR4
IQSEC3
KDR
MYOC
PDGFRA
PEAK1
SDHA
YLPM1
CHD1
CNTN6
CR1|SELP
FRG1
HMGB2|A
IL7R
KMT5C
LINC01237
MYC
OR4F21
PARD3|S
RAD21
VAMP4
ARHGEF10
ASXL1
BCL11B
CIPM
COBN
DDR2
ESR1
GBX2
KMT2B
KMT2D
LINC02465
NTRK1
PARD6G-AS1
PIK3R1
PRKDC|A
PTPN14
RNF43
ROS1
SMARCA2
SMARCA4
SMARCD2
UAP1
WNK2
ARID1B
CCSER1
DNPH2
DIP2A
FBXL2
FSIP2
MACROD2
MDN1
MYO5B
NAALAD2
NOTCH1
NOTCH3
NSD1
PRKN
SETD2
SMARCA1
SPTA1
STK11
TCF3
TMEM132
AKT1
ARHGAP35
CFAP299
CRB1
CSTF2T
DAZAP1
DCC
EBF1
EFNA5
ERBB4
FANCD2
FAT3
FH
FLT4
IGSF10
JAK1
KRAS
MAP3K4
MAX
MCL1
MDM4
PIK3CA
PMS2
PRKG1
RECK
SDHC
SMAD3
SPEN
TET2
TNFAIP3
TSC2
UBASH3A
VDAC2
ZFPM1
ZNF598
ZNF703
ANHX
APOB
AR
BCORL1
CCNE1
COMTD1
DICER1
DNMT3A
DST
ERBB2
FGFR1
PUBP1
IRF4
LAMA4
LINC00321
LINC00689
MAP2K4
MED12
MUC4
NCOR1
PCSK7
PHF10
PPP1R1B
RNL5
RXRA
SETD1B
SMTNL2
TCF7L1
TRAF7
TSC1
TUBB8
USP22
ZNF500-AS1
pTERT mut.
Chromothripsis
Kataegis
CHORD
Subtype
Mutational category
Deep Amplifications
Deep Deletions
Structural variants
Splicing mutations
Coding mutations
Mutant
GIST
LMS
Other
Deep Gain n=
Deep Del n=
dNdS q < 0.1
GIST
LMS
Other
mSarcoma Cohort (n = 122)
Background
Deep Deletion
Deep Gain
Shallow Deletion
Shallow Gain
Inframe Insertion/Deletion
Missense Variant
Multiple Mutations
Frameshift Variant
Splice Acceptor Variant
Splice Donor Variant
Splice Region Variant
Stop Gained
Structural Variant
Synonymous Variant
Neutral
Mutational Signatures (COSMIC v3)
Deamination of 5-methylcytosine (Sig. 1)
APOBEC activity (Sig. 2 & 13)
Defective HR DNA repair: BRCA1/2 mutation (Sig. 3)
Tobacco smoking (Sig. 4)
Putative clock-like (Sig. 5)
Defective DNA MMR (Sig. 6, 15, 21, 26, 44)
Ultraviolet light exposure (Sig. 7a-d)
Polymerase η activity (Sig. 9)
Alkylating agents (Sig. 11)
5-Fluorouracil (Sig. 17a-b)
Reactive oxygen species (Sig. 18)
Aristolochic acid exposure (Sig. 22)
Aflatoxin exposure (Sig. 24)
Tobacco chewing (Sig. 29)
Defective BER; NTHL1 mutation (Sig. 30)
Platinum treatment (Sig. 31 & 35)
Azathioprine treatment (Sig. 32)
Defective BER; MUTYH mutation (Sig. 36)
AID activity (Sig. 84 & 85)
Possible sequencing artefact
Unknown aetiology
Filtered (<5%)
Subtype
Angiosarcoma
Atypical fibroxanthoma
Clear cell sarcoma
Endometrial stromal sarcoma
Fibrosarcoma
GIST
Leiomyosarcoma
Liposarcoma
MPNST
Myofibroblastic sarcoma
NOS
Pleomorphic dermal sarcoma
Rhabdomyosarcoma
Spindle cell sarcoma
Synovial sarcoma

◂ Supp. Figure 10 - Putative drivers and soft tissue sarcoma associated genes within the mSTS cohort as detected by unbiased discovery (dN/dS, GISTIC2) and literature.
Overview of putative drivers harboring coding mutations within mSTS. Depicted are putative drivers as detected by dN/dS and/or GISTIC2 and supplemented with additional STS-associated drivers from the literature. mSTS and genes are sorted based on mutual exclusivity of the depicted putative drivers. Only GISTIC2 focal peaks with deep amplifications and deletions are shown.

a) Number of genomic mutations per megabase over the entire genome (TMB); SNV, InDel and MNV are depicted in blue, orange and salmon respectively. Y-axis is shown in log10-scale.
b) Mean genome-wide ploidy, ranging from 0 to 8 (octaploid). Diploidy is shown in white.
c) Relative contribution of the COSMIC single-base substitution mutational signatures (v3; N=67). Signatures with less than 5 percent overall contribution within the entire mSTS cohort were categorized under the "Filtered (<5%)" category. The proposed etiology of the signatures is denoted below.
d) Overview of coding mutation(s) per mSTS, background colors depict copy-number aberrations whilst the inner square depicts the type of (coding) mutation(s). The adjacent bar plots represent the relative proportions of mutational categories (coding mutations (SNVs, InDels and MNVs), splicing mutations, SVs, deep gains (high-level amplifications resulting in many additional copies) and deep deletions (high-level losses resulting in (near) homozygous losses) per gene. The middle-outer barplot depicts the percentage of GIST (green), LMS (red) and other (grey) mSTS which harbored a mutation. In addition, dN/dS and/or GISTIC2 support are shown on the outer-right bar plot for either the entire mSTS cohort (all) or separate subcohorts (GIST, LMS, other); GISTIC2 results are colored light purple if these genes were detected within a recurrent focal deletion and salmon if detected within a recurrent focal gain.
e) Presence of TERT promoter mutations; mSTS with TERT mutations are shown in blue.
f) Presence of chromothripsis; mSTS with chromothripsis are shown in pink.
g) Presence of kataegis; mSTS with kataegis are shown in red.
h) Status of homologous recombination deficiency (HRD) as determined by CHORD, mSTS with BRCA1/2-associated HRD ($p \geq 0.5$) are shown in red, otherwise colored white.
i) Soft tissue sarcoma subtypes are indicated below.



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.

Abstract

Background: Liposarcomas are heterogeneous soft tissue sarcomas of adipocytic origin. Well-differentiated liposarcoma (WDLPS) and dedifferentiated liposarcoma (DDLPS) are the most common liposarcoma subtypes and are molecularly characterized by the presence of a neo-chromosome which consists of the amplified 12q13-15 region. This region includes well-known oncogenes such as *MDM2* and *CDK4* as well as microRNAs. As microRNAs can be closely involved in sarcomagenesis, we investigated the expression levels and functional roles of microRNAs located in the 12q13-15 region in WDLPS and DDLPS.

Methods: RT-PCR was used to quantitatively measure the expression of miRNAs in normal fat (n=17), lipomas (n=14), WDLPS (n=35), DDLPS (n=31) and relevant cell lines. Transient transfections using microRNA mimics and microRNA LNA™ inhibitors were used to modulate microRNA levels in WDLPS and DDLPS cell lines after which cellular proliferation and apoptosis induction were determined. Western blotting and RT-PCR were used to verify potential mRNA targets of selected microRNAs.

Results: Based on their location on 12q13-15 and proximity to either *MDM2* or *CDK4* miR-26a-5p, miR-616-3p, miR-1228-5p, miR-1279-5p, miR-3913 and miR-6759-5p were examined. MiR-26a-5p was found to be overexpressed in DDLPS compared to fat, lipomas and WDLPS. In addition, miR-3913-3p and miR-3913-5p were overexpressed in WDLPS and in DDLPS compared to fat and lipomas. None of the other miRNAs was consistently differentially expressed between tumor and control tissues. In cell lines, miR-26a-5p was found to be expressed both in WDLPS and DDLPS cells, although a 13.5-fold higher expression was observed in DDLPS cells. MiR-3913-3p, but not miR-3913-5p, was expressed in both WDLPS and DDLPS cell lines at approximately equal levels. Inhibition of miR-26a-5p and miR-3913-3p decreased cellular proliferation of both WDLPS and DDLPS cell lines, whereas microRNA mimics had no effect. Inhibition of miR-3913-3p induced apoptosis in WDLPS cell lines but not in DDLPS. In contrast, inhibition of miR-26a-5p did not affect apoptosis. MiR-26a-5p was suspected to regulate the tumor suppressor PTEN in the context of WDLPS and DDLPS.

Conclusion: MiR-26a and miR-3913 overexpression, most likely due to their amplification on the neo-chromosome in WDLPS and DDLPS, promotes sarcoma development by stimulating cellular proliferation and – to a lesser extent – suppressing apoptosis. MiR-26a-5p is suspected to target the tumor suppressor PTEN. It is concluded that in addition to the protein coding genes present on the neo-chromosome in WDLPS and DDLPS also non-protein coding genes, like microRNAs, may play essential roles in sarcomagenesis of WDLPS and DDLPS.

Introduction

Liposarcoma is one of the most common soft tissue sarcomas (STS), accounting for approximately 20% of all STS and 10-15% of all mesenchymal neoplasms [1-3]. Based on morphological and genetic features, four subtypes can be distinguished: well-differentiated liposarcoma/atypical lipomatous tumors (WDLPS/ALT), dedifferentiated liposarcoma (DDLPS), myxoid liposarcoma (MLPS) and pleomorphic liposarcoma (PLPS) [4]. WDLPS and DDLPS differ from each other in terms of clinical behavior: WDLPS can be locally aggressive but barely has any metastatic potential and WDLPS patients generally have a good prognosis. In contrast, DDLPS does have metastatic capabilities and therefore a more dismal prognosis [4]. At a genetic level, WDLPS and DDLPS are characterized by the presence of a neochromosome which consists of amplified 12q13-15 chromosomal regions and includes the oncogenes *MDM2*, *CDK4* and *HMGA2* amongst others [5, 6]. The 12q13-15 region also harbors several microRNA (miRNA) genes that may be co-amplified, overexpressed and possibly play a role in liposarcoma development.

MiRNAs are a class of small single-stranded non-coding RNAs of approximately 20-22 nucleotides, involved in post-transcriptional regulation of gene expression [7]. Most commonly, miRNAs bind in the context of the RNA-induced silencing complex (RISC) to the 3' untranslated region (3'UTR) of target mRNAs. The miRNA-mRNA interaction causes translational inhibition and/or mRNA degradation, thereby reducing target gene expression levels. Since one miRNA may regulate multiple mRNAs and one mRNA can be regulated by multiple miRNAs, miRNAs can form regulatory networks. MiRNAs have proven to be dysregulated in cancer and to play essential roles in tumorigenesis and tumor progression [8-11], also in liposarcomas [12-16].

Previous research showed that miR-26a-2, located on 12q near *CDK4*, was amplified and upregulated in WDLPS and DDLPS [12, 17, 18]. Overexpression of miR-26a-2 was associated with poor survival, enhanced proliferation and migration, and induced resistance to apoptosis *in vitro* [17]. In addition to miR-26a, a total of 5 other miRNAs (miR-616, miR-1228, miR-1279, miR-3913, miR-6759) were found to be located in the proximity of the protein coding genes known to be recurrently amplified and overexpressed in WDLPS an DDLPS (Supplementary Figure S1). The aim of this study was to confirm the overexpression of miR-26a-2, to evaluate the expression levels of other potentially amplified miRNAs in the 12q13-15 region in WDLPS and DDLPS, and to assess the cellular effects of these miRNAs *in vitro*.

Materials and methods

Tumor and control tissue samples

From the Erasmus MC tissue bank, fresh frozen samples of human liposarcomas (WDLPS, n=35 and DDLPS, n=31) and control tissue (normal fat, n=16 and lipomas, n=14) were obtained. Patient, tumor and control tissue characteristics are listed in Table 1. Hematoxylin-eosin stained sections of all samples were examined by expert pathologists to verify the liposarcoma subtype, lipoma and normal fat classification. The percentage of tumor cells in the liposarcoma tissues was estimated at >90%. The experimental protocol was submitted for review to and approved by the Medical Ethics Committee Erasmus MC of Rotterdam (MEC-2016-213). All experimental procedures, including the use of human tissues samples, were performed in accordance with the relevant guidelines and regulations, adhering to the code of conduct for medical research as laid out by the council of the Federation of Dutch Medical Scientific Societies (https://www.federa.org/codes-conduct). The use of anonymous or coded left-over material for scientific purposes is part of the standard treatment agreement with patients and therefore informed consent was not required according to Dutch law.

Table 1. Patient, tumor and control tissue characteristics

Tissue/tumor type	Sex	N	Age at time of diagnosis*	Location	N
Normal adipose tissue (N=16)	Male	4	58.5 (48.8-61.5)	Extremity	1
				Retroperitoneal/abdominal	5
	Female	12		Trunk	5
				Scrotum/inguinal	5
Lipoma (N=14)	Male	9	59 (48.5-65.5)	Extremity	9
	Female	5		Trunk	5
Well-differentiated liposarcoma (N=35)	Male	19	59 (45-64)	Extremity	21
				Retroperitoneal/abdominal	11
	Female	16		Trunk	1
				Scrotum/inguinal	2
Dedifferentiated liposarcoma (N=31)	Male	16	59 (52-68.5)	Extremity	5
				Retroperitoneal/abdominal	23
	Female	15		Head	1
				Scrotum/inguinal	2

*Presented as median (interquartile range)

Cell culture

The WDLPS cell lines 93T449, 94T778 and 95T1000 were a kind gift from Dr. F. Pedeutour (Nice University Hospital, Nice, France). The DDLPS cell lines LPS224, LPS246 and LPS863 were acquired from Dr. Y Lu (M.D. Anderson Cancer Center, Houston, Texas, USA) and the

DDLPS cell line FU-DDLS-1 was obtained from Dr. J. Nishio (Fukuoka University, Fukuoka, Japan). The WDLPS cell lines and FU-DDLS-1 were maintained in RPMI 1640 (Gibco Life Technologies) supplemented with 10% fetal calf serum and a final concentration of 2mM ALA-GLN (L-alanyl-L-glutamine; Sigma, cat. No. G8541) for FU-DDLS-1. LPS224, LPS246 and LPS863 were cultured in DMEM (Gibco Life Technologies) with 10% fetal calf serum. All culture media contained 100IU/ml penicillin and 100µg/mL streptomycin. The cell lines were routinely cultured in a humidified incubator at 37°C in a 5% CO_2 atmosphere. At regular intervals the cell lines were tested for mycoplasma infections.

RNA isolation

Total RNA from the frozen tissue samples and from the cell lines was isolated using RNAbee (Tel Test Inc.) according to the manufacturer's recommendations. RNA quantity and quality were determined using a Nanodrop-2000 (Thermo Fisher Scientific).

MicroRNA qPCR

The expression of selected miRNAs was assessed by RT-PCR using commercially available Taqman® MicroRNA assays (Applied Biosystems/ThermoFisher Scientific). Based on the expression information given in miRBase (www.mirbase.org) we opted to quantify the most abundant strand of each miRNA. When miRbase contained inconclusive data or when dealing with a relatively unknown miRNA, we quantified both the 5p and 3p mature strands. In brief, 50ng of isolated total RNA was reverse transcribed using the Taqman® MicroRNA reverse transcription kit (Applied Biosystems/ThermoFisher Scientific) and specific miRNA primers from the Taqman® MicroRNA assays according to recommended protocol, with each sample analyzed in duplicate. The resulting cDNA was used in a quantitative real-time PCR (qPCR) using a miRNA specific primer/probe mix together with the Taqman® Universal PCR Master Mix No AmpErase® UNG (Applied Biosystems) in a 7500 Fast Real-Time PCR system (Applied Biosystems). qPCR data was analyzed using SDS software (version 2.4, Applied Biosystems). A standard dilution series of a cDNA sample-pool was included on every plate allowing for the absolute quantification of the miRNA expression.

mRNA qPCR

cDNA was synthesized from 1µg total RNA using the High Capacity cDNA reverse Transcription Kit (Applied Biosystems/ThermoFisher Scientific) according to the manufacturer's protocol. mRNA expression levels were determined by RT-PCR using Taqman® Universal PCR Master mix and the specific Assay-On-Demand products (Applied Biosystems/ThermoFisher Scientific) on an ABI 7500 Real-Time PCR machine (Applied Biosystems). Each sample was analyzed in duplicate using the following assays: *PTEN* (*Hs02621230_s1*), *GAPDH* (*Hs99999905_m1*), *HPRT*

(*Hs02800695_m1*) and *PPIA* (*Hs99999904_m1*). *PTEN* expression was normalized against the average of *GAPDH, HPRT* and *PPIA* expression using the comparative C_T-method [19]. qPCR data was analyzed using SDS software (Applied Biosystems).

MicroRNA transfections

The cells were plated in 96-well or 24-well plates with a concentration of 7,500 or 40,000 cells/well in a total volume of 200µL or 500µL of standard cell culture medium without antibiotics. Cells were transfected after 24 hours with 50nM MiRDIAN miRNA mimics (Dharmacon) or 50nM miRCURY LNA™ inhibitors (Exiqon/Qiagen). As controls, a scrambled miRNA mimic Negative control #1 (Dharmacon) and the miRCURY LNA™ inhibitor Negative Control (Exiqon/Qiagen) were used. DharmaFECT 1 (Dharmacon) was used as transfection agent. For each cell line the transfection conditions were optimized (transfection efficiency > 90%) with a fluorescently labeled miRNA (MiRDIAN mimic transfection control DY547, Dharmacon).

SRB cell proliferation assay

Cell proliferation was assessed by a sulforodamine B (SRB) assay at 48, 72 and 96 hours post-transfection essentially as described by Keepers et al [20]. In short, the cells were fixed by 10% TCA (Merck) in PBS for 1h at 4°C, washed and stained with 0.4% SRB (Sigma-Aldrich) in 1% acetic acid for 15 minutes. Next, cells were washed in 1% acetic acid and air dried. Subsequently, the stain was released from the cells by 10mM Tris-Base after which the absorption was measured at 540nm by a spectrophotometer (Tecan).

Apoptosis assay

Cells were harvested by trypsinization 48 hours after miRNA inhibitor transfection. Apoptotic markers were quantified using the FITC Annexin V Apoptosis detection kit (Biolegend) according to the manufacturer's protocol. In short: the cells were washed in PBS, resuspended in Annexin V Binding buffer and stained with FITC Annexin V for 30min on ice. This was followed by staining with propidium iodide (PI) for 15min in the dark. The cells were analyzed by FACS using a 488nm laser with the emission filters 502LP and BP530/30nm for FITC and 655LP and BP695/40nm for PI on a FACS Aria III Flow Cytometer (BD Bioscience). Dead cells were defined as $PI^+/AnnexV^-$ cells, late apoptotic cells as $PI^+/AnnexV^+$ cells, early apoptotic cells as $PI^-/AnnexV^+$ cells and live cells as $PI^-/AnnexV^-$ cells.

Protein lysates, SDS-PAGE and Western Blotting

Total protein was extracted from cells 72 hours post-transfection using MCB lysis buffer (50mM Tris-HCl pH 7.5, 50mM NaCl, 10 % (v/v) glycerol, 1% (v/v) NP-40, 0.5% (w/v) Na-deoxycholate, 1mM Na_3VO_4, 20mM NaF) supplemented with a cocktail of protease and

phosphatase inhibitors. Lysates were thoroughly vortexed and further lysed by 2 freeze-thaw cycles. The protein concentration of lysates was determined with a Bradford assay (Bio-Rad). Equal amounts of total protein (8-15µg/lane) were subjected to SDS-PAGE and subsequently transferred to a PVDF membrane by electroblotting. The membrane was blocked with 5% (w/v) BSA in TBS-0.1% (v/v) Tween'20 or 5% (w/v) non-fat dry milk in TBS-0.1% (v/v) Tween'20 to saturate remaining protein binding sites on the PVDF membrane. Incubation with primary antibodies was executed in the same buffers using the following antibodies: rabbit monoclonal anti-PTEN (1:1,000, D4.3 XP®, Cell Signaling Technology) and mouse monoclonal anti-β-Actin (1:10,000, A5441, Sigma-Aldrich). As secondary antibody, HRP conjugated goat-anti-rabbit (1:10,000, Jackson ImmunoResearch) and goat-anti-mouse (1:10,000, Jackson ImmunoResearch) were used. Bound antibodies were visualized using enhanced chemiluminescence (SuperSignal West Pico Chemiluminescent substrate, ThermoFisher) and the ChemiDoc MP Imager (Bio-Rad). Protein expression was quantified using ImageLab Software (version 5.0, Bio-Rad).

Statistics

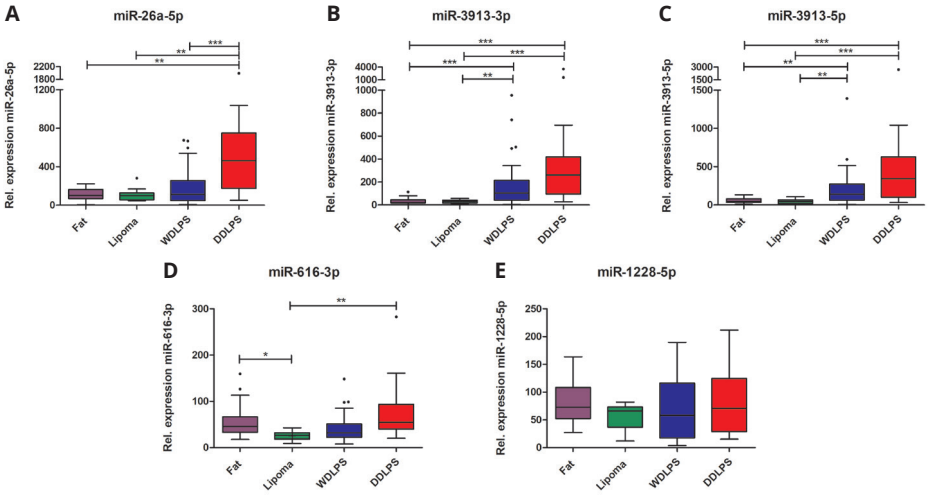
Kruskal-Wallis tests were used to assess the differences in miRNA expression between the liposarcoma subtypes and control tumor samples. The p-values were adjusted for multiple testing using the Bonferroni correction. Differences in miRNA expression in the WDLPS and DDLPS cell-lines were evaluated by Mann-Whitney U tests. The effect of miRNA modulation on cell-viability was tested with the one-way ANOVA with Bonferroni correction. Spearman's correlation was used to test the correlation between *PTEN* mRNA expression and miR-26a expression. For all statistical tests, a two-sided p-value ≤0.05 was considered statistically significant and marked by a single asterisk (*). P-values ≤0.01 were marked by double asterisks (**) and ≤0.001 by triple asterisks (***). All statistical tests were performed with SPSS (IBM).

Results

miR-26a-5p, miR-3913-3p and miR-3913-5p are overexpressed in liposarcomas

First, it was determined which of the selected miRNAs were overexpressed in WDLPS and DDLPS compared to control tissues such as lipomas and normal adipose tissue by using RT-PCR assays. The expression of miR-26a-5p was significantly higher in the DDLPS samples compared to WDLPS, lipoma and fat (Figure 1). Both miR-3913-3p and miR-3913-5p were found to be overexpressed in WDLPS/DDLPS compared to the control tissues (Figure 1). miR-616-3p expression levels were significantly higher in DDLPS and normal fat tissue than

in lipoma, but no consistent differences in expression were detected between control and tumor samples (Figure 1). The expression levels of miR-1228-5p between the tumor groups and control tissues were not different (Figure 1). No expression of miR-1279-5p and miR-6759-5p could be detected (C_T-values >35).

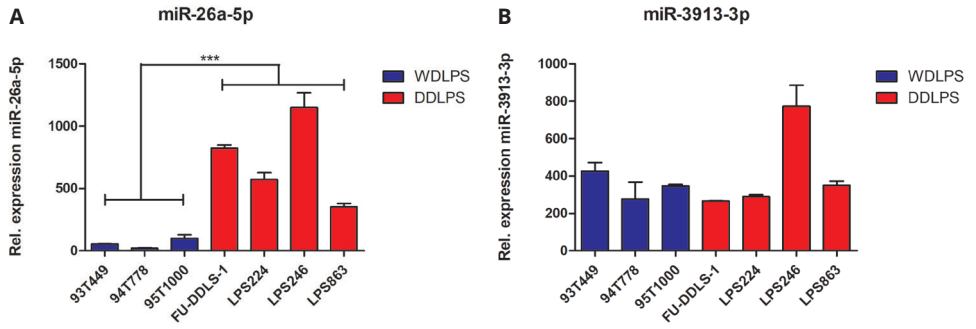


▲Figure 1. miR-26a-5p, miR-3913-3p and miR-3913-5p are overexpressed in liposarcomas.
A-C: The box plots represent the expression levels of miR-26a-5p (**A**), miR-3913-3p (**B**), miR-3913-5p (**C**) as determined by qPCR in an extended sample set of fresh frozen normal adipose tissue (fat) (n=16), lipomas (n=14), WDLPS (n=35) and DDLPS (n=31). **D-E:** The box plot represents the expression levels of miR-616-3p (**D**) and miR-1228 (**E**) as determined by qPCR in fresh frozen normal adipose tissue (fat) (n=16), lipomas (n=14), WDLPS (n=23) and DDLPS (n=15). Boxes indicate the 25th–75th percentile, the line in the box represents the median, whiskers show the lowest and highest values within the 1.5x interquartile range, black dots are outliers. Kruskal-Wallis tests were used to assess statistical differences, p values were adjusted for multiple testing by Bonferroni correction. P-value ≤0.05 (*); p-value ≤0.01 (**); p-value≤0.001 (***).

Effect of inhibiting miR-26a and miR-3913-3p expression on proliferation and apoptosis

Next, the expression levels of miR-26a-5p, miR-3919-3p and miR-3913-5p were measured in three WDLPS and four DDLPS cell lines to validate the expression of these miRNAs in cultured cells. In line with the tumor samples, miR-26a-5p was found significantly higher expressed in DDLPS cells than in WDLPS cells (Figure 2). Regarding the expression of miR-3913-3p, no significant differences in expression between the WDLPS and DDLPS cell lines were observed (Figure 2). Despite the results obtained in the tumor samples, miR-3913-5p could not be detected in the cell lines examined.

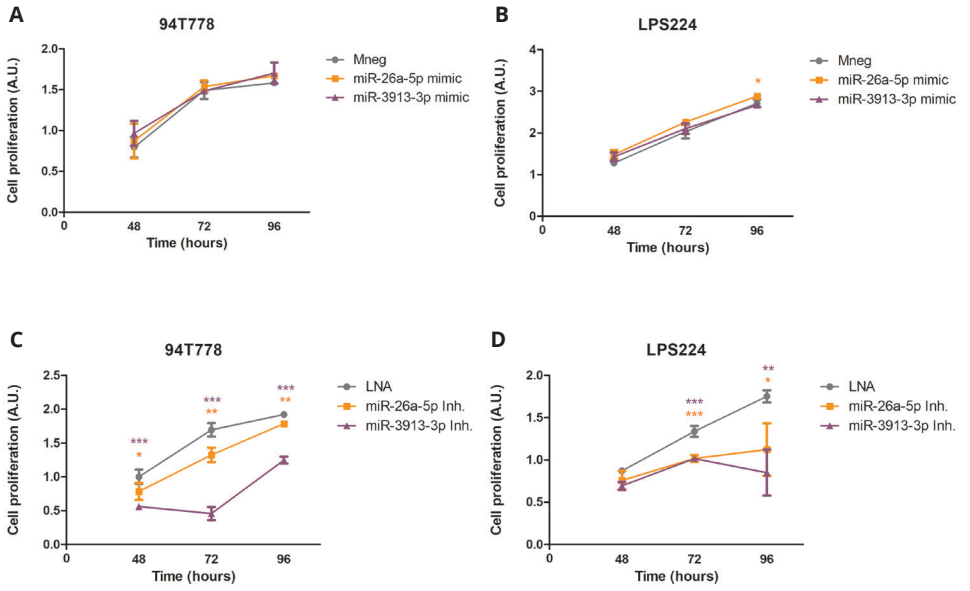
The cell lines 94T778 (WDLPS), 95T1000 (WDLPS), LPS224 (DDLPS) and LPS246 (DDLPS) were used for experiments in which expression of miR-26a-5p and miR-3913-3p levels were transiently modulated by transfection of specific miRNA mimics or miRNA antisense



▲Figure 2. miR-26a-5p and miR-3913-3p expression in WDLPS and DDLPS cell lines.
The expression of miR-26a-5p (**A**) and miR-3913-3p (**B**) was determined by qPCR in a panel of WDLPS cell lines (93T449, 94T778 an 95T100) and DDLPS cell lines (FU-DDLS-1, LPS224, LPS246, LPS863). The bar graph depicts mean values ±SD. Mann-Whitney U tests were used to determine statistical significance; p-value ≤0.001 (***).

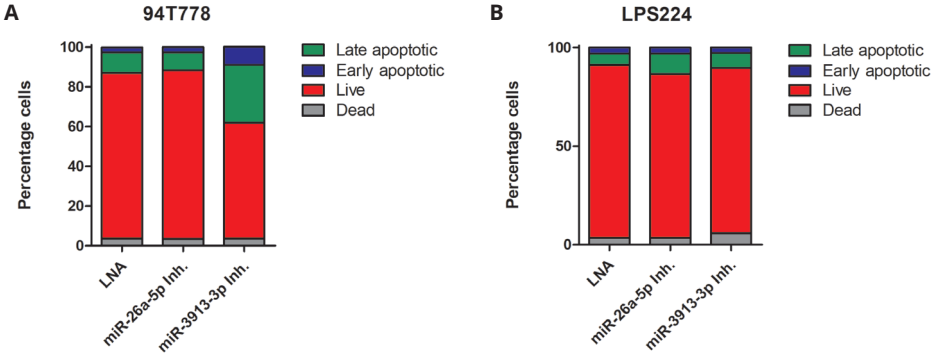
inhibitors. When the amount of miR-26a-5p or miR-3913-3p were increased through mimics, only a significant difference was observed in the cellular proliferation rate of the LPS224 cells after 96 hours of transfection, but not in the 94T778 cells (Figure 3A, Figure 3B). In contrast, when miR-26a-5p and miR-3913-3p expression levels were decreased by inhibitors, the proliferation rates were significantly reduced, with the strongest effects observed at 72 and 96 hours post-transfection (Figure 3C, Figure 3D). In the 95T1000 cell line, inhibition of miR-26a-5p also reduced cellular proliferation, although the reduction was not statistically significant, and miR-3913-3p inhibition did significantly block cell proliferation (Supplementary Figure S2A). In the DDLPS cell line LPS246 inhibition of miR-26a-5p and miR-3913-3p reduced cellular proliferation somewhat, particularly at the 96h time point, but only reached statistical significance for miR-26a-5p after 72h of transfection (Supplementary Figure S2B).

In order to determine whether the observed decrease in cellular proliferation upon miR-3913-3p and miR-26a-5p inhibition was the result of apoptosis induction, an apoptosis assay was performed following exposure to antisense miRNA inhibitors. A reduction of miR-3913-3p expression levels resulted in an increase of early and late apoptotic cells in the WDLPS cell lines 94T778 and 95T1000 at 48h post-transfection, whereas no induction of apoptosis was observed in the DDLPS cell lines LPS224 and LPS246 (Figure 4, Supplementary Figure S3). Also, no induction of apoptosis was detected upon miR-26a-5p inhibition (Figure 4, Supplementary Figure S3). Based on these findings it is concluded that the reduced cellular proliferation after lowering miR-26a-5p expression is not caused by the induction of apoptosis. Conversely, a reduction of miR-3913-3p levels did result in apoptosis induction, thereby explaining the severe inhibition of proliferation observed in the cell lines.



▲Figure 3. Inhibition of miR-26a-5p and miR-3913-3p affects proliferation rate of WDLPS and DDLPS cell lines.

The miRNA levels of miR-26a-5p and miR-3913-3p were modulated by transfecting the WDLPS cell line 94T778 (**A**) and DDLPS cell line LPS224 (**B**) with miR-26a-5p or miR-3913-3p mimics at t=0h. Secondly, the miRNA levels of miR-26a-5p and miR-3913-3p were modulated by transfecting the WDLPS cell line 94T778 (**C**) and DDLPS cell line LPS224 (**D**) with miR-26a-5p or miR-3913-3p inhibitors at t=0h. Upon transfection, cell viability was checked by SRB assay at 48h, 72h and 96h post-transfection as an indicator for cellular proliferation. A one-way ANOVA with Bonferroni correction was used to determine statistical significance. P-value ≤0.05 (*); p-value ≤0.01 (**); p-value≤0.001 (***). Data on the WDLPS cell line 95T1000 and DDLPS cell line LPS246 are presented in Supplementary Figure S2.

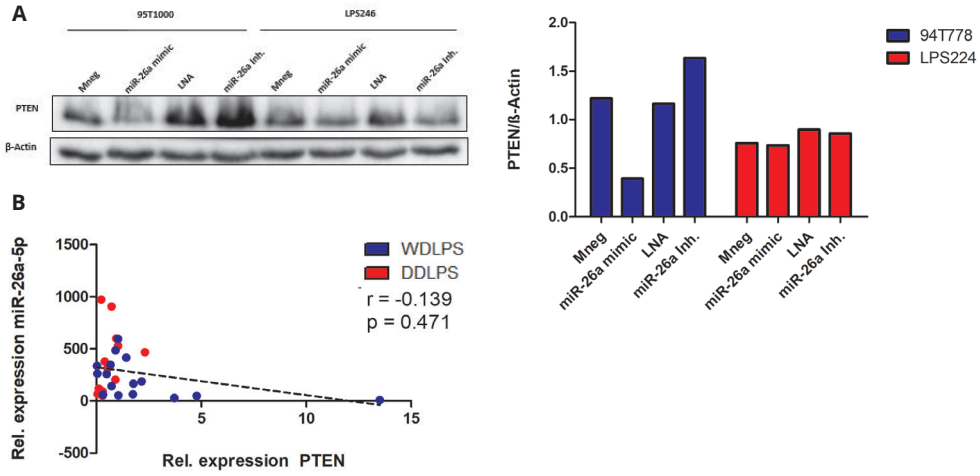


▲Figure 4. Effects of miR-26a-5p and miR-3913-3p inhibition on apoptosis induction in WDLPS and DDLPS cells.

FACS analysis of Annexin V/PI stained liposarcoma cell lines 94T778 (**A**) and LPS224 (**B**) transfected with miR-26a-5p or miR-3913-3p antisense inhibitors or scrambled control inhibitor (LNA) at 48h post-transfection. Live cells are presented by the Annexin V^-/PI^- fraction, early apoptotic cells by the Annexin V^+/PI^- fraction, late apoptotic cells by the Annexin V^+/PI^+ fraction and dead cells in the Annexin V^-/PI^+ fraction. Results of the cell lines 95T1000 and LPS246 are presented in Supplementary Figure S3.

miR-26a-5p potentially targets PTEN in WDLPS cells

As miR-26a-5p is known to regulate the tumor suppressor gene *phosphatase and tensin homolog* (*PTEN*) [21], it was investigated whether this miRNA targets PTEN in liposarcoma cell lines. First, the PTEN protein levels were determined in cell lysates of the WDLPS cell lines 94T778 and 95T1000, and the DDLPS cell lines LPS224 and LPS246 after transfection with either a miR-26a-5p mimic or antisense inhibitor. Figure 5A indicates that in 94T778 the PTEN levels were reduced when miR-26a is overexpressed and that PTEN protein levels were increased when miR-26a-5p is downregulated. A similar effect was observed in the WDLPS cell line 95T1000 (Supplementary Figure S4A). When DDLPS cell lines were used, the outcome was less consistent. MiR-26a-5p level modulations in LPS224 did not affect the PTEN protein levels (Figure 5A), while the miR-26a-5p mimic reduced PTEN levels in LPS246. However, miR-26a-5p inhibition did not result in a PTEN increase (Supplementary Figure S4A). When the miR-26a-5p expression values (measured by RT-PCR) in the WDLPS and DDLPS cell lines were plotted against the PTEN transcript levels, a significant negative correlation was observed confirming that miR-26a-5p targets PTEN mRNA, also in liposarcomas (Figure 5B). Similarly, in WDLPS and DDLPS tumor samples a negative correlation was observed between miR-26a-5p and PTEN mRNA expression which failed to reach significance (Supplementary Figure S4B).



▲Figure 5. miR-26a-5p expression levels exhibit inverse correlation with PTEN mRNA and protein levels.
(**A**) Cell lysates were prepared of the WDLPS cell line 94T778 and the DDLPS cell line LPS224 transfected with either miR-26a-5p mimics, miR-26a-5p antisense inhibitors or the appropriate scrambled controls (mneg for the mimics and LNA for the inhibitors). PTEN protein level in each of the lysates was measured by immunoblotting. The bar chart shows the quantified results of the immune-blot in which PTEN levels are normalized using the β-actin loading control. (**B**) miR-26a-5p and PTEN transcript levels were determined by RT-PCR in duplicate samples of 4 DDLPS cell lines (FU-DDLS-1; LPS224; LPS246; LPS863) and 3 WDLPS cell lines (93T449; 94T778; 95T1000). A Spearman's correlation was used to determine statistical significance.

Discussion

This study showed that co-amplified miRNAs, like miR-26a-5p and miR-3913-3p, located at the chromosome 12q13-15 region and present on the neo-chromosome in WDLPS and DDLPS, affect cancer-related processes like cellular proliferation and apoptosis. MiR-26a-5p was previously reported to be overexpressed in liposarcoma, notably WDLPS, DDLPS and myxoid/round cell liposarcoma [17]. The authors demonstrated that overexpression of miR-26a in liposarcoma cell lines enhanced growth and survival, whereas miR-26a inhibition resulted in opposite responses. Overexpression of miR-26a-5p correlated with poor patient survival in both WDLPS and DDLPS [17]. It is suggested that these effects are mediated through inhibition of *RCBTB1* by miR-26a. A more recent report from the same group implicated *HOXA5* as target for miR-26a in DDLPS: the downregulation of *HOXA5* observed in liposarcoma cell lines appeared to cause resistance to apoptotic death [22]. Our reports generally confirm the findings by Lee et al. [17], although in our experiments not all WDLPS and DDLPS cell lines respond equally strong to miR-26a-5p modulation. We provide evidence that miR-26a-5p targets the tumor suppressor *PTEN*, which has a key regulatory role in the PI3K/AKT/mTOR pathway, with *PTEN* loss-of-function promoting cell survival through PI3K/AKT signaling. Of note, the PI3K/AKT pathway, in particular AKT activation, has been implicated as a driver pathway in WDLPS and possibly DDLPS [23]. A recent overview on *PTEN* expression in soft tissue sarcomas also highlights its role in liposarcomas [24]. The other miRNA that was found overexpressed, miR-3913, is functionally rather ill-defined due to lack of studies and has not been linked to cancer before. It is of interest that the strongest cellular effects were seen when miR-3913-3p was inhibited, particularly in WDLPS cell lines. It might be that the dedifferentiation that occurred in DDLPS and the accompanying deregulated gene expression makes this subtype less dependent on miR-3913-3p overexpression. An intriguing observation was the lack of expression of miR-3913-5p in the cell lines, whereas expression of this miRNA was easily detected in clinical tumor samples. It is of relevance to functionally characterize miR-3913, both the 3p and 5p mature forms, in the liposarcoma context as it may reveal novel drug targets and further elucidate the full significance of the expression of amplified miRNAs for sarcomagenesis, tumor progression and liposarcoma treatment.

With regard to the specific genetic alterations observed in WDLPS and DDLPS, most attention has been given to protein coding genes like *MDM2* and *CDK4* that were found overexpressed. *MDM2*, murine double minute 2, is a negative regulator of TP53. Clinical trials in which MDM2 inhibitors were tested had limited success due to bone marrow toxicity and resulting thrombocytopenia [25, 26]. *CDK4*, cyclin-dependent kinase 4, is amplified in up to 90% of WDLPS and DDLPS. Initial clinical trials with CDK4/6 inhibitors, like palbociclib

and abemaciclib, displayed anti-tumor activity with prolonged disease stabilization with a median progression-free survival up to 30 weeks [27-29]. Inhibition of CDK4 activity prevents phosphorylation of RB1, thereby blocking cell cycle progression from the G1-phase to the S-phase. Follow-up phase 2 trails are ongoing [27]. Effective therapeutic treatments for WDLPS and particularly DDLPS are urgently needed, and it might be worthwhile to further explore miRNA-based therapeutics specifically targeting miR-26a-5p and/or miR-3913 [30].

Although the involvement of miR-26a-5p and miR-3913 in WDLPS and DDLPS is implied, this study has some limitations. First, expression levels of miR-26a-5p, miR-3913-3p and miR-3913-5p should be correlated to progression-free survival and overall survival in an appropriately sized WDLPS and DDLPS patient cohort. Secondly, the cellular phenotype of miR-3913 modulation should be investigated in greater detail using additional, well-characterized cell lines. These experiments can be exploited in a full functional characterization of miR-3913.

Conclusion

The findings reported in this study highlight the potentially essential role that non-coding RNAs may play in liposarcoma development. Further studies into the function of these transcripts in WDLPS and DDLPS may reveal novel biology in these tumors and point to novel therapeutic opportunities.

Acknowledgements

We would like to thanks G.J.L.H. van Leenders and M. den Bakker, whose expert knowledge of liposarcoma pathological features was very helpful to assess the fresh frozen tumor samples.

Funding

None.

Conflicts of interest

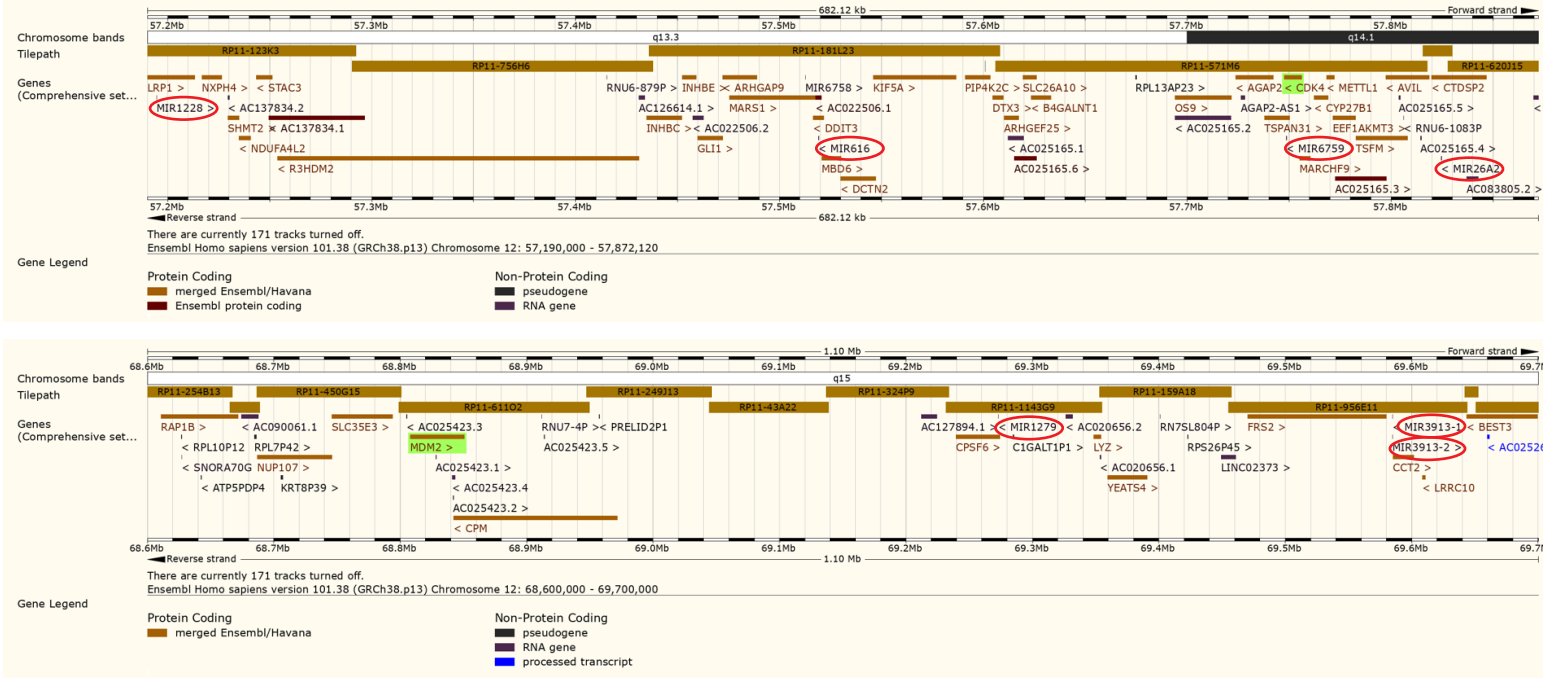
None of the authors declare any conflict of interest.

References

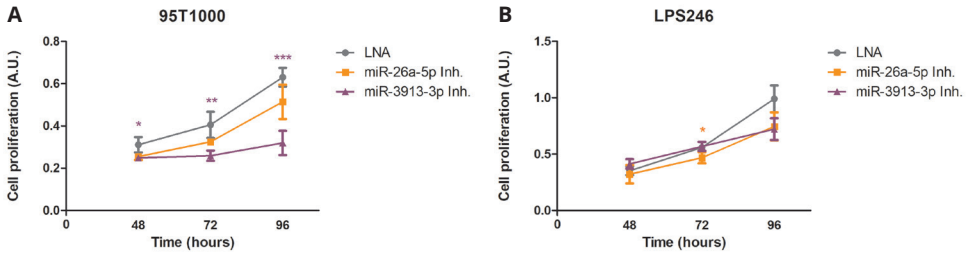
1. Amadeo B, Penel N, Coindre JM, Ray-Coquard I, Ligier K, Delafosse P, et al. Incidence and time trends of sarcoma (2000-2013): results from the French network of cancer registries (FRANCIM). BMC Cancer. 2020;20:190.
2. Ducimetiere F, Lurkin A, Ranchere-Vince D, Decouvelaere AV, Isaac S, Claret-Tournier C, et al. [Incidence rate, epidemiology of sarcoma and molecular biology. Preliminary results from EMS study in the Rhone-Alpes region]. Bull Cancer. 2010;97:629-41.
3. Toro JR, Travis LB, Wu HJ, Zhu K, Fletcher CD, Devesa SS. Incidence patterns of soft tissue sarcomas, regardless of primary site, in the surveillance, epidemiology and end results program, 1978-2001: An analysis of 26,758 cases. Int J Cancer. 2006;119:2922-30.
4. Fletcher CDM, Bridge JA, Hogendoorn PCW, Mertens F, World Health Organization, International Agency for Research on Cancer. WHO classification of tumours of soft tissue and bone. Lyon: IARC Press; 2013.
5. Dei Tos AP, Doglioni C, Piccinin S, Sciot R, Furlanetto A, Boiocchi M, et al. Coordinated expression and amplification of the MDM2, CDK4, and HMGI-C genes in atypical lipomatous tumours. J Pathol. 2000;190:531-6.
6. Garsed DW, Marshall OJ, Corbin VD, Hsu A, Di Stefano L, Schroder J, et al. The architecture and evolution of cancer neochromosomes. Cancer Cell. 2014;26:653-67.
7. Bartel DP. Metazoan MicroRNAs. Cell. 2018;173:20-51.
8. Wiemer EA. The role of microRNAs in cancer: no small matter. Eur J Cancer. 2007;43:1529-44.
9. Lujambio A, Lowe SW. The microcosmos of cancer. Nature. 2012;482:347-55.
10. Di Leva G, Garofalo M, Croce CM. MicroRNAs in cancer. Annu Rev Pathol. 2014;9:287-314.
11. Hayes J, Peruzzi PP, Lawler S. MicroRNAs in cancer: biomarkers, functions and therapy. Trends in Molecular Medicine. 2014;20:460-9.
12. Gits CM, van Kuijk PF, Jonkers MB, Boersma AW, Smid M, van Ijcken WF, et al. MicroRNA expression profiles distinguish liposarcoma subtypes and implicate miR-145 and miR-451 as tumor suppressors. Int J Cancer. 2014;135:348-61.
13. Kapodistrias N, Mavridis K, Batistatou A, Gogou P, Karavasilis V, Sainis I, et al. Assessing the clinical value of microRNAs in formalin-fixed paraffin-embedded liposarcoma tissues: Overexpressed miR-155 is an indicator of poor prognosis. Oncotarget. 2016.
14. Vincenzi B, Iuliani M, Zoccoli A, Pantano F, Fioramonti M, De Lisi D, et al. Deregulation of dicer and mir-155 expression in liposarcoma. Oncotarget. 2015;6:10586-91.
15. Sun R, Shen JK, Choy E, Yu Z, Hornicek FJ, Duan Z. The emerging roles and therapeutic potential of microRNAs (miRs) in liposarcoma. Discov Med. 2015;20:311-24.
16. Zhang P, Bill K, Liu J, Young E, Peng T, Bolshakov S, et al. MiR-155 is a liposarcoma oncogene that targets casein kinase-1alpha and enhances beta-catenin signaling. Cancer Res. 2012;72:1751-62.
17. Lee DH, Amanat S, Goff C, Weiss LM, Said JW, Doan NB, et al. Overexpression of miR-26a-2 in human liposarcoma is correlated with poor patient survival. Oncogenesis. 2013;2:e47.
18. Ugras S, Brill E, Jacobsen A, Hafner M, Socci ND, Decarolis PL, et al. Small RNA sequencing and functional characterization reveals MicroRNA-143 tumor suppressor activity in liposarcoma. Cancer Res. 2011;71:5659-69.
19. Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C(T) method. Nat Protoc. 2008;3:1101-8.

20. Keepers YP, Pizao PE, Peters GJ, van Ark-Otte J, Winograd B, Pinedo HM. Comparison of the sulforhodamine B protein and tetrazolium (MTT) assays for in vitro chemosensitivity testing. Eur J Cancer. 1991;27:897-900.
21. Huse JT, Brennan C, Hambardzumyan D, Wee B, Pena J, Rouhanifard SH, et al. The PTEN-regulating microRNA miR-26a is amplified in high-grade glioma and facilitates gliomagenesis in vivo. Genes Dev. 2009;23:1327-37.
22. Lee DH, Forscher C, Di Vizio D, Koeffler HP. Induction of p53-independent apoptosis by ectopic expression of HOXA5 in human liposarcomas. Sci Rep. 2015;5:12580.
23. Gutierrez A, Snyder EL, Marino-Enriquez A, Zhang YX, Sioletic S, Kozakewich E, et al. Aberrant AKT activation drives well-differentiated liposarcoma. Proc Natl Acad Sci U S A. 2011;108:16386-91.
24. Stefano S, Giovanni S. The PTEN Tumor Suppressor Gene in Soft Tissue Sarcoma. Cancers (Basel). 2019;11.
25. de Jonge M, de Weger VA, Dickson MA, Langenberg M, Le Cesne A, Wagner AJ, et al. A phase I study of SAR405838, a novel human double minute 2 (HDM2) antagonist, in patients with solid tumours. Eur J Cancer. 2017;76:144-51.
26. Jung J, Lee JS, Dickson MA, Schwartz GK, Le Cesne A, Varga A, et al. TP53 mutations emerge with HDM2 inhibitor SAR405838 treatment in de-differentiated liposarcoma. Nat Commun. 2016;7:12609.
27. Assi T, Kattan J, Rassy E, Nassereddine H, Farhat F, Honore C, et al. Targeting CDK4 (cyclin-dependent kinase) amplification in liposarcoma: A comprehensive review. Crit Rev Oncol Hematol. 2020;153:103029.
28. Dickson MA, Schwartz GK, Keohan ML, D'Angelo SP, Gounder MM, Chi P, et al. Progression-Free Survival Among Patients With Well-Differentiated or Dedifferentiated Liposarcoma Treated With CDK4 Inhibitor Palbociclib: A Phase 2 Clinical Trial. JAMA Oncol. 2016;2:937-40.
29. Dickson MA, Tap WD, Keohan ML, D'Angelo SP, Gounder MM, Antonescu CR, et al. Phase II trial of the CDK4 inhibitor PD0332991 in patients with advanced CDK4-amplified well-differentiated or dedifferentiated liposarcoma. J Clin Oncol. 2013;31:2024-8.
30. Slack FJ, Chinnaiyan AM. The Role of Non-coding RNAs in Oncology. Cell. 2019;179:1033-55.

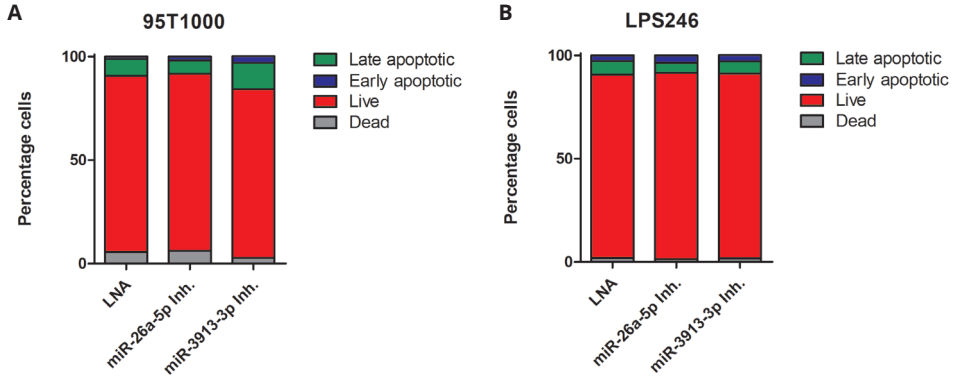
Supplemental information



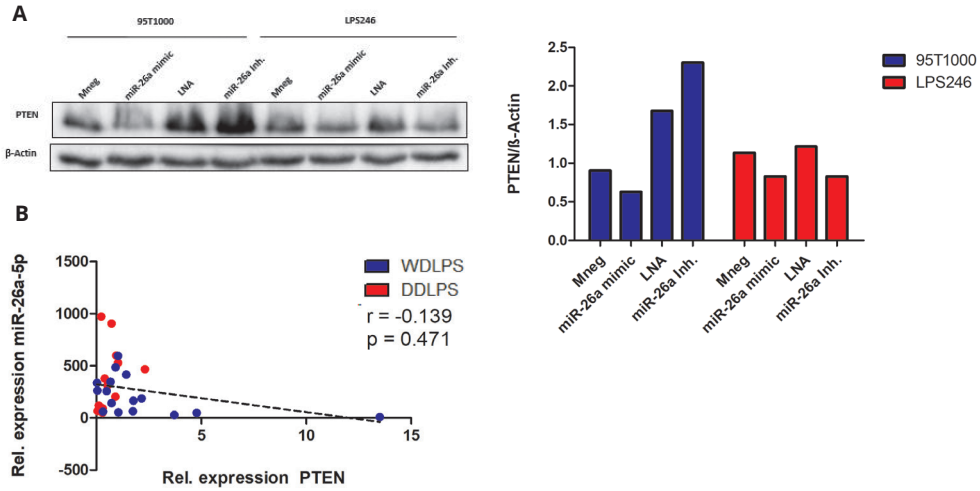
▲Supplemental Figure S1. Chromosomal location of selected miRNAs on chromosome 12q13-15 which is amplified in WDLPS and DDLPS.
Schematic overview of two parts of the chromosomal 12q13-15 region in humans derived from the ENSEMBL Genome Browser (release 101, August 2020). Indicated are the location and coding strands of protein and non-protein coding genes, including miRNAs, located in the proximity of the *CDK4* and *MDM2* genes, both highlighted in green. Red encircled are miRNAs selected for our study: miR-1228; miR-616; miR-6759; miR-26a-2; miR-1279 and miR-3913-1 and miR-3913-2.



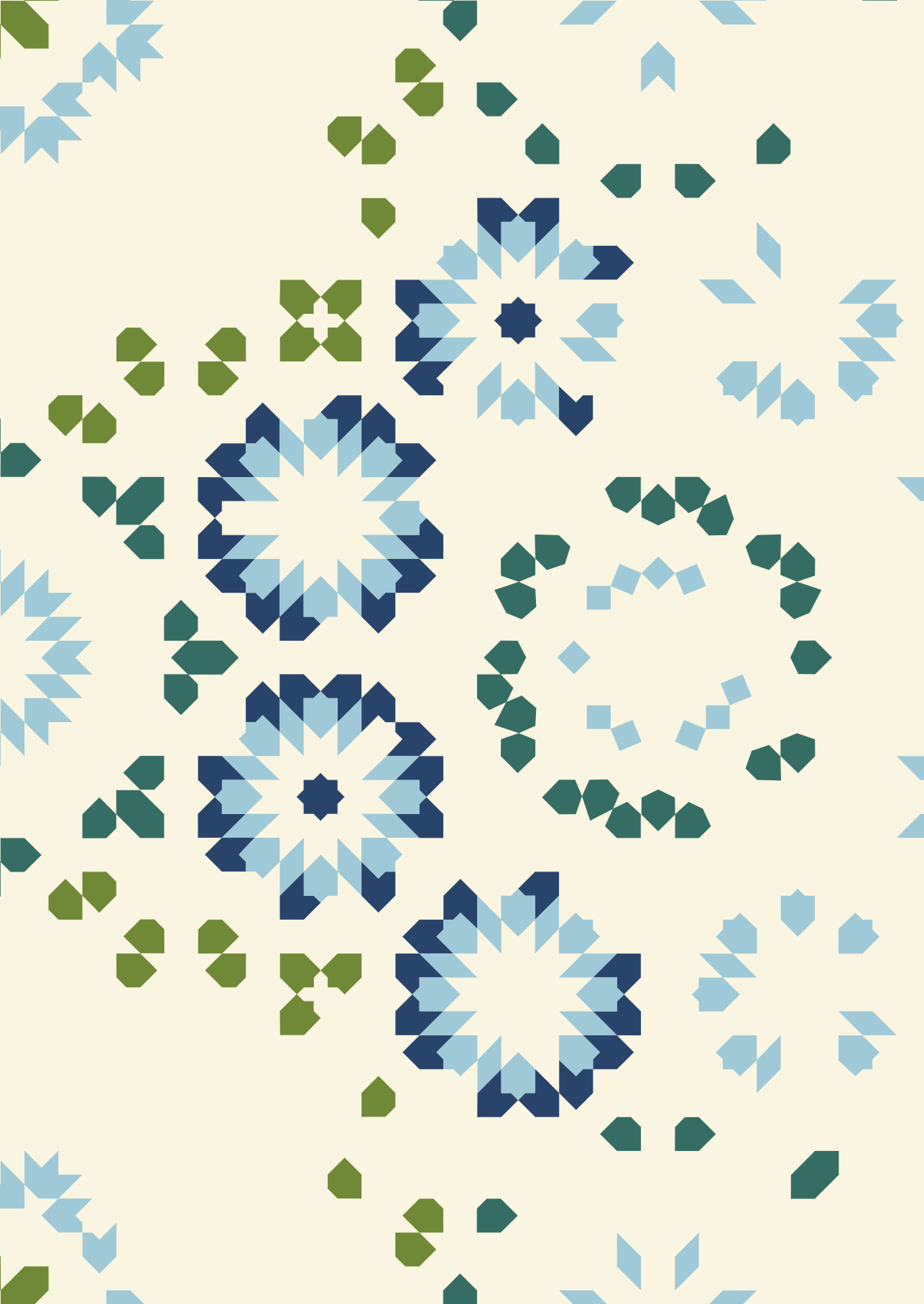
▲Supplemental Figure S2. Effects of inhibition of miR-26a-5p and miR-3913-3p on 95T1000 and LPS246 proliferation. The miRNA levels of miR-26a-5p and miR-3913-3p were modulated by transfecting the WDLPS cell line 95T1000 (**A**) and DDLPS cell line LPS246 (**B**) with miR-26a-5p or miR-3913-3p inhibitors at t=0h. Upon transfection, cell viability was checked by SRB assay at 48h, 72h and 96h post-transfection as an indicator for cellular proliferation. A one-way ANOVA with Bonferroni correction was used to determine statistical significance. P-value ≤0.05 (*); p-value ≤0.01 (**); p-value≤0.001 (***).



▲Supplemental Figure S3. Effects of miR-26a-5p and miR-3913-3p inhibition on apoptosis induction in WDLPS and DDLPS cells. FACS analysis of Annexin V/PI stained liposarcoma cell lines 95T1000 (**A**) and LPS246 (**B**) transfected with miR-26a-5p or miR-3913-3p antisense inhibitors, or scrambled control inhibitor (LNA) at 48h post-transfection. Live cells are presented by the Annexin V^-/PI^- fraction, early apoptotic cells by the Annexin V^+/PI^- fraction, late apoptotic cells by the Annexin V^+/PI^+ fraction and dead cells in the Annexin V^-/PI^+ fraction.



►Supplemental Figure S4. miR-26a-5p expression levels exhibit inverse correlation with PTEN protein levels. (**A**) Cell lysates were prepared of the WDLPS cell line 95T1000 and the DDLPS cell line LPS246 transfected with either miR-26a-5p mimics, miR-26a-5p antisense inhibitors or the appropriate scrambled controls (mneg for the mimics and LNA for the inhibitors). PTEN protein level in each of the lysates was measured by immunoblotting. The bar chart shows the quantified results of the immune-blot in which PTEN levels are normalized using the β-actin loading control. (**B**) miR-26a-5p and PTEN transcript levels were determined by RT-PCR in WDLPS (n=16) and DDLPS (n=13) samples. A Spearman's correlation was used to determine statistical significance.



CHAPTER 4

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.

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.

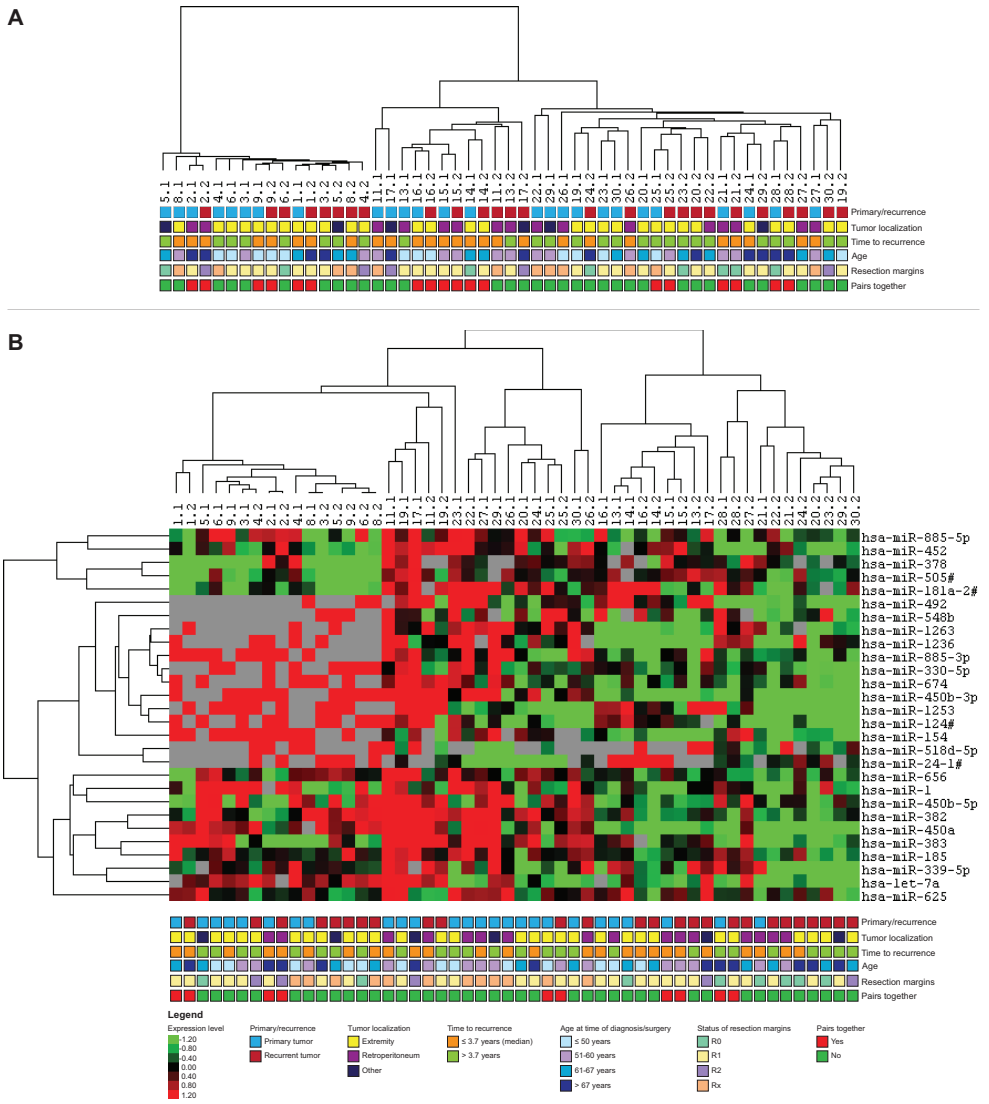
Sample	Age†	Sex	Localization	Resection margins	Time to recurrence‡	No. of DMRs
1.1	64	Female	Upper leg	R1	3.7	32,854
1.2	68			R1		
2.1	78	Male	Retroperitoneal	R1	1.9	2,430
2.2	79			R2		
3.1	58	Female	Upper leg	R1	10.6	4,410
3.2	69			R1		
4.1	50	Male	Upper leg	Rx	8.3	1,061
4.2	59			R2		
5.1	62	Male	Axilla	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.

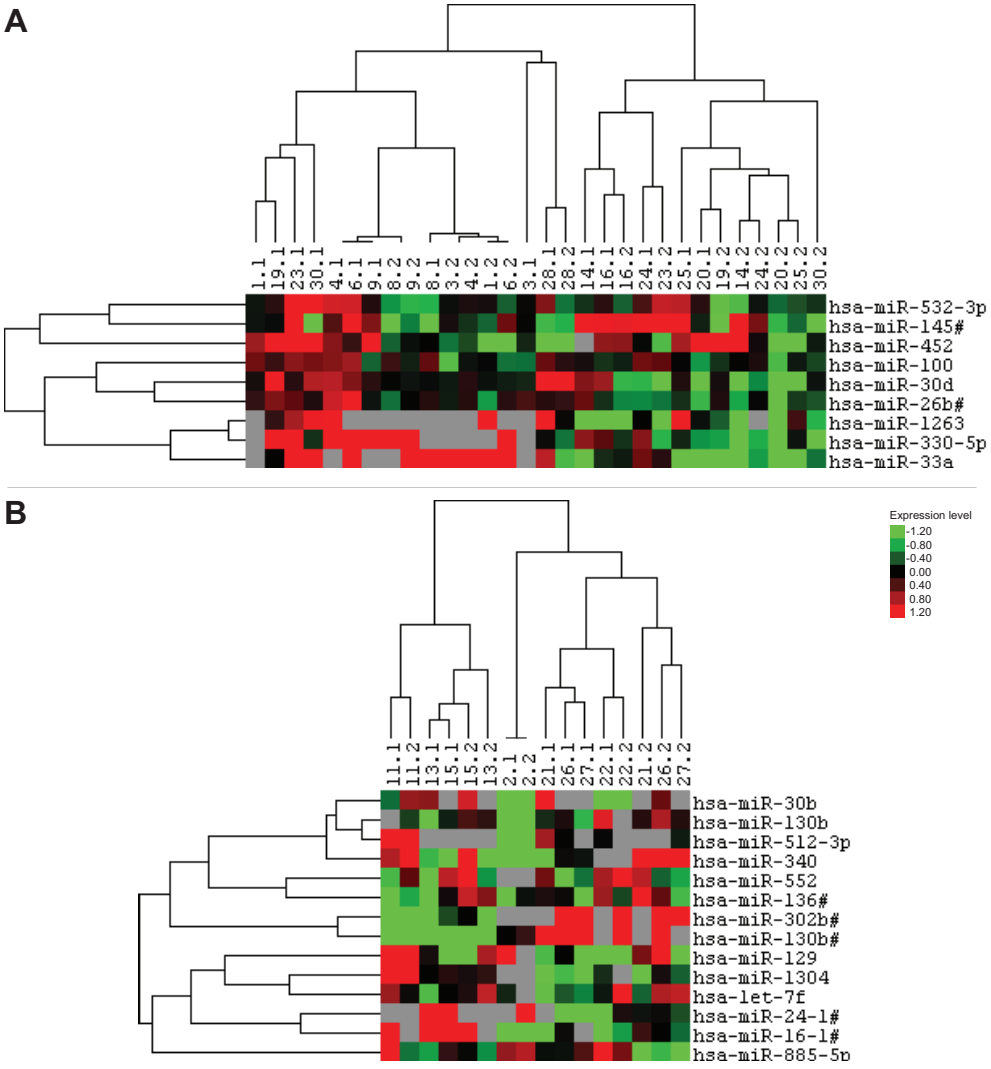
Next, a supervised analysis was performed based on the expression levels of the 28 significant differentially expressed microRNAs ($p<0.05$, FDR<0.25)(Fig 1B, S1 Table). The heat map indicated no clear discriminative pattern between primary and recurrent WDLPS, nor a distinction based on tumor localization, 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.

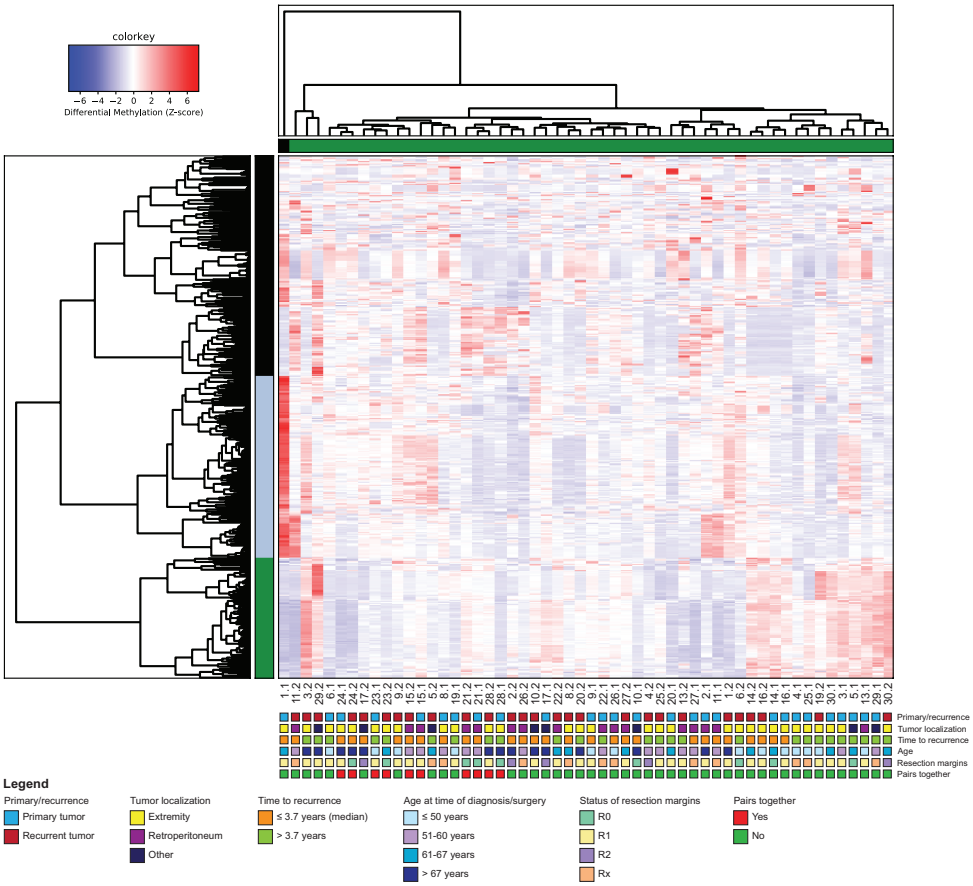
A relatively high number of the observed 470 DMRs was located at chromosome 12 (S3



▲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

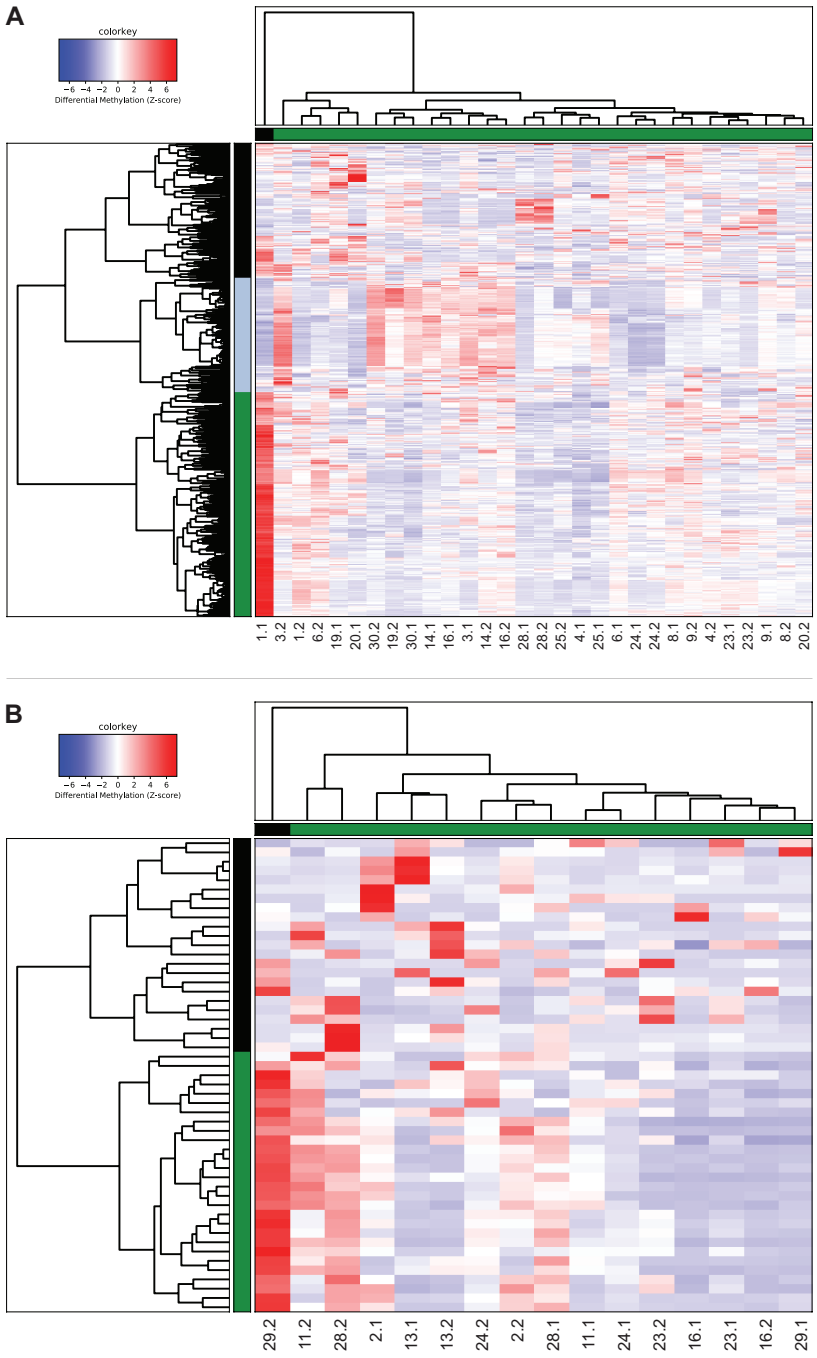
localized in the retroperitoneum, 1,071 DMRs were identified. To prevent the clustering from being blurred by background noise due to the higher number of DMRs, the clustering analysis for the retroperitoneal tumors was based on the DMRs with a fold change >2 (N = 53 DMRs). Again, this did not lead to 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 WDPLS 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.

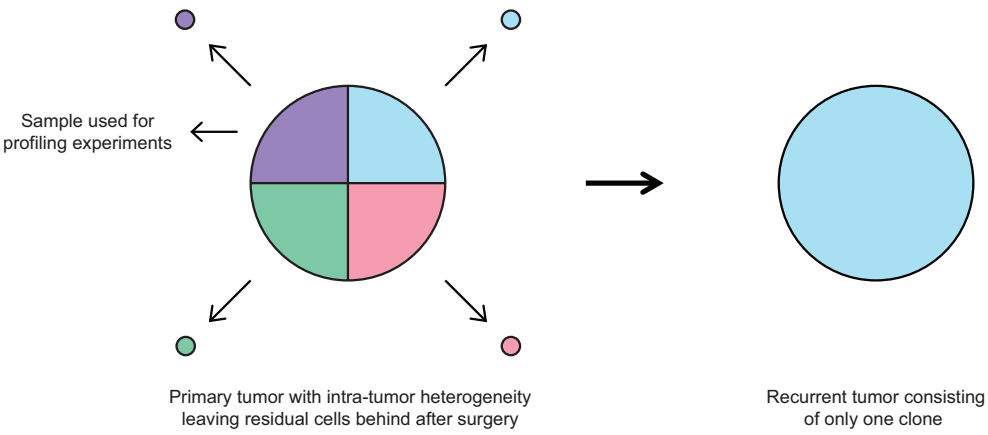
In the unsupervised microRNA clustering analysis, 7 of the 13 pairs (54%) with a short time to recurrence (before median time to recurrence) clustered together, compared to 2 of the 13 pairs (15%) with a longer time to recurrence. This might point towards a recurrence through the outgrowth of a residue 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.

Acknowledgments

We would like to thank Marcel Smid for his expert help with the bioinformatics and statistical analysis of the microRNA expression data.

Funding

The author(s) received no specific funding for this work.

Competing interests

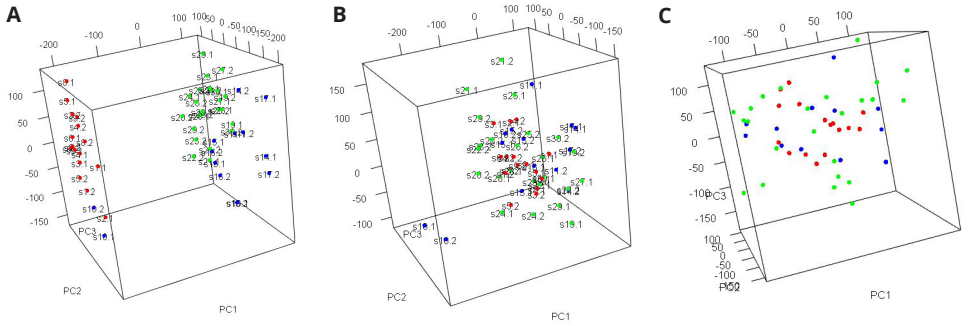
R. Boers, J. Boers, W.F.J. van IJcken and J. Gribnau declare a conflict of interest as shareholders of Methylomics B.V. This does not alter our adherence to PLOS ONE policies on sharing data and materials. The other authors declare no conflicts of interest.

References

1. Fletcher CDM, Bridge JA, Hogendoorn PCW, Mertens F, World Health Organization, International Agency for Research on Cancer. WHO classification of tumours of soft tissue and bone. Lyon: IARC Press; 2013.
2. The Netherlands Comprehensive Cancer Registry. Bijlage D Deelrapportage voor wekedelensarcomen. The Netherlands Comprehensive Cancer Organisation (IKNL); 2014.
3. Hayes J, Peruzzi PP, Lawler S. MicroRNAs in cancer: biomarkers, functions and therapy. Trends in Molecular Medicine. 2014;20:460-9.
4. Di Leva G, Garofalo M, Croce CM. MicroRNAs in cancer. Annu Rev Pathol. 2014;9:287-314.
5. Lujambio A, Lowe SW. The microcosmos of cancer. Nature. 2012;482:347-55.
6. Gits CM, van Kuijk PF, Jonkers MB, Boersma AW, Smid M, van Ijcken WF, et al. MicroRNA expression profiles distinguish liposarcoma subtypes and implicate miR-145 and miR-451 as tumor suppressors. Int J Cancer. 2014;135:348-61.
7. Sun R, Shen JK, Choy E, Yu Z, Hornicek FJ, Duan Z. The emerging roles and therapeutic potential of microRNAs (miRs) in liposarcoma. Discov Med. 2015;20:311-24.
8. Ugras S, Brill E, Jacobsen A, Hafner M, Socci ND, Decarolis PL, et al. Small RNA sequencing and functional characterization reveals MicroRNA-143 tumor suppressor activity in liposarcoma. Cancer Res. 2011;71:5659-69.
9. Vincenzi B, Iuliani M, Zoccoli A, Pantano F, Fioramonti M, De Lisi D, et al. Deregulation of dicer and mir-155 expression in liposarcoma. Oncotarget. 2015;6:10586-91.
10. Kapodistrias N, Mavridis K, Batistatou A, Gogou P, Karavasilis V, Sainis I, et al. Assessing the clinical value of microRNAs in formalin-fixed paraffin-embedded liposarcoma tissues: Overexpressed miR-155 is an indicator of poor prognosis. Oncotarget. 2016.
11. Lee DH, Amanat S, Goff C, Weiss LM, Said JW, Doan NB, et al. Overexpression of miR-26a-2 in human liposarcoma is correlated with poor patient survival. Oncogenesis. 2013;2:e47.
12. Renner M, Czwan E, Hartmann W, Penzel R, Brors B, Eils R, et al. MicroRNA profiling of primary high-grade soft tissue sarcomas. Genes Chromosomes Cancer. 2012;51:982-96.
13. Subramanian S, Lui WO, Lee CH, Espinosa I, Nielsen TO, Heinrich MC, et al. MicroRNA expression signature of human sarcomas. Oncogene. 2008;27:2015-26.
14. Zhang P, Bill K, Liu J, Young E, Peng T, Bolshakov S, et al. MiR-155 is a liposarcoma oncogene that targets casein kinase-1alpha and enhances beta-catenin signaling. Cancer Res. 2012;72:1751-62.
15. Nezu Y, Hagiwara K, Yamamoto Y, Fujiwara T, Matsuo K, Yoshida A, et al. miR-135b, a key regulator of malignancy, is linked to poor prognosis in human myxoid liposarcoma. Oncogene. 2016;35:6177-88.
16. Schubeler D. Function and information content of DNA methylation. Nature. 2015;517:321-6.
17. Portela A, Esteller M. Epigenetic modifications and human disease. Nat Biotechnol. 2010;28:1057-68.
18. De Carvalho DD, Sharma S, You JS, Su SF, Taberlay PC, Kelly TK, et al. DNA methylation screening identifies driver epigenetic events of cancer cell survival. Cancer Cell. 2012;21:655-67.
19. Laird PW. The power and the promise of DNA methylation markers. Nat Rev Cancer. 2003;3:253-66.
20. Heyn H, Esteller M. DNA methylation profiling in the clinic: applications and challenges. Nat Rev Genet. 2012;13:679-92.
21. Figueroa ME, Lugthart S, Li Y, Erpelinck-Verschueren C, Deng X, Christos PJ, et al. DNA Methylation Signatures Identify Biologically Distinct Subtypes in Acute Myeloid Leukemia. Cancer cell. 2010;17:13-27.

22. Noushmehr H, Weisenberger DJ, Diefes K, Phillips HS, Pujara K, Berman BP, et al. Identification of a CpG island methylator phenotype that defines a distinct subgroup of glioma. Cancer Cell. 2010;17:510-22.
23. Brock MV, Hooker CM, Ota-Machida E, Han Y, Guo M, Ames S, et al. DNA methylation markers and early recurrence in stage I lung cancer. N Engl J Med. 2008;358:1118-28.
24. Boers R, Boers J, de Hoon B, Kockx C, Ozgur Z, Molijn A, et al. Genome-wide DNA methylation profiling using the methylation-dependent restriction enzyme LpnPI. Genome Res. 2018;28:88-99.
25. Cancer Genome Atlas Research Network. Electronic address edsc, Cancer Genome Atlas Research N. Comprehensive and Integrated Genomic Characterization of Adult Soft Tissue Sarcomas. Cell. 2017;171:950-65 e28.
26. Davidovic R, Sopta J, Mandusic V, Krajnovic M, Stanojevic M, Tulic G, et al. p14(ARF) methylation is a common event in the pathogenesis and progression of myxoid and pleomorphic liposarcoma. Med Oncol. 2013;30:682.
27. Taylor BS, DeCarolis PL, Angeles CV, Brenet F, Schultz N, Antonescu CR, et al. Frequent alterations and epigenetic silencing of differentiation pathway genes in structurally rearranged liposarcomas. Cancer Discov. 2011;1:587-97.
28. Mazzu YZ, Hu Y, Soni RK, Mojica KM, Qin LX, Agius P, et al. miR-193b-Regulated Signaling Networks Serve as Tumor Suppressors in Liposarcoma and Promote Adipogenesis in Adipose-Derived Stem Cells. Cancer Res. 2017;77:5728-40.
29. Johnson WE, Li C, Rabinovic A. Adjusting batch effects in microarray expression data using empirical Bayes methods. Biostatistics. 2007;8:118-27.
30. Wienholds E, Kloosterman WP, Miska E, Alvarez-Saavedra E, Berezikov E, de Bruijn E, et al. MicroRNA expression in zebrafish embryonic development. Science. 2005;309:310-1.
31. Zhou J, Sears RL, Xing X, Zhang B, Li D, Rockweiler NB, et al. Tissue-specific DNA methylation is conserved across human, mouse, and rat, and driven by primary sequence conservation. BMC genomics. 2017;18:724-.
32. Zhang B, Zhou Y, Lin N, Lowdon RF, Hong C, Nagarajan RP, et al. Functional DNA methylation differences between tissues, cell types, and across individuals discovered using the M&M algorithm. Genome research. 2013;23:1522-40.
33. Su M, Qin B, Liu F, Chen Y, Zhang R. miR-885-5p upregulation promotes colorectal cancer cell proliferation and migration by targeting suppressor of cytokine signaling. Oncol Lett. 2018;16:65-72.
34. Lam CS, Ng L, Chow AK, Wan TM, Yau S, Cheng NS, et al. Identification of microRNA 885-5p as a novel regulator of tumor metastasis by targeting CPEB2 in colorectal cancer. Oncotarget. 2017;8:26858-70.
35. Liu Y, Bao Z, Tian W, Huang G. miR-885-5p suppresses osteosarcoma proliferation, migration and invasion through regulation of β-catenin. Oncology letters. 2019;17:1996-2004.
36. Garsed DW, Marshall OJ, Corbin VD, Hsu A, Di Stefano L, Schroder J, et al. The architecture and evolution of cancer neochromosomes. Cancer Cell. 2014;26:653-67.
37. Hoekstra HJ, Haas RLM, Verhoef C, Suurmeijer AJH, van Rijswijk CSP, Bongers BGH, et al. Adherence to Guidelines for Adult (Non-GIST) Soft Tissue Sarcoma in the Netherlands: A Plea for Dedicated Sarcoma Centers. Ann Surg Oncol. 2017;24:3279-88.
38. Otsu H, Watanabe M, Inoue N, Masutani R, Iwatani Y. Intraindividual variation of microRNA expression levels in plasma and peripheral blood mononuclear cells and the associations of these levels with the pathogenesis of autoimmune thyroid diseases. Clin Chem Lab Med. 2017;55:626-35.
39. Flanagan JM, Popendikyte V, Pozdniakovaite N, Sobolev M, Assadzadeh A, Schumacher A, et al. Intra- and interindividual epigenetic variation in human germ cells. American journal of human genetics. 2006;79:67-84.

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

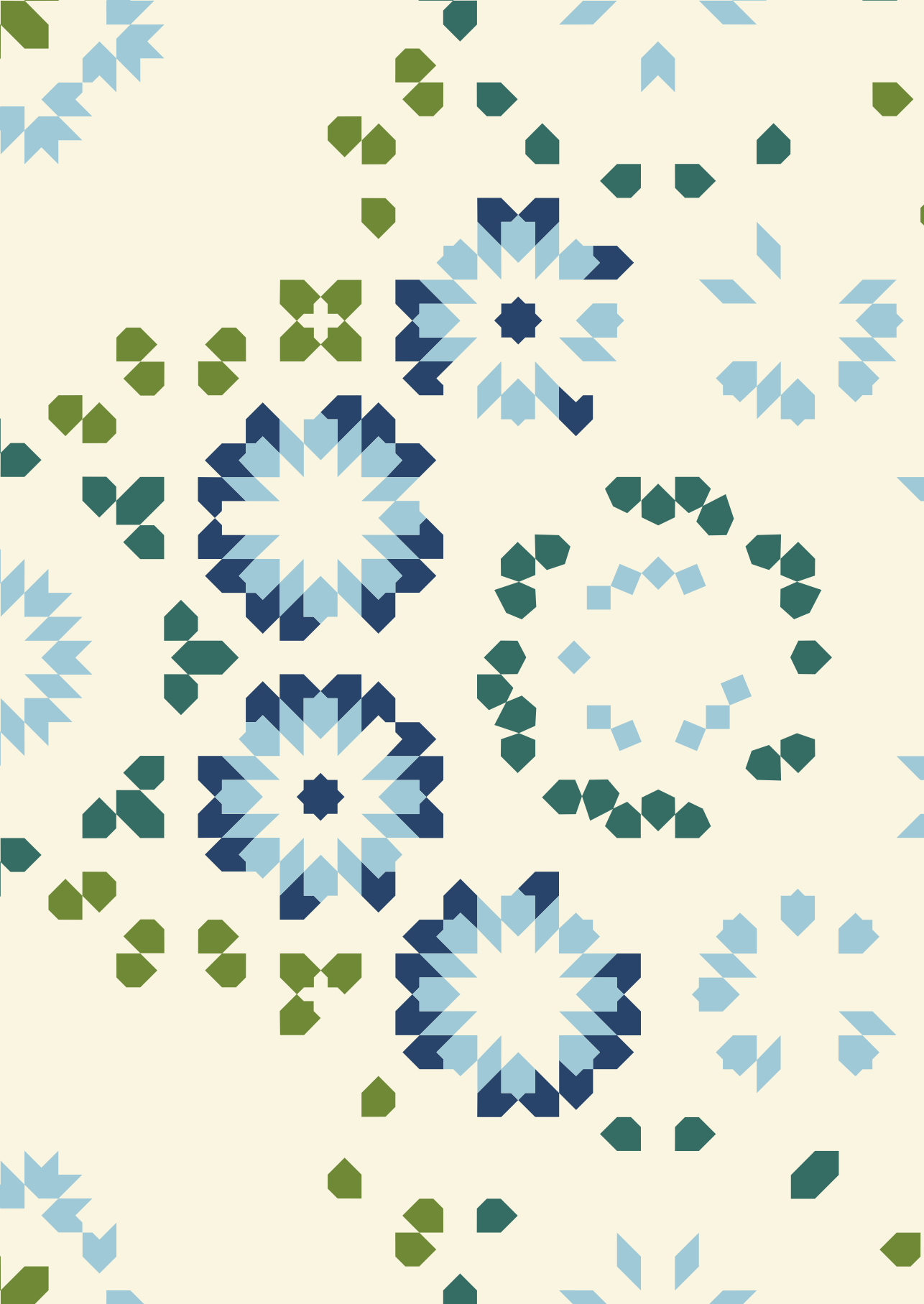
S4 Table. Top 100 genes with a DMR. Top 100 genes that contain at least one differentially methylated DNA region (DMR) after Bonferroni correction, excluding genes/DMRs located on the sex chromosomes, found by MeD-seq on 27 paired primary and recurrent WDLPS tumor samples.

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	RNA5S17	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	HMGA2	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	RNA5S10	2	1.085	Primary	chr1
87	RNA5S11	2	1.085	Primary	chr1
88	RNA5S12	2	1.085	Primary	chr1
89	RNA5SP19	1	1.082	Primary	chr1

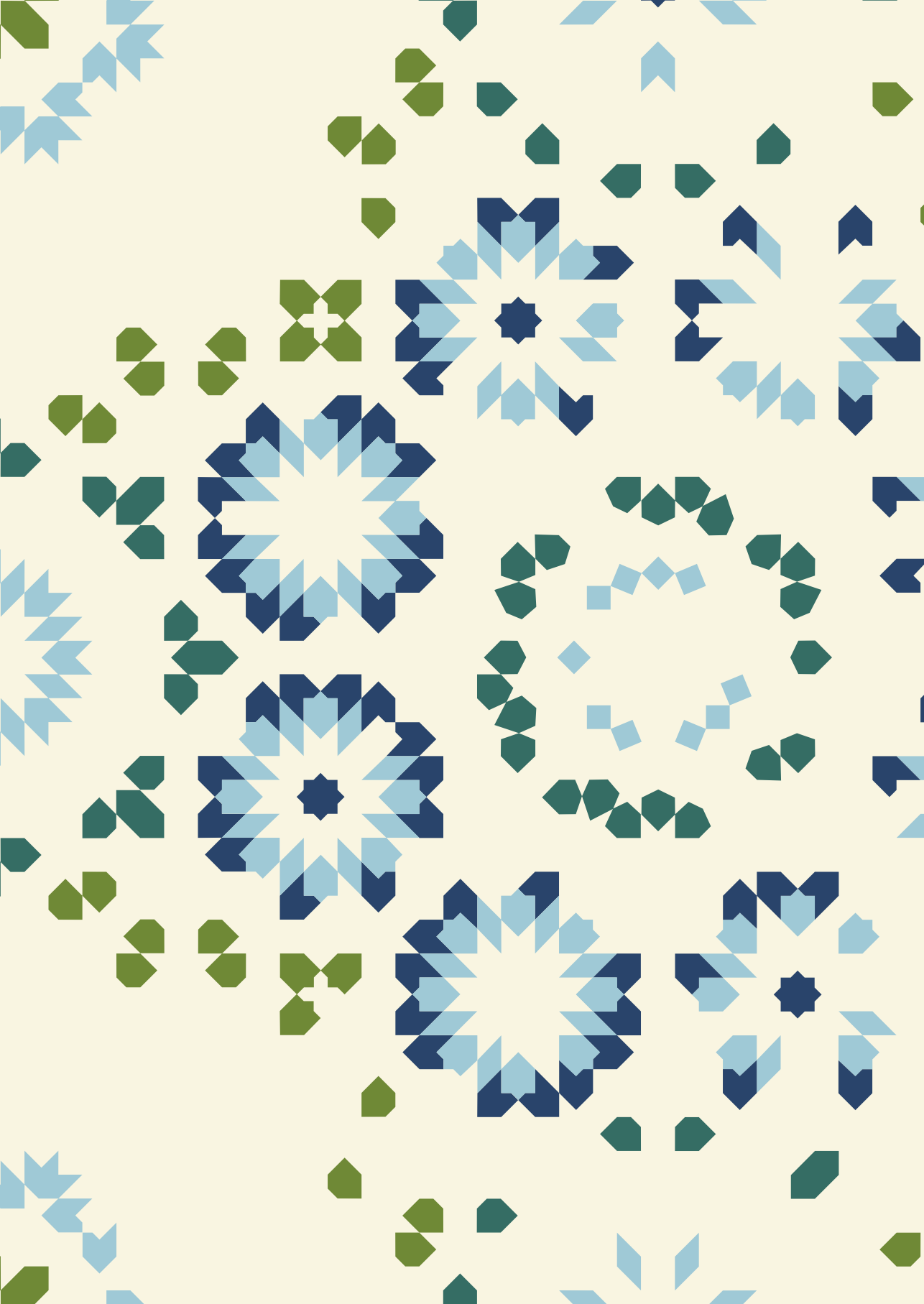
No.	Gene	No. of DMRs	Fold Change*	Hypermethylated in	Location
90	RNA5SP162	1	1.082	Primary	chr1
91	DUX4L23	1	1.082	Primary	chr10
92	CTD-3162L10.1	5	1.080	Primary	chr19
93	TMEM242	2	1.078	Recurrence	chr6
94	AL671532.2	1	1.076	Recurrence	chr14
95	DLGAP2	1	1.076	Recurrence	chr8
96	CPSF6	1	1.074	Primary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

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.

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.

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-TI 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*

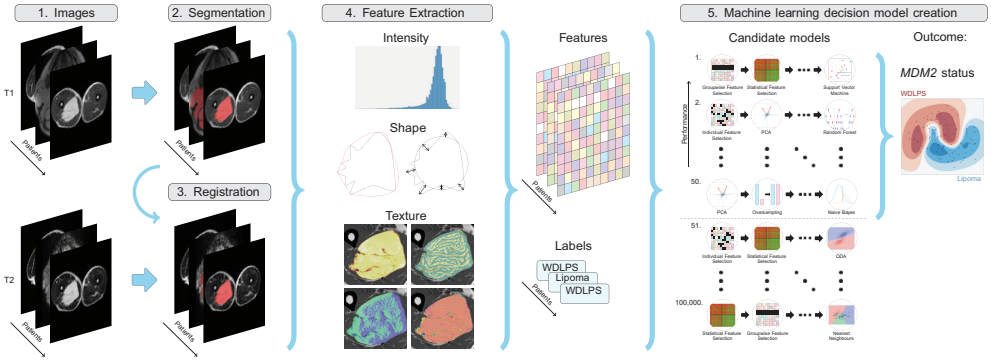
(supporting information). The shape features quantified were morphological properties such as volume and similarity to a circle. Intensity features were quantified using first-order statistics such as the mean and standard deviation. Texture features quantified more complex properties, such as the presence 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).

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 a DDLPS 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).

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)

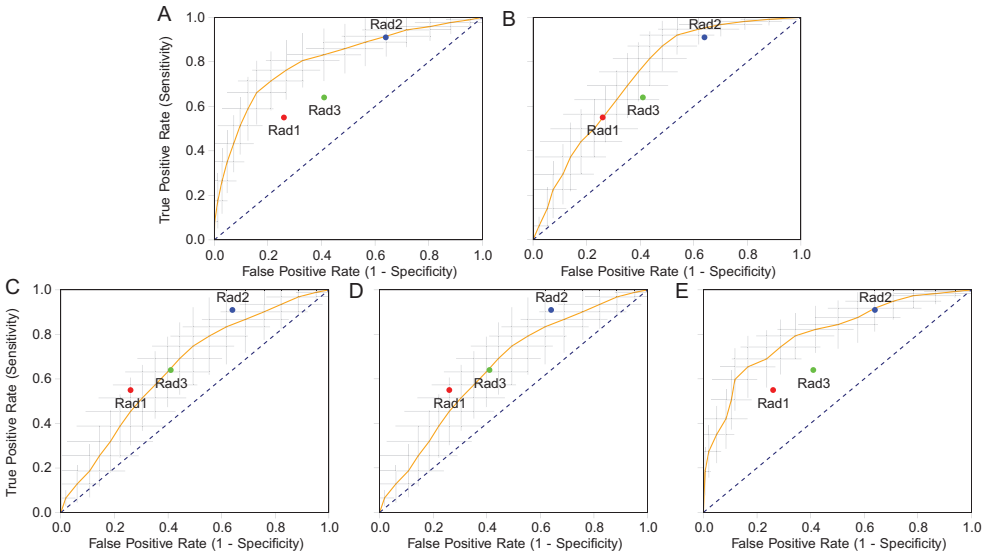
*With percentages in parentheses unless indicated otherwise; †values are median (i.q.r.). WDLPS, well differentiated liposarcoma; DDLPS, dedifferentiated liposarcoma.

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.

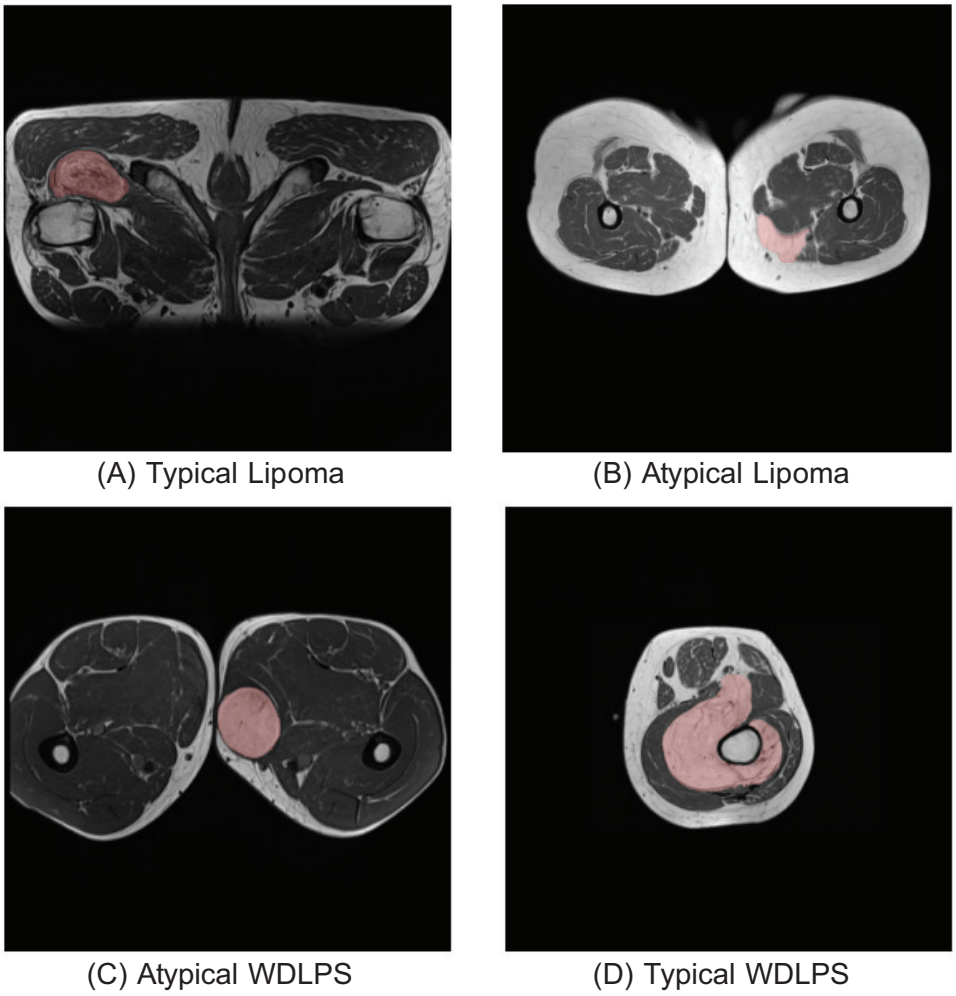
	T1 imaging features only	T1 + T2 imaging features	Patient features only	Manually scored features only	Volume only
AUC	0.69 [0.58, 0.80]	0.81 [0.72, 0.90]	0.74 [0.64, 0.84]	0.67 [0.56, 0.77]	0.64 [0.53, 0.74]
Accuracy	0.67 [0.57, 0.76]	0.75 [0.66, 0.83]	0. 66 [0.56, 0.75]	0.60 [0.51, 0.69]	0.66 [0.57, 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.



▲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 with DDLPS to evaluate whether DDLPS 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 distinguishing DDLPS 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 between DDLPS and non-DDLPS, they were outperformed by the T1 and T1+T2 imaging models in differentiating WDLPS 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 as DDLPS 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.

Acknowledgements

M.V. and M.P.A.S. contributed equally to this study. The authors thank E.H.G. Oei and D.F. Hanff for classifying the lipomatous tumours. This study was financed by the Stichting Coolsingel (reference no. 567), a Dutch non-profit foundation. M.P.A.S. acknowledges funding from the research programme STRaTeGy (project no. 14929-14930), which is partly financed by the Netherlands Organization for Scientific Research (NWO). W.J.N. is founder, scientific lead and stock holder of Quantib.

Disclosure

The authors declare no other conflicts of interest.

References

1. Fletcher CDM, Bridge JA, Hogendoorn PCW, Mertens F, World Health Organization, International Agency for Research on Cancer. WHO classification of tumours of soft tissue and bone. Lyon: IARC Press; 2013.
2. Brisson M, Kashima T, Delaney D, Tirabosco R, Clarke A, Cro S, et al. MRI characteristics of lipoma and atypical lipomatous tumor/well-differentiated liposarcoma: retrospective comparison with histology and MDM2 gene amplification. Skeletal Radiol. 2013;42:635-47.
3. Kransdorf MJ, Bancroft LW, Peterson JJ, Murphey MD, Foster WC, Temple HT. Imaging of fatty tumors: distinction of lipoma and well-differentiated liposarcoma. Radiology. 2002;224:99-104.
4. Gupta P, Potti TA, Wuertzer SD, Lenchik L, Pacholke DA. Spectrum of fat-containing soft-tissue masses at MR imaging: the common, the uncommon, the characteristic, and the sometimes confusing. Radiographics. 2016;36:753-66.
5. Drevelegas A, Pilavaki M, Chourmouzi D. Lipomatous tumors of soft tissue: MR appearance with histological correlation. Eur J Radiol. 2004;50:257-67.
6. O'Donnell PW, Griffin AM, Eward WC, Sternheim A, White LM, Wunder JS, et al. Can experienced observers differentiate between lipoma and well-differentiated liposarcoma using only MRI? Sarcoma. 2013;2013:982784.
7. Kransdorf MJ, Meis JM, Jelinek JS. Dedifferentiated liposarcoma of the extremities: imaging findings in four patients. American Journal of Roentgenology. 1993;161:127-30.
8. Tateishi U, Hasegawa T, Beppu Y, Satake M, Moriyama N. Primary dedifferentiated liposarcoma of the retroperitoneum. Prognostic significance of computed tomography and magnetic resonance imaging features. J Comput Assist Tomogr. 2003;27:799-804.
9. Yun JS, Chung HW, Song JS, Lee SH, Lee MH, Shin MJ. Dedifferentiated liposarcoma of the musculoskeletal system: expanded MR imaging spectrum from predominant fatty mass to non-fatty mass. Acta Radiol. 2019;[Epub ahead of print]:284185119833060.
10. Murphey MD, Arcara LK, Fanburg-Smith J. From the archives of the AFIP: imaging of musculoskeletal liposarcoma with radiologic-pathologic correlation. Radiographics. 2005;25:1371-95.
11. ESMO/European Sarcoma Network Working Group. Soft tissue and visceral sarcomas: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2014;25 Suppl 3:iii102-12.
12. Thway K, Wang J, Swansbury J, Min T, Fisher C. Fluorescence in situ hybridization for MDM2 amplification as a routine ancillary diagnostic tool for suspected well-differentiated and dedifferentiated liposarcomas: experience at a tertiary center. Sarcoma. 2015;2015:812089.
13. Kimura H, Dobashi Y, Nojima T, Nakamura H, Yamamoto N, Tsuchiya H, et al. Utility of fluorescence in situ hybridization to detect MDM2 amplification in liposarcomas and their morphological mimics. Int J Clin Exp Pathol. 2013;6:1306-16.
14. Lambin P, Rios-Velazquez E, Leijenaar R, Carvalho S, van Stiphout RG, Granton P, et al. Radiomics: extracting more information from medical images using advanced feature analysis. Eur J Cancer. 2012;48:441-6.
15. Juntu J, Sijbers J, De Backer S, Rajan J, Van Dyck D. Machine learning study of several classifiers trained with texture analysis features to differentiate benign from malignant soft-tissue tumors in T1-MRI images. J Magn Reson Imaging. 2010;31:680-9.
16. Corino VDA, Montin E, Messina A, Casali PG, Gronchi A, Marchiano A, et al. Radiomic analysis of soft tissues sarcomas can distinguish intermediate from high-grade lesions. J Magn Reson Imaging. 2018;47:829-40.

17. Vallieres M, Freeman CR, Skamene SR, El Naqa I. A radiomics model from joint FDG-PET and MRI texture features for the prediction of lung metastases in soft-tissue sarcomas of the extremities. Phys Med Biol. 2015;60:5471-96.
18. Starmans MPA, Miclea RL, van der Voort SR, Niessen WJ, Thomeer MG, Klein S. Classification of malignant and benign liver tumors using a radiomics approach. SPIE Medical Imaging: Image Processing; 10574D; 2018.
19. Klein S, Staring M, Murphy K, Viergever MA, Pluim JP. Elastix: a toolbox for intensity-based medical image registration. IEEE Trans Med Imaging. 2010;29:196-205.
20. van der Voort SR, Starmans MPA. Predict a Radiomics Extensive Differentiable Interchangable Classification Toolkit (PREDICT). 2018.
21. van Griethuysen JJM, Fedorov A, Parmar C, Hosny A, Aucoin N, Narayan V, et al. Computational radiomics system to decode the radiographic phenotype. Cancer Res. 2017;77:e104-e7.
22. Starmans MPA. Workflow for Optimal Radiomics Classification (WORC). 2018.
23. Nadeau C, Bengio Y. Inference for the generalization error. Advances in neural information processing systems2000. p. 307-13.
24. Sanchez MR, Golomb FM, Moy JA, Potozkin JR. Giant lipoma: case report and review of the literature. J Am Acad Dermatol. 1993;28:266-8.
25. Smith CA, Martinez SR, Tseng WH, Tamurian RM, Bold RJ, Borys D, et al. Predicting survival for well-differentiated liposarcoma: the importance of tumor location. J Surg Res. 2012;175:12-7.
26. Thornhill RE, Golfam M, Sheikh A, Cron GO, White EA, Werier J, et al. Differentiation of lipoma from liposarcoma on MRI using texture and shape analysis. Academic Radiology. 2014;21:1185-94.
27. Echegaray S, Gevaert O, Shah R, Kamaya A, Louie J, Kothary N, et al. Core samples for radiomics features that are insensitive to tumor segmentation: method and pilot study using CT images of hepatocellular carcinoma. J Med Imaging (Bellingham). 2015;2:041011.
28. Lu L, Lv W, Jiang J, Ma J, Feng Q, Rahmim A, et al. Robustness of radiomic features in [(11)C]choline and [(18)F]FDG PET/CT imaging of nasopharyngeal carcinoma: impact of segmentation and discretization. Mol Imaging Biol. 2016;18:935-45.
29. Parmar C, Rios Velazquez E, Leijenaar R, Jermoumi M, Carvalho S, Mak RH, et al. Robust radiomics feature quantification using semiautomatic volumetric segmentation. PLoS One. 2014;9:e102107.
30. Vos M, Grünhagen DJ, Koseła-Paterczyk H, Rutkowski P, Sleijfer S, Verhoef C. Natural history of well-differentiated liposarcoma of the extremity compared to patients treated with surgery. Surgical Oncology. 2019;29:84-9.

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) toolbox[1] makes us 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) SMOTE[2], which creates new synthetic patients using a combination of the patients in the minority class.

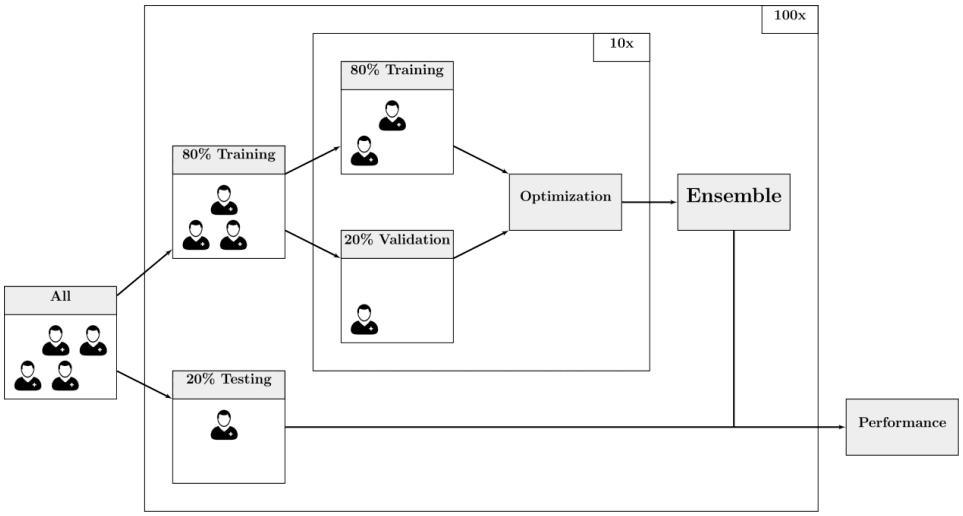
Lastly, machine learning methods were used to determine a decision rule to distinguish between WDLPS and lipoma. These included 1) logistic regression; 2) support vector machines; 3) random forests; 4) naive Bayes; and 5) linear and quadratic discriminant analysis.

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.

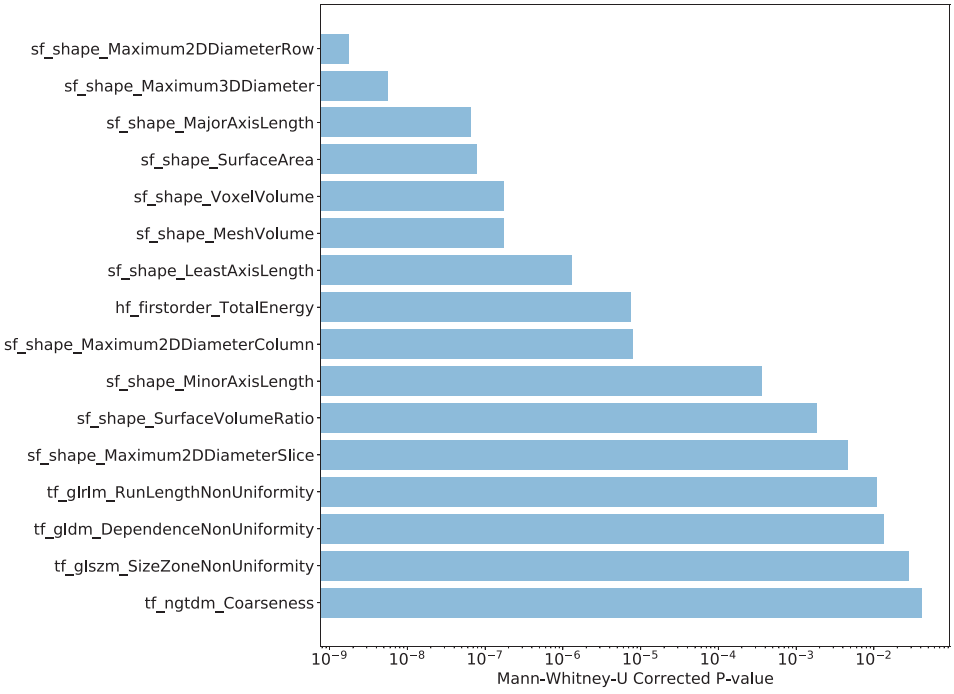
In the training phase, a total of 100,000 pseudo-randomly generated workflows is created and executed. The workflows are ranked from best to worst based on the F1-score, which is the harmonic average of precision and recall. Due to the large number of workflows executed, there is a chance that the best 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.
2. Han H, Wang W-Y, Mao B-H. Borderline-SMOTE: a new over-sampling method in imbalanced data sets learning. In; 2005; Berlin, Heidelberg: Springer Berlin Heidelberg; 2005. p. 878-887.

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.

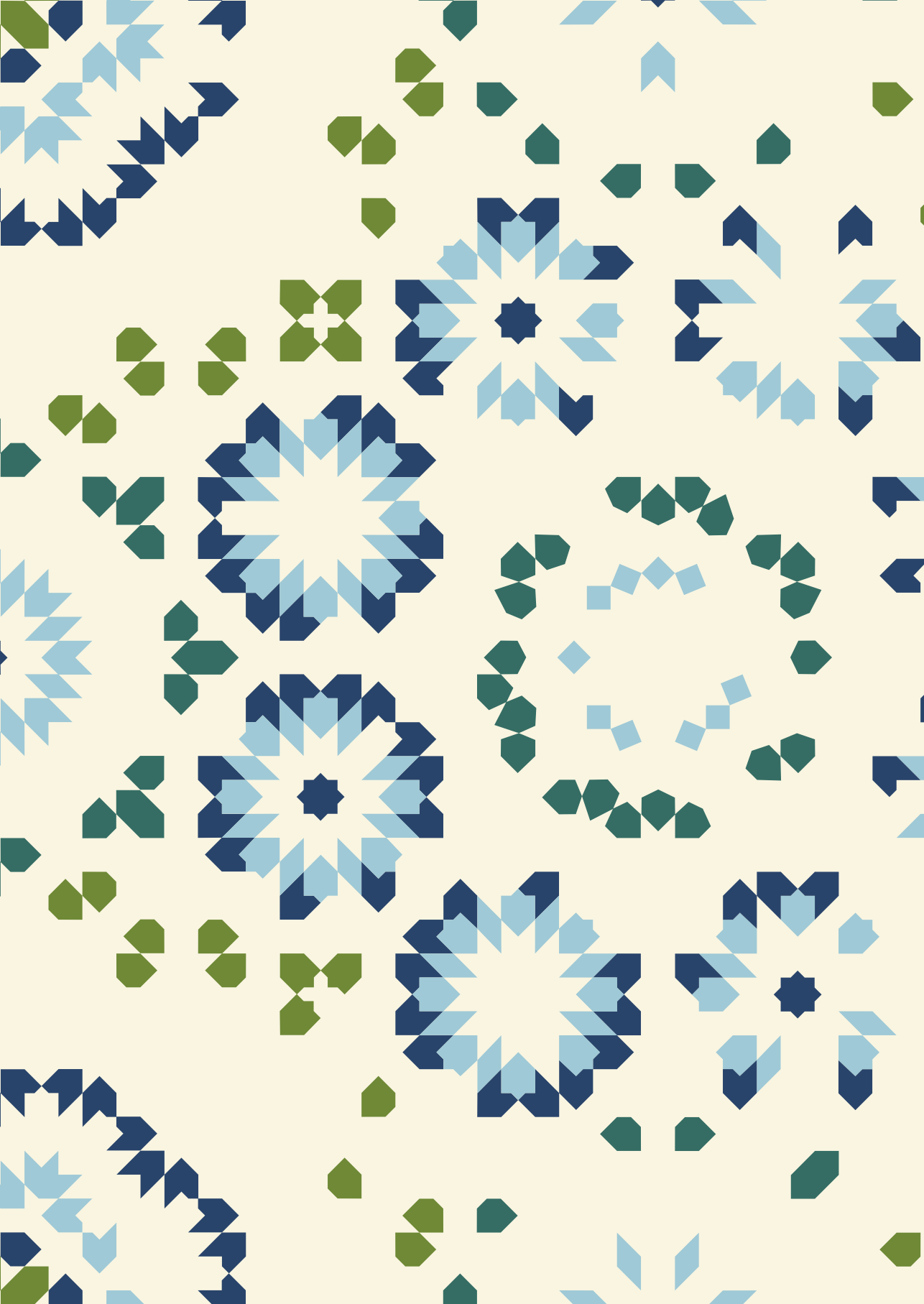
	T1	T1 + T1-FS	T1 + T1-GD	T1 + T1-FS-GD	T1 + T2	T1 + T2-FS
AUC	0.83 [0.75, 0.90]	0.84 [0.75, 0.92]	0.81 [0.72, 0.90]	0.81 [0.73, 0.89]	0.89 [0.83, 0.95]	0.81 [0.73, 0.88]
Accuracy	0.68 [0.67, 0.84]	0.77 [0.69, 0.85]	0.76 [0.67, 0.84]	0.75 [0.66, 0.83]	0.81 [0.74, 0.87]	0.74 [0.66, 0.81]
Sensitivity	0.68 [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

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.

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 or DDLPS ($p = 0.014$), 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.

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 were DDLPS (7%) or PLPS (8%). Retroperitoneal/intrathoracic LPS were mostly of the DDLPS (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 mainly DDLPS (54%) and WDLPS (37%), and rarely PLPS (6%) or MLPS (3%)(Table 2).

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/intrathoracic DDLPS experienced significantly more often a local recurrence (62%) than patients with other locations of DDLPS (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
	TLR (months), median (IQR)	23 (10.8-58)	
Distant metastases	No	428	82.6
	Yes	90	17.4
	TSD (months), median (IQR)	23.5 (8.8-58.5)	
Survival	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)	

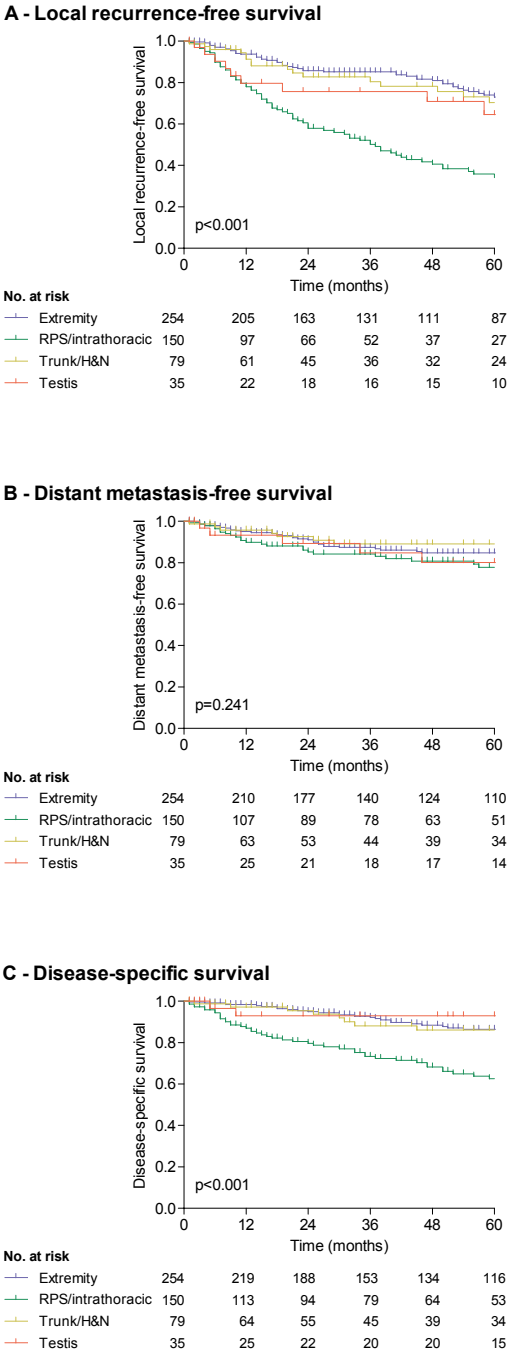
WDLPS, well-differentiated liposarcoma; DDLPS, dedifferentiated liposarcoma; MLPS, myxoid liposarcoma; PLPS, pleomorphic liposarcoma; RPS, retroperitoneal sarcoma; IQR, interquartile range; RTx, radiotherapy; ILP, isolated limb perfusion; CTx, chemotherapy; TLR, time to local recurrence; TSD, time to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



▲**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 ration; 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 LFRS, 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).

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.

Funding

None.

Acknowledgements

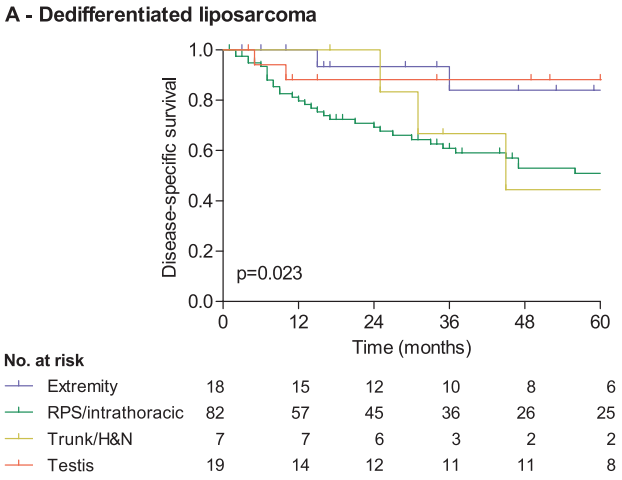
None.

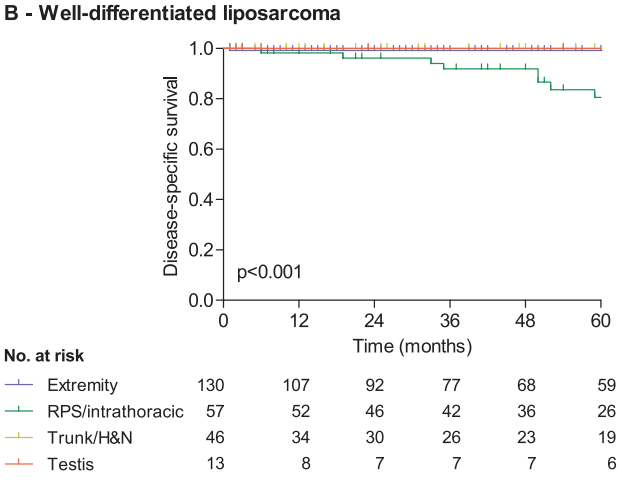
References

1. The Netherlands Comprehensive Cancer Registry. Bijlage D Deelrapportage voor wekedelensarcomen. The Netherlands Comprehensive Cancer Organisation (IKNL); 2014.
2. Fletcher CDM, Bridge JA, Hogendoorn PCW, Mertens F, World Health Organization, International Agency for Research on Cancer. WHO classification of tumours of soft tissue and bone. Lyon: IARC Press; 2013.
3. Cahlon O, Brennan MF, Jia X, Qin LX, Singer S, Alektiar KM. A postoperative nomogram for local recurrence risk in extremity soft tissue sarcomas after limb-sparing surgery without adjuvant radiation. Ann Surg. 2012;255:343-7.
4. Callegaro D, Miceli R, Bonvalot S, Ferguson P, Strauss DC, Levy A, et al. Development and external validation of two nomograms to predict overall survival and occurrence of distant metastases in adults after surgical resection of localised soft-tissue sarcomas of the extremities: a retrospective analysis. Lancet Oncol. 2016;17:671-80.
5. Gronchi A, Casali PG, Mariani L, Miceli R, Fiore M, Lo Vullo S, et al. Status of surgical margins and prognosis in adult soft tissue sarcomas of the extremities: a series of patients treated at a single institution. J Clin Oncol. 2005;23:96-104.
6. Gronchi A, Lo Vullo S, Colombo C, Collini P, Stacchiotti S, Mariani L, et al. Extremity soft tissue sarcoma in a series of patients treated at a single institution: local control directly impacts survival. Ann Surg. 2010;251:506-11.
7. Gronchi A, Strauss DC, Miceli R, Bonvalot S, Swallow CJ, Hohenberger P, et al. Variability in Patterns of Recurrence After Resection of Primary Retroperitoneal Sarcoma (RPS): A Report on 1007 Patients From the Multi-institutional Collaborative RPS Working Group. Ann Surg. 2016;263:1002-9.
8. Gronchi A, Casali PG, Fiore M, Mariani L, Lo Vullo S, Bertulli R, et al. Retroperitoneal soft tissue sarcomas: patterns of recurrence in 167 patients treated at a single institution. Cancer. 2004;100:2448-55.
9. Rutkowski P, Trepka S, Ptaszynski K, Kolodziejczyk M. Surgery quality and tumor status impact on survival and local control of resectable liposarcomas of extremities or the trunk wall. Clin Orthop Relat Res. 2013;471:860-70.
10. Trovik CS, Bauer HC, Alvegard TA, Anderson H, Blomqvist C, Berlin O, et al. Surgical margins, local recurrence and metastasis in soft tissue sarcomas: 559 surgically-treated patients from the Scandinavian Sarcoma Group Register. Eur J Cancer. 2000;36:710-6.
11. Singer S, Antonescu CR, Riedel E, Brennan MF. Histologic subtype and margin of resection predict pattern of recurrence and survival for retroperitoneal liposarcoma. Ann Surg. 2003;238:358-70; discussion 70-1.
12. Baxter KJ, Govsyeyev N, Namm JP, Gonzalez RJ, Roggin KK, Cardona K. Is multimodality therapy necessary for the management of pure myxoid liposarcomas? A multi-institutional series of pure myxoid liposarcomas of the extremities and torso. J Surg Oncol. 2015;111:146-51.
13. Cassier PA, Kantor G, Bonvalot S, Lavergne E, Stoeckle E, Le Pechoux C, et al. Adjuvant radiotherapy for extremity and trunk wall atypical lipomatous tumor/well-differentiated LPS (ALT/WD-LPS): a French Sarcoma Group (GSF-GETO) study. Ann Oncol. 2014;25:1854-60.
14. Kooby DA, Antonescu CR, Brennan MF, Singer S. Atypical lipomatous tumor/well-differentiated liposarcoma of the extremity and trunk wall: importance of histological subtype with treatment recommendations. Ann Surg Oncol. 2004;11:78-84.
15. Smith CA, Martinez SR, Tseng WH, Tamurian RM, Bold RJ, Borys D, et al. Predicting survival for well-differentiated liposarcoma: the importance of tumor location. J Surg Res. 2012;175:12-7.

16. Dalal KM, Kattan MW, Antonescu CR, Brennan MF, Singer S. Subtype specific prognostic nomogram for patients with primary liposarcoma of the retroperitoneum, extremity, or trunk. Ann Surg. 2006;244:381-91.
17. Knebel C, Lenze U, Pohlig F, Lenze F, Harrasser N, Suren C, et al. Prognostic factors and outcome of Liposarcoma patients: a retrospective evaluation over 15 years. BMC Cancer. 2017;17:410.
18. Committee EG, Euracan, Gronchi A, Frezza AM, Casali PG, De Álava E, et al. Soft tissue and visceral sarcomas: ESMO–EURACAN Clinical Practice Guidelines for diagnosis, treatment and follow-up†. Annals of Oncology. 2018;29:iv51-iv67.
19. Schemper M, Smith TL. A note on quantifying follow-up in studies of failure time. Controlled Clinical Trials. 1996;17:343-6.
20. Bonvalot S, Rivoire M, Castaing M, Stoeckle E, Le Cesne A, Blay JY, et al. Primary retroperitoneal sarcomas: a multivariate analysis of surgical factors associated with local control. J Clin Oncol. 2009;27:31-7.
21. Gronchi A, Miceli R, Colombo C, Stacchiotti S, Collini P, Mariani L, et al. Frontline extended surgery is associated with improved survival in retroperitoneal low- to intermediate-grade soft tissue sarcomas. Ann Oncol. 2012;23:1067-73.
22. Gronchi A, Lo Vullo S, Fiore M, Mussi C, Stacchiotti S, Collini P, et al. Aggressive surgical policies in a retrospectively reviewed single-institution case series of retroperitoneal soft tissue sarcoma patients. J Clin Oncol. 2009;27:24-30.
23. Vos M, Kosela-Paterczyk H, Rutkowski P, van Leenders GJLH, Normantowicz M, Lecyk A, et al. Differences in recurrence and survival of extremity liposarcoma subtypes. Eur J Surg Oncol. 2018;44:1391-7.
24. Bonvalot S, Gronchi A, Le Pechoux C, Swallow CJ, Strauss DC, Meeus P, et al. STRASS (EORTC 62092): A phase III randomized study of preoperative radiotherapy plus surgery versus surgery alone for patients with retroperitoneal sarcoma. Journal of Clinical Oncology. 2019;37:11001-.

Appendix A. Supplementary data





▲**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 ration; 95% CI, 95% confidence interval.

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

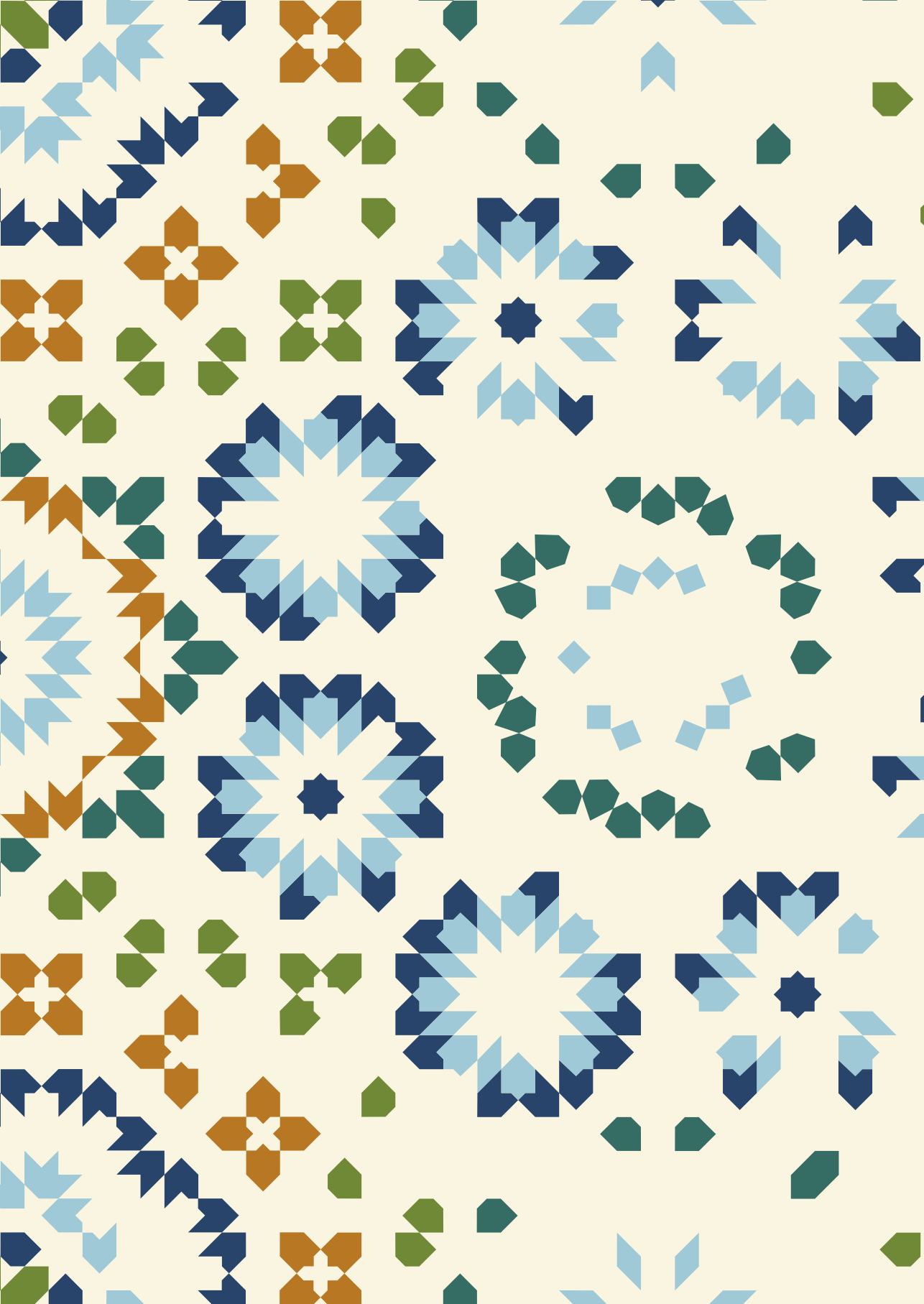
		Univariable				Multivariable			
		N	**HR**	**95% CI**	**p**	**N**	**HR**	**95% CI**	**p**
Age at time of diagnosis		518	0.99	0.98-1.01	0.180				
Gender	Male	290	Ref						
	Female	228	0.71	0.46-1.08	0.108				
Subtype	WDLPS	246	Ref			246	Ref		
	DDLPS	126	13.08	5.80-29.52	<0.001	126	6.74	2.46-18.44	<0.001
	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				Multivariable			
		N	**HR**	**95% CI**	**p**	**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

M. Vos, H. Koseła-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.

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 with DDLPS (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	
	Unknown	81	17.8	35	15.3	46	20.3	
Resection margins	Radical (R0)	285	62.5	94	41.0	191	84.1	<0.001[b]
	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	
	Unknown	2	0.4	2	0.9	0	0	
Adjuvant therapy	None	370	81.1	160	69.9	210	92.5	<0.001[b,c]
	RTx	82	18.0	65	28.4	17	7.5	
	CTx	1	0.2	1	0.4	0	0	
	Unknown	3	0.7	3	1.3	0	0	
Local recurrence (LR)	Yes	88	19.3	61	26.6	27	11.9	<0.001[b]
	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)		0.210

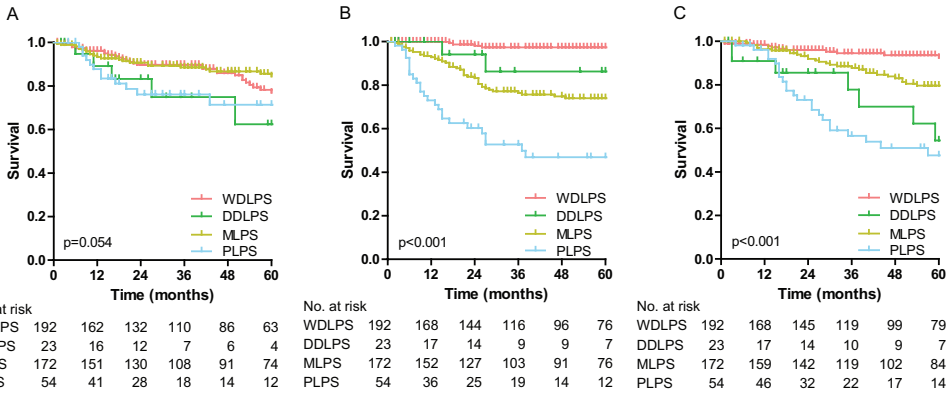
		Total (N=456)		Rotterdam (N=229)		Warsaw (N=227)		p-value[a]
		N	% of total	N	% of total	N	% of total	
Survival	Alive	357	78.3	170	74.2	187	82.4	0.035[b]
	Dead	99	21.7	59	25.8	40	17.6	
	- Death of disease	-	-	38	16.6	-	-	
	- Death of other/ unknown causes	-	-	21	9.2	-	-	
	Follow-up time (mo.), median (IQR)	61 (32-109)		68 (31-126)		57 (32-84)		<0.001[b,d]

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			**5y-DMFS**			**5y-OS**		
	Rotterdam	Warsaw	p-value	Rotterdam	Warsaw	p-value	Rotterdam	Warsaw	p-value
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

[a] Significant 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 subtypes DDLPS 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). [a] χ^2-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%)**	

[a] χ^2-test, [b] Fisher'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 the DDLPS, 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 be 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.

Acknowledgements

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.

References

1. Integraal Kankercentrum Nederland. Richtlijn wekedelen tumoren. IKNL; 2011.
2. The Netherlands Comprehensive Cancer Registry. Bijlage D Deelrapportage voor wekedelensarcomen. The Netherlands Comprehensive Cancer Organisation (IKNL); 2014.
3. Fletcher CDM, Bridge JA, Hogendoorn PCW, Mertens F, World Health Organization, International Agency for Research on Cancer. WHO classification of tumours of soft tissue and bone. Lyon: IARC Press; 2013.
4. Baxter KJ, Govsyeyev N, Namm JP, Gonzalez RJ, Roggin KK, Cardona K. Is multimodality therapy necessary for the management of pure myxoid liposarcomas? A multi-institutional series of pure myxoid liposarcomas of the extremities and torso. J Surg Oncol. 2015;111:146-51.
5. Cahlon O, Brennan MF, Jia X, Qin LX, Singer S, Alektiar KM. A postoperative nomogram for local recurrence risk in extremity soft tissue sarcomas after limb-sparing surgery without adjuvant radiation. Ann Surg. 2012;255:343-7.
6. Callegaro D, Miceli R, Bonvalot S, Ferguson P, Strauss DC, Levy A, et al. Development and external validation of two nomograms to predict overall survival and occurrence of distant metastases in adults after surgical resection of localised soft-tissue sarcomas of the extremities: a retrospective analysis. Lancet Oncol. 2016;17:671-80.
7. Cassier PA, Kantor G, Bonvalot S, Lavergne E, Stoeckle E, Le Pechoux C, et al. Adjuvant radiotherapy for extremity and trunk wall atypical lipomatous tumor/well-differentiated LPS (ALT/WD-LPS): a French Sarcoma Group (GSF-GETO) study. Ann Oncol. 2014;25:1854-60.
8. Dalal KM, Kattan MW, Antonescu CR, Brennan MF, Singer S. Subtype specific prognostic nomogram for patients with primary liposarcoma of the retroperitoneum, extremity, or trunk. Ann Surg. 2006;244:381-91.
9. Gronchi A, Casali PG, Mariani L, Miceli R, Fiore M, Lo Vullo S, et al. Status of surgical margins and prognosis in adult soft tissue sarcomas of the extremities: a series of patients treated at a single institution. J Clin Oncol. 2005;23:96-104.
10. Gronchi A, Lo Vullo S, Colombo C, Collini P, Stacchiotti S, Mariani L, et al. Extremity soft tissue sarcoma in a series of patients treated at a single institution: local control directly impacts survival. Ann Surg. 2010;251:506-11.
11. Lemeur M, Mattei JC, Souteyrand P, Chagnaud C, Curvale G, Rochwerger A. Prognostic factors for the recurrence of myxoid liposarcoma: 20 cases with up to 8 years follow-up. Orthop Traumatol Surg Res. 2015;101:103-7.
12. Rutkowski P, Trepka S, Ptaszynski K, Kolodziejczyk M. Surgery quality and tumor status impact on survival and local control of resectable liposarcomas of extremities or the trunk wall. Clin Orthop Relat Res. 2013;471:860-70.
13. Smith HG, Memos N, Thomas JM, Smith MJ, Strauss DC, Hayes AJ. Patterns of disease relapse in primary extremity soft-tissue sarcoma. Br J Surg. 2016;103:1487-96.
14. Trovik CS, Bauer HC, Alvegard TA, Anderson H, Blomqvist C, Berlin O, et al. Surgical margins, local recurrence and metastasis in soft tissue sarcomas: 559 surgically-treated patients from the Scandinavian Sarcoma Group Register. Eur J Cancer. 2000;36:710-6.
15. Gronchi A, Casali PG, Fiore M, Mariani L, Lo Vullo S, Bertulli R, et al. Retroperitoneal soft tissue sarcomas: patterns of recurrence in 167 patients treated at a single institution. Cancer. 2004;100:2448-55.
16. Gronchi A, Strauss DC, Miceli R, Bonvalot S, Swallow CJ, Hohenberger P, et al. Variability in Patterns of Recurrence After Resection of Primary Retroperitoneal Sarcoma (RPS): A Report on 1007 Patients From the Multi-institutional Collaborative RPS Working Group. Ann Surg. 2016;263:1002-9.

17. Singer S, Antonescu CR, Riedel E, Brennan MF. Histologic subtype and margin of resection predict pattern of recurrence and survival for retroperitoneal liposarcoma. Ann Surg. 2003;238:358-70; discussion 70-1.
18. Schemper M, Smith TL. A note on quantifying follow-up in studies of failure time. Controlled Clinical Trials. 1996;17:343-6.
19. Nishida Y, Tsukushi S, Nakashima H, Ishiguro N. Clinicopathologic prognostic factors of pure myxoid liposarcoma of the extremities and trunk wall. Clin Orthop Relat Res. 2010;468:3041-6.
20. Yang JC, Chang AE, Baker AR, Sindelar WF, Danforth DN, Topalian SL, et al. Randomized prospective study of the benefit of adjuvant radiation therapy in the treatment of soft tissue sarcomas of the extremity. J Clin Oncol. 1998;16:197-203.
21. Suit HD, Russell WO, Martin RG. Sarcoma of soft tissue: clinical and histopathologic parameters and response to treatment. Cancer. 1975;35:1478-83.
22. Jebsen NL, Trovik CS, Bauer HC, Rydholm A, Monge OR, Hall KS, et al. Radiotherapy to improve local control regardless of surgical margin and malignancy grade in extremity and trunk wall soft tissue sarcoma: a Scandinavian sarcoma group study. Int J Radiat Oncol Biol Phys. 2008;71:1196-203.
23. Zagars GK, Goswitz MS, Pollack A. Liposarcoma: outcome and prognostic factors following conservation surgery and radiation therapy. Int J Radiat Oncol Biol Phys. 1996;36:311-9.
24. Pitson G, Robinson P, Wilke D, Kandel RA, White L, Griffin AM, et al. Radiation response: An additional unique signature of myxoid liposarcoma. International Journal of Radiation Oncology*Biology*Physics. 2004;60:522-6.
25. Chung PW, Deheshi BM, Ferguson PC, Wunder JS, Griffin AM, Catton CN, et al. Radiosensitivity translates into excellent local control in extremity myxoid liposarcoma: a comparison with other soft tissue sarcomas. Cancer. 2009;115:3254-61.
26. Koseła-Paterczyk H, Szumera-Ciećkiewicz A, Szacht M, Haas R, Morysiński T, Dziewirski W, et al. Efficacy of neoadjuvant hypofractionated radiotherapy in patients with locally advanced myxoid liposarcoma. European Journal of Surgical Oncology (EJSO). 2016;42:891-8.
27. O'Sullivan B, Davis AM, Turcotte R, Bell R, Catton C, Chabot P, et al. Preoperative versus postoperative radiotherapy in soft-tissue sarcoma of the limbs: a randomised trial. Lancet. 2002;359:2235-41.
28. Hui AC, Ngan SY, Wong K, Powell G, Choong PFM. Preoperative radiotherapy for soft tissue sarcoma: The Peter MacCallum Cancer Centre experience. European Journal of Surgical Oncology (EJSO). 2006;32:1159-64.
29. Cai L, Mirimanoff R-O, Mouhsine E, Guillou L, Leyvraz P-F, Leyvraz S, et al. Prognostic Factors in Adult Soft Tissue Sarcoma Treated with Surgery Combined with Radiotherapy: A Retrospective Single-Center Study on 164 Patients. Rare Tumors. 2013;5:e55.
30. McGee L, Indelicato DJ, Dagan R, Morris CG, Knapik JA, Reith JD, et al. Long-Term Results Following Postoperative Radiotherapy for Soft Tissue Sarcomas of the Extremity. International Journal of Radiation Oncology*Biology*Physics. 2012;84:1003-9.
31. Haas RLM, DeLaney TF, O'Sullivan B, Keus RB, Le Pechoux C, Olmi P, et al. Radiotherapy for Management of Extremity Soft Tissue Sarcomas: Why, When, and Where? International Journal of Radiation Oncology*Biology*Physics. 2012;84:572-80.
32. El-Bared N, Wong P, Wang D. Soft tissue sarcoma and radiation therapy advances, impact on toxicity. Curr Treat Options Oncol. 2015;16:19.
33. Felderhof JM, Creutzberg CL, Putter H, Nout RA, Bovee JV, Dijkstra PD, et al. Long-term clinical outcome of patients with soft tissue sarcomas treated with limb-sparing surgery and postoperative radiotherapy. Acta Oncol. 2013;52:745-52.

34. Ortiz-Ibanez B, Amaya JV, Baixauli F, Angulo M, Mayordomo-Aranda E, Barrios C. Surgical resection of massive liposarcomas at the extremities: a 10-year experience in a referral musculoskeletal sarcoma unit. World J Surg Oncol. 2015;13:206.
35. Kooby DA, Antonescu CR, Brennan MF, Singer S. Atypical lipomatous tumor/well-differentiated liposarcoma of the extremity and trunk wall: importance of histological subtype with treatment recommendations. Ann Surg Oncol. 2004;11:78-84.
36. Smith CA, Martinez SR, Tseng WH, Tamurian RM, Bold RJ, Borys D, et al. Predicting survival for well-differentiated liposarcoma: the importance of tumor location. J Surg Res. 2012;175:12-7.

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**
Physical examination		
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
Imaging		
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**
Physical examination		
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
Imaging		
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%**

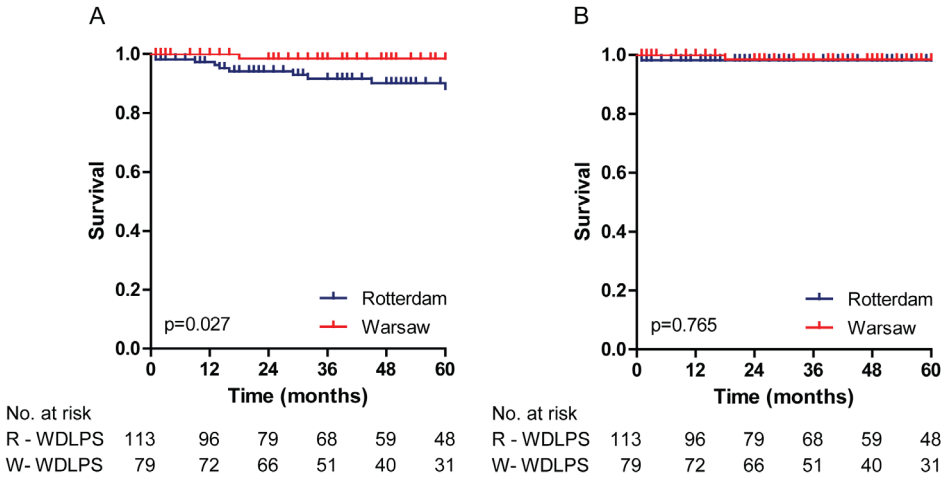
[a] Difference between subtypes significant with p=0.005
[b] Difference between subtypes significant with p<0.001
5y-OS: 5-year overall survival, 5y-DSS: 5-year disease specific survival, SE: Standard Error

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

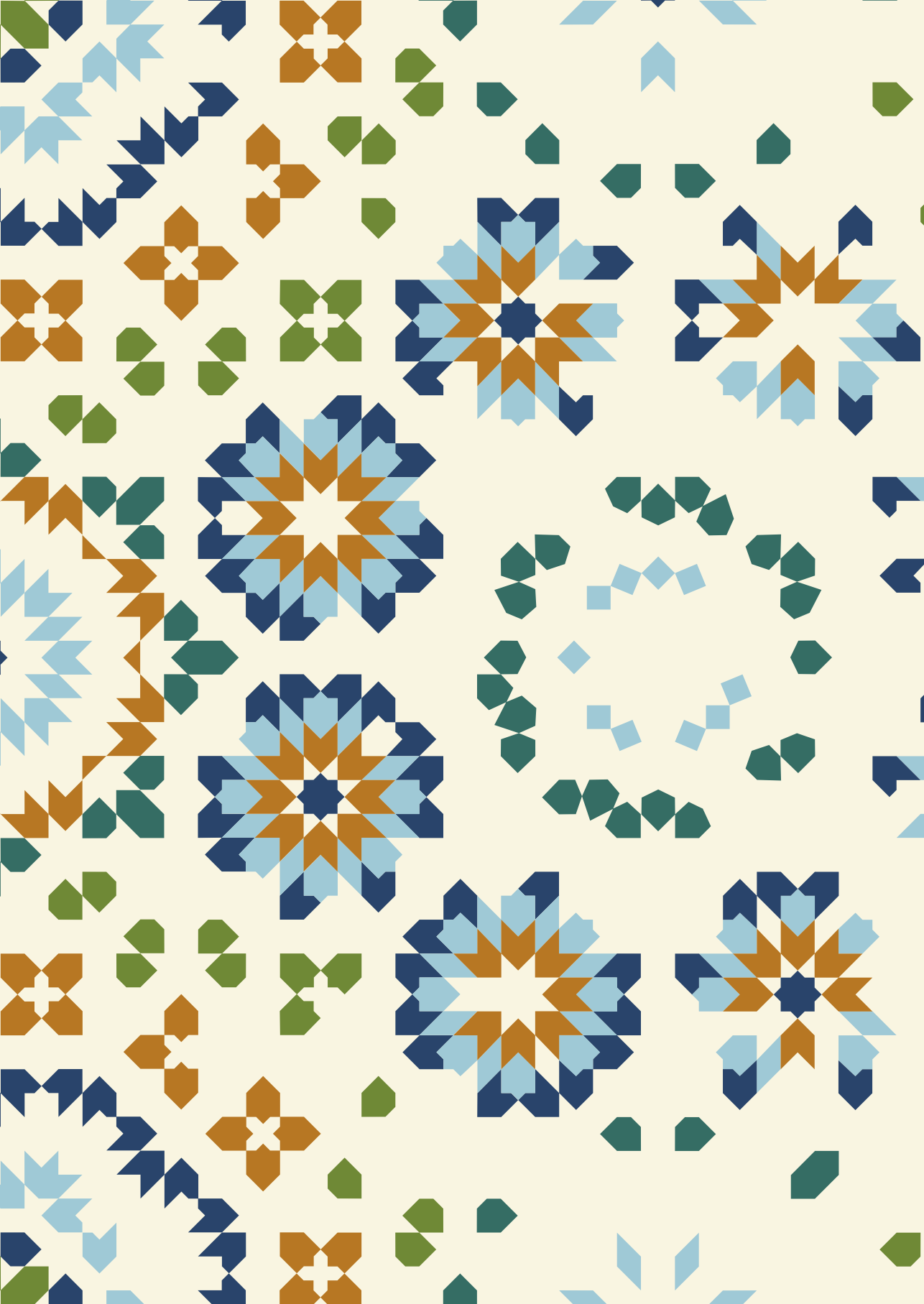
		Local recurrence				Distant metastasis				Overall survival			
		N	HR	CI (95%)	p-value	N	HR	CI (95%)	p-value	N	HR	CI (95%)	p-value
Age at diagnosis (in years)		441	1.016	0.999-1.033	0.069	434	0.996	0.980-1.012	0.600	441	1.031	1.014-1.048	<0.001*
Gender	Female	215	1			215	1			215	1		
	Male	226	1.725	1.057-2.816	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)		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
Radicality	R0	273	1			273	1			273	1		
	R1/R2	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
	Unknown	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
Neoadj. treatment	None	234	1			234	1			234	1		
	RTx	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
	Other	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‡
Adjuvant treatment	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

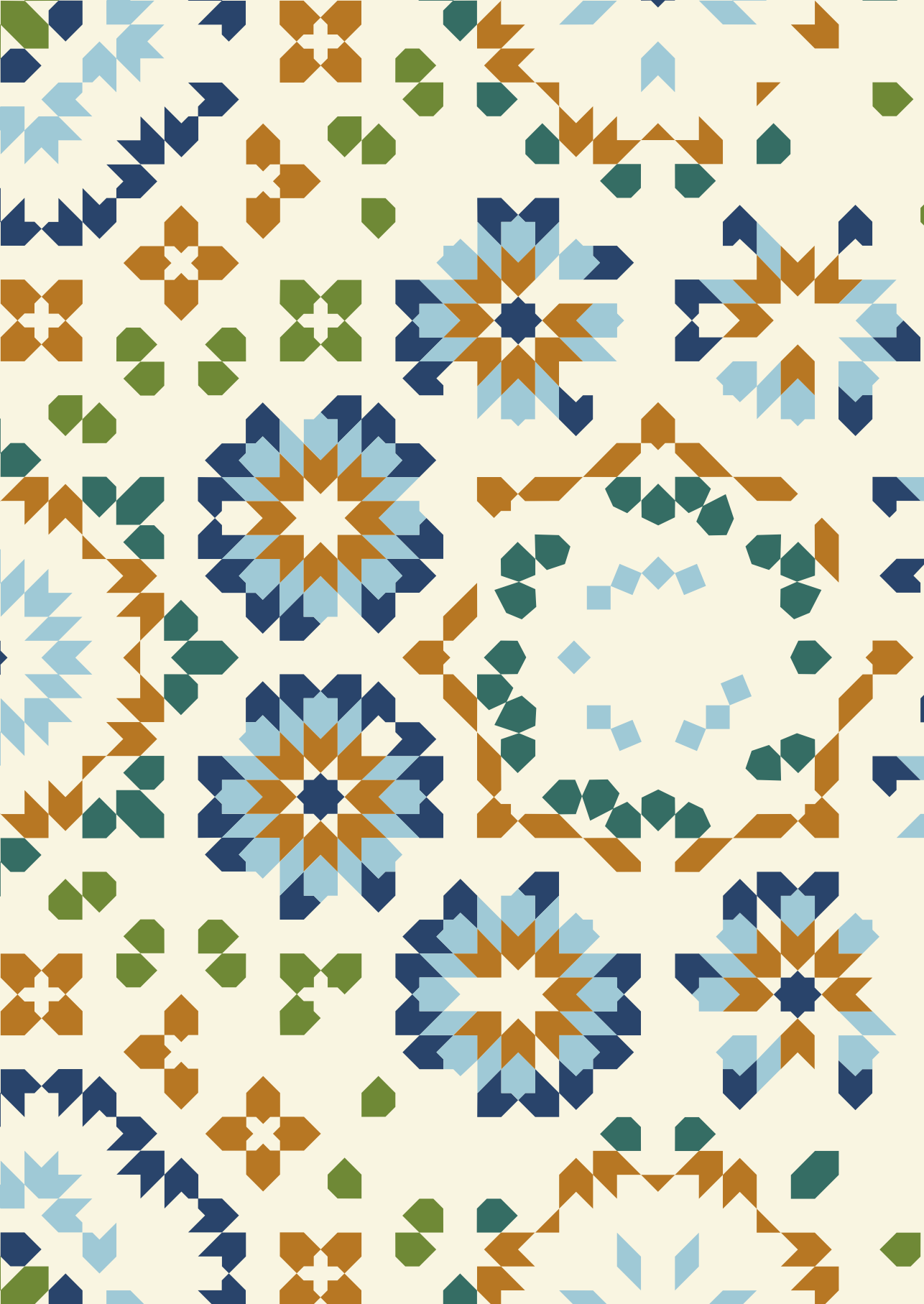


▲**Figure S1.** 5-year overall survival (**A**) and 5-year disease specific survival (**B**) compared between the two centres for the subgroup of patients with WDLPS.



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

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

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

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)[a]		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)[a]		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)[a]	41 (15-57)		41 (15-57)		43 (21.5-64)		0.869
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)[a]	24 (18-26)		24 (21-25)		68.5 (17-120)		1.000
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)[a]		49 (24-75.5)		49.5 (19-82.5)		48 (27-74)		0.949

[a] 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.

			Primary tumor		Local Recurrence				Distant Metastases			Follow-up			
No.	Age[a]	R/W	Margins	RTx	LR	TLR	Dediff.	Treatment	TSD	Metastatic sites	Treatment	Survival	Total FU[‡]	FU since DM[◊]	Remarks
1	48	R	R1	No RTx	Yes	5	Yes	Surgery + RTx	26	Lungs, later multiple	CTx + RTx	DOD	75	49	
2	42	R	Unknown	No RTx	Yes	19	Yes	Surgery + RTx	24	Multiple sites (subcutaneous)	Surgery + CTx (CR)	Alive	198	174	
3	47	W	R0	Neoadj. RTX	Yes	45	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	18	No	None	18	Paravertebral	RTx (24Gy)	Alive	60	42	No biopsy of metastatic site
5	64	W	R0	Neoadj. RTX	No	NA	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.
[a] Age 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.

			Primary tumor		Local Recurrence				Distant metastasis			Follow-up			
	Age[a]	R/W	Margins	(Neo)adj. treatment	LR	TLR	Dediff.	Treatment	DM	TSD	Treatment	Survival	FU since DM[◊]	Total FU[‡]	Remarks
1	64	W	R0	Neoadj. RTx	No	-	-	-	Yes	17	None	DOD	1	18	Unfit for CTx
2	48	R	R1	None	Yes	5	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

R/W: 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). [‡]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 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

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.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

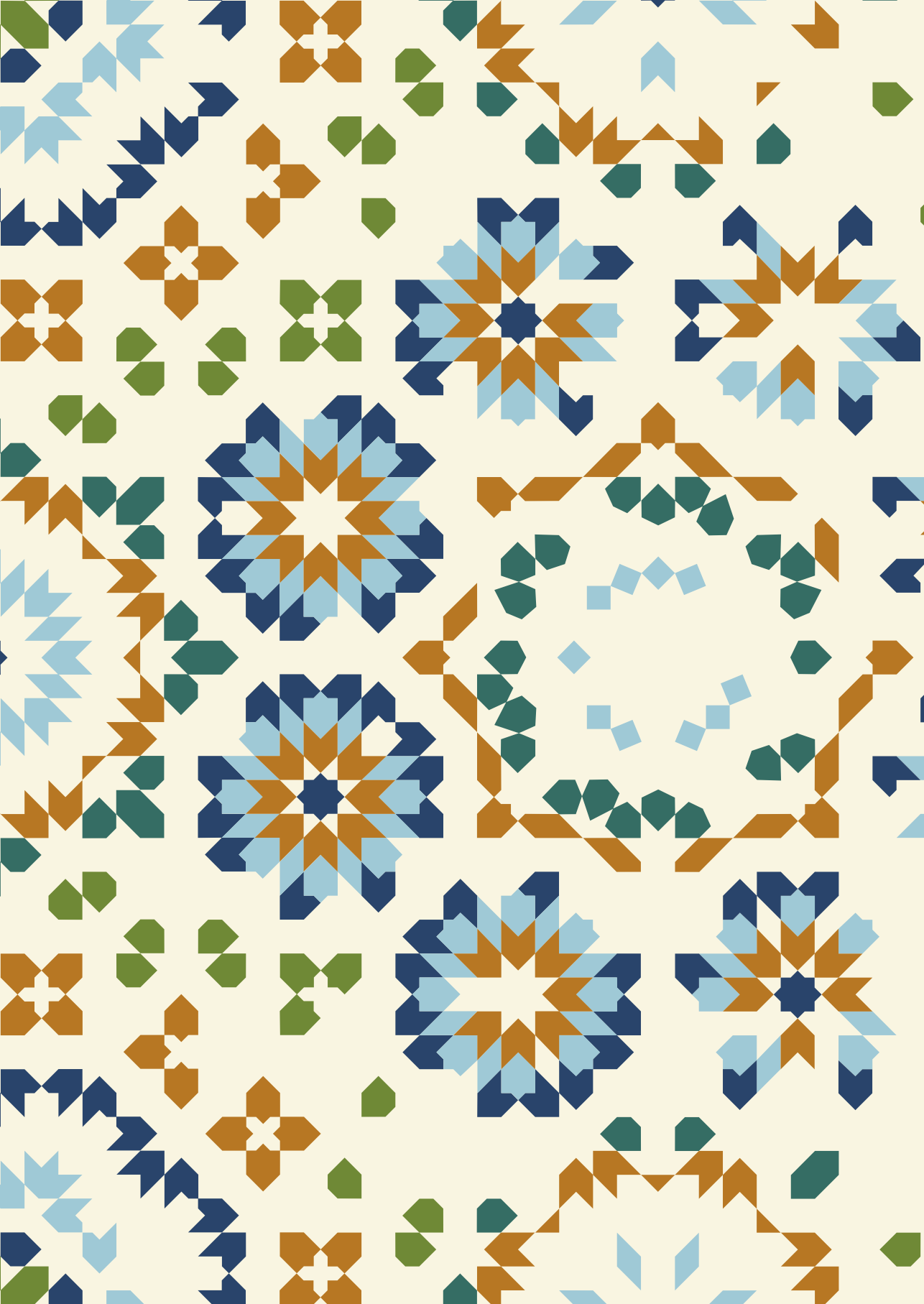
Acknowledgements

None.

References

1. Fletcher CDM, Bridge JA, Hogendoorn PCW, Mertens F, World Health Organization, International Agency for Research on Cancer. WHO classification of tumours of soft tissue and bone. Lyon: IARC Press; 2013.
2. Evans HL, Soule EH, Winkelmann RK. Atypical lipoma, atypical intramuscular lipoma, and well differentiated retroperitoneal liposarcoma: a reappraisal of 30 cases formerly classified as well differentiated liposarcoma. Cancer. 1979;43:574-84.
3. Gatta G, Capocaccia R, Botta L, Mallone S, De Angelis R, Ardanaz E, et al. Burden and centralised treatment in Europe of rare tumours: results of RARECAREnet-a population-based study. Lancet Oncol. 2017;18:1022-39.
4. Yamamoto N, Hayashi K, Tanzawa Y, Kimura H, Takeuchi A, Igarashi K, et al. Treatment strategies for well-differentiated liposarcomas and therapeutic outcomes. Anticancer Res. 2012;32:1821-5.
5. Kubo T, Sugita T, Shimose S, Arihiro K, Ochi M. Conservative surgery for well-differentiated liposarcomas of the extremities adjacent to major neurovascular structures. Surg Oncol. 2006;15:167-71.
6. Sommerville SM, Patton JT, Luscombe JC, Mangham DC, Grimer RJ. Clinical outcomes of deep atypical lipomas (well-differentiated lipoma-like liposarcomas) of the extremities. ANZ J Surg. 2005;75:803-6.
7. Bonvalot S, Ternes N, Fiore M, Bitsakou G, Colombo C, Honore C, et al. Spontaneous regression of primary abdominal wall desmoid tumors: more common than previously thought. Ann Surg Oncol. 2013;20:4096-102.
8. Fiore M, Rimareix F, Mariani L, Domont J, Collini P, Le Pechoux C, et al. Desmoid-type fibromatosis: a front-line conservative approach to select patients for surgical treatment. Ann Surg Oncol. 2009;16:2587-93.
9. Bonvalot S, Eldweny H, Haddad V, Rimareix F, Missenard G, Oberlin O, et al. Extra-abdominal primary fibromatosis: Aggressive management could be avoided in a subgroup of patients. Eur J Surg Oncol. 2008;34:462-8.
10. van Broekhoven DL, Grunhagen DJ, van Dalen T, van Coevorden F, Bonenkamp HJ, Been LB, et al. Tailored Beta-catenin mutational approach in extra-abdominal sporadic desmoid tumor patients without therapeutic intervention. BMC Cancer. 2016;16:686.
11. Kasper B, Baumgarten C, Bonvalot S, Haas R, Haller F, Hohenberger P, et al. Management of sporadic desmoid-type fibromatosis: A European consensus approach based on patients' and professionals' expertise – A Sarcoma Patients EuroNet and European Organisation for Research and Treatment of Cancer/Soft Tissue and Bone Sarcoma Group initiative. European Journal of Cancer. 2015;51:127-36.
12. Vos M, Kosela-Paterczyk H, Rutkowski P, van Leenders GJLH, Normantowicz M, Lecyk A, et al. Differences in recurrence and survival of extremity liposarcoma subtypes. Eur J Surg Oncol. 2018;44:1391-7.
13. Integraal Kankercentrum Nederland. Richtlijn wekedelen tumoren. IKNL; 2011.
14. Mavrogenis AF, Lesensky J, Romagnoli C, Alberghini M, Letson GD, Ruggieri P. Atypical lipomatous tumors/well-differentiated liposarcomas: clinical outcome of 67 patients. Orthopedics. 2011;34:e893-8.
15. Kooby DA, Antonescu CR, Brennan MF, Singer S. Atypical lipomatous tumor/well-differentiated liposarcoma of the extremity and trunk wall: importance of histological subtype with treatment recommendations. Ann Surg Oncol. 2004;11:78-84.

16. Zagars GK, Goswitz MS, Pollack A. Liposarcoma: outcome and prognostic factors following conservation surgery and radiation therapy. Int J Radiat Oncol Biol Phys. 1996;36:311-9.
17. Dalal KM, Kattan MW, Antonescu CR, Brennan MF, Singer S. Subtype specific prognostic nomogram for patients with primary liposarcoma of the retroperitoneum, extremity, or trunk. Ann Surg. 2006;244:381-91.
18. Chang DH, Ma H, Liao WC, Huang MH, Wu PS. Atypical Lipomatous Tumors of the Extremities and Trunk Wall-The First Case Series of Chinese Population With 45 Cases. Ann Plast Surg. 2016;76 Suppl 1:S8-12.
19. Kito M, Yoshimura Y, Isobe K, Aoki K, Momose T, Suzuki S, et al. Clinical outcome of deep-seated atypical lipomatous tumor of the extremities with median-term follow-up study. Eur J Surg Oncol. 2015;41:400-6.
20. Rutkowski P, Trepka S, Ptaszynski K, Kolodziejczyk M. Surgery quality and tumor status impact on survival and local control of resectable liposarcomas of extremities or the trunk wall. Clin Orthop Relat Res. 2013;471:860-70.
21. Al-Refaie WB, Habermann EB, Jensen EH, Tuttle TM, Pisters PW, Virnig BA. Surgery alone is adequate treatment for early stage soft tissue sarcoma of the extremity. Br J Surg. 2010;97:707-13.
22. Cassier PA, Kantor G, Bonvalot S, Lavergne E, Stoeckle E, Le Pechoux C, et al. Adjuvant radiotherapy for extremity and trunk wall atypical lipomatous tumor/well-differentiated LPS (ALT/WD-LPS): a French Sarcoma Group (GSF-GETO) study. Ann Oncol. 2014;25:1854-60.
23. Rothermundt C, Whelan JS, Dileo P, Strauss SJ, Coleman J, Briggs TW, et al. What is the role of routine follow-up for localised limb soft tissue sarcomas? A retrospective analysis of 174 patients. Br J Cancer. 2014;110:2420-6.
24. Puri A, Gulia A, Hawaldar R, Ranganathan P, Badwe RA. Does Intensity of Surveillance Affect Survival After Surgery for Sarcomas? Results of a Randomized Noninferiority Trial. Clinical Orthopaedics and Related Research®. 2014;472:1568-75.
25. Italiano A, Toulmonde M, Cioffi A, Penel N, Isambert N, Bompas E, et al. Advanced well-differentiated/dedifferentiated liposarcomas: role of chemotherapy and survival. Annals of Oncology. 2012;23:1601-7.
26. Dangoor A, Seddon B, Gerrand C, Grimer R, Whelan J, Judson I. UK guidelines for the management of soft tissue sarcomas. Clinical Sarcoma Research. 2016;6:20.
27. Manji GA, Schwartz GK. Managing Liposarcomas: Cutting Through the Fat. Journal of Oncology Practice. 2016;12:221-7.
28. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer. 2009;45:228-47.
29. Gronchi A, Colombo C. Tailored Beta-catenin Mutational Approach in Extra-abdominal Sporadic Desmoids Tumor Patients (ClinicalTrials.gov Identifier: NCT02547831). NIH U.S. National Libraby of Medicine (ClinicalTrial.gov); 2015.
30. Bonvalot S, Motreff T. Peripheral Primitive Fibromatosis (WS-RT Fibro) (ClinicalTrials.gov Identifier: NCT01801176). NIH U.S. National Libraby of Medicine (ClinicalTrial.gov); 2013.



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

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.

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

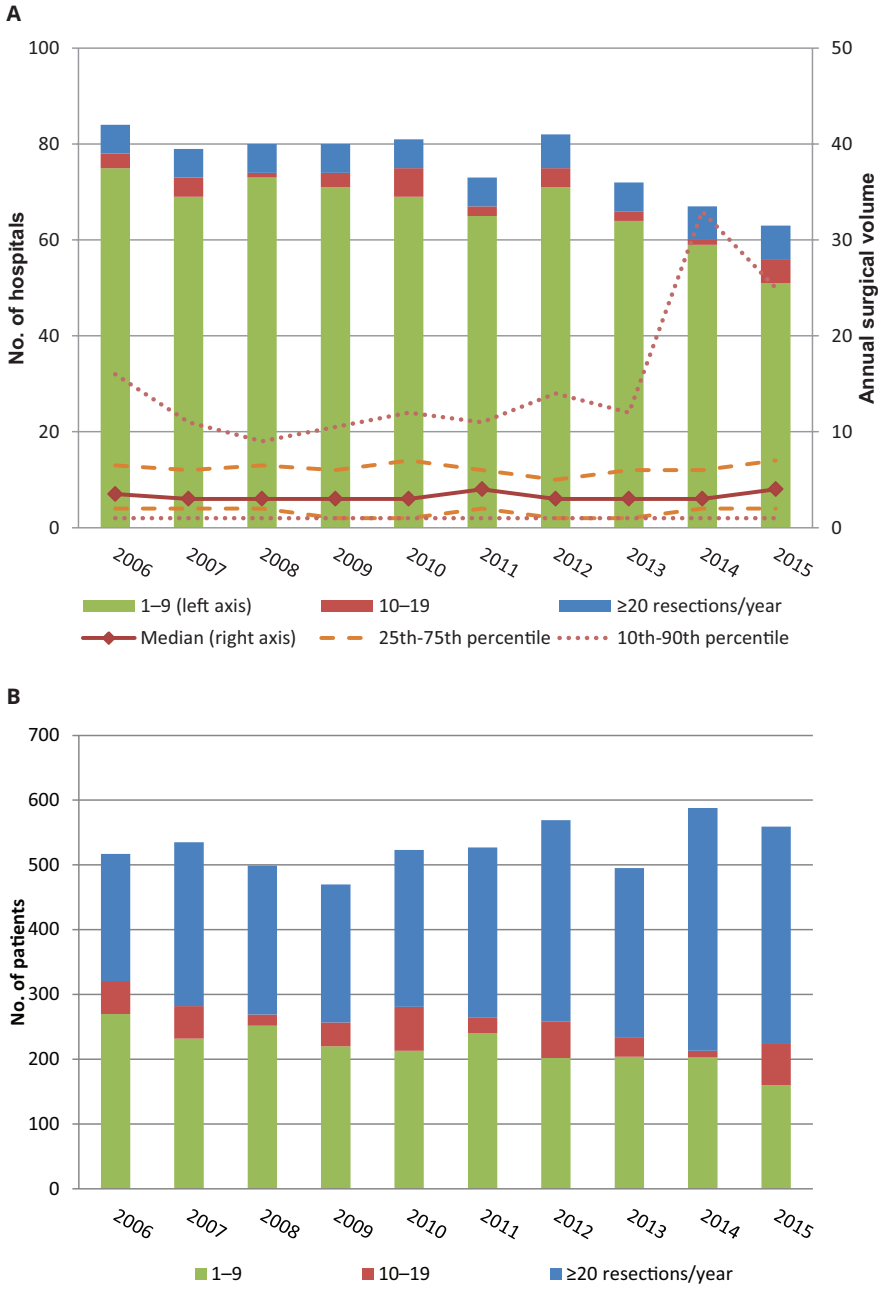
On annual average, in 76 hospitals STS surgery was performed. This number decreased from 82 hospitals in 2006-2007 to 66 in 2014-2015 ($p = 0.05$), mainly because of a decrease in the number of low-volume hospitals (72 to 56) (Table 1). Of the hospitals in which STS surgery was performed, 88% of the 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

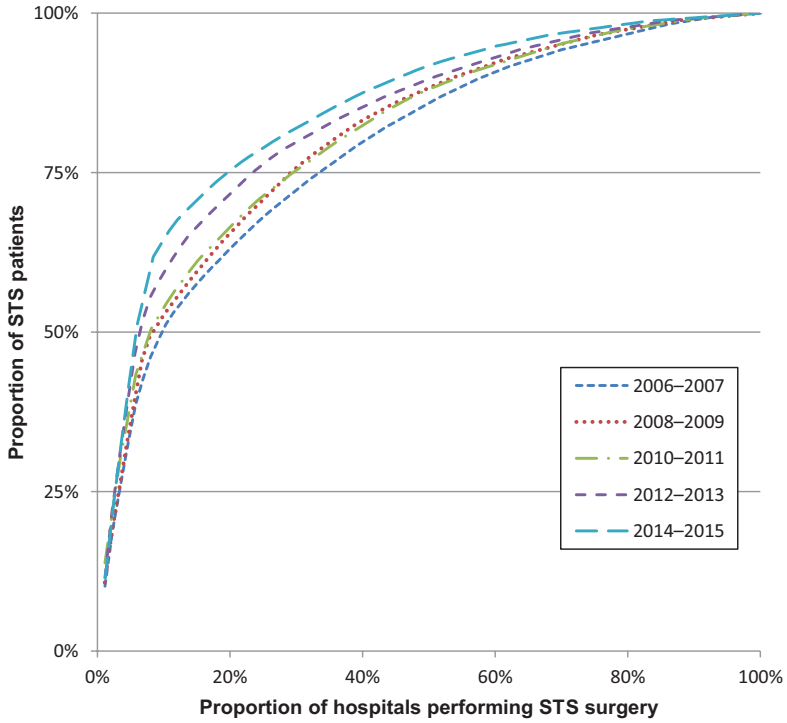
Table 2. Tumour characteristics and surgical characteristics of patients undergoing surgery for soft tissue sarcoma in the Netherlands from 2006 to 2015 stratified by hospital volume.

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 to2015, 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-

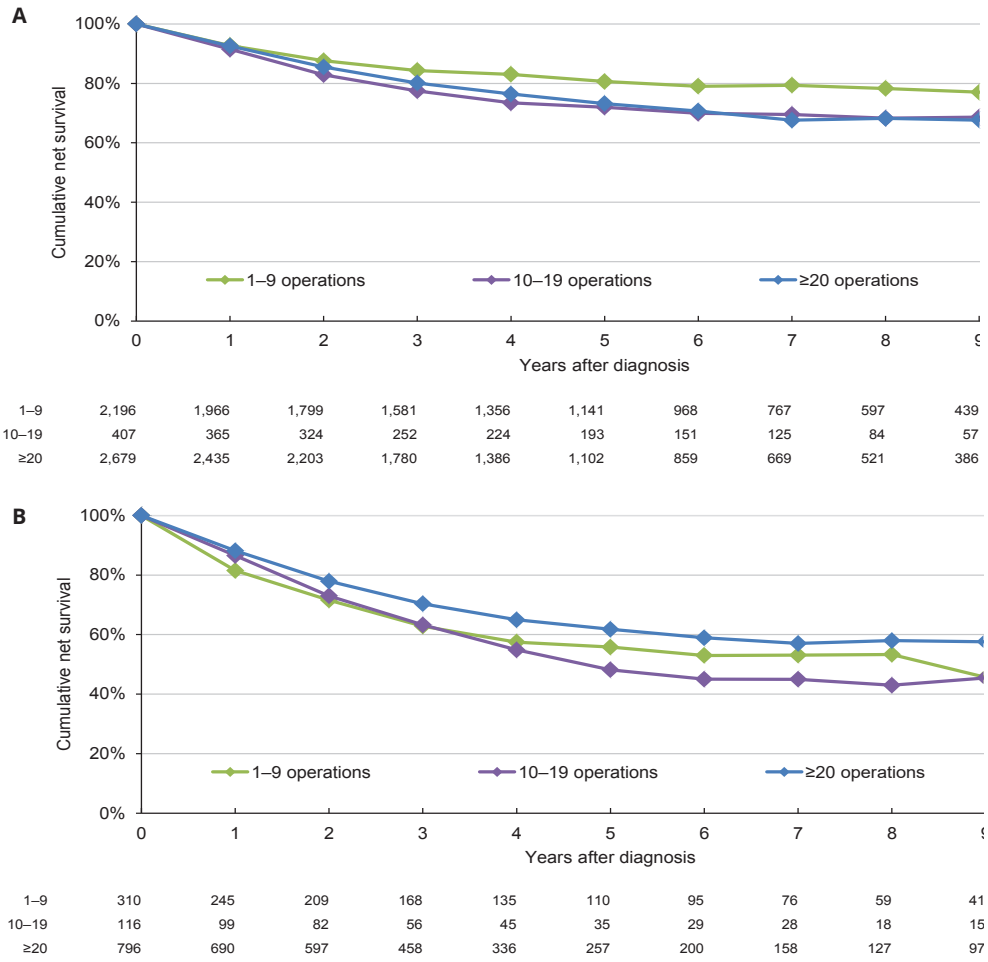
volume versus low-volume, 95% CI 1.3-1.7, p < 0.001) (Fig. 3A). However, after adjustment for other prognostic factors, a multivariable Poisson regression analysis did not show any impact of surgical volume on net survival (medium-volume versus high-volume: relative rate [RR] 1.2, 95% CI 0.93-1.4, p = 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 ≤ 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/adjuvant radiotherapy, instead of surgery alone.

It has been shown that simple referral guidelines (referral of all deep-seated tumours and all superficial tumours ≥5cm) 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

The authors thank the registration team of the Netherlands Comprehensive Cancer Organisation (IKNL) for collecting data in the Netherlands Cancer Registry.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

Conflicts of interest statement

None of the authors has any conflicts of interest.

Appendix A. Supplementary data

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

References

1. Fletcher CDM, Bridge JA, Hogendoorn PCW, Mertens F, World Health Organization, International Agency for Research on Cancer. WHO classification of tumours of soft tissue and bone. Lyon: IARC Press; 2013.
2. The Netherlands Comprehensive Cancer Registry. Bijlage D Deelrapportage voor wekedelensarcomen. The Netherlands Comprehensive Cancer Organisation (IKNL); 2014.
3. Hoekstra HJ, Haas RLM, Verhoef C, Suurmeijer AJH, van Rijswijk CSP, Bongers BGH, et al. Adherence to Guidelines for Adult (Non-GIST) Soft Tissue Sarcoma in the Netherlands: A Plea for Dedicated Sarcoma Centers. Annals of Surgical Oncology. 2017.
4. Stepniewska KA. snp4: Non-parametric test for trend across ordered groups. Stata technical bulletin. 1992;9:21.
5. Schaffar R, Rachet B, Belot A, Woods LM. Estimation of net survival for cancer patients: Relative survival setting more robust to some assumption violations than cause-specific setting, a sensitivity analysis on empirical data. Eur J Cancer. 2017;72:78-83.
6. Perme MP, Stare J, Estève J. On Estimation in Relative Survival. Biometrics. 2012;68:113-20.
7. Ederer F, Heise H. Instructions to IBM 650 programmers in processing survival computations. Methodological note no. 10, end results evaluation section. Bethesda. Bethesda MD, National Cancer Institute. 1959.
8. Maurice MJ, Yih JM, Ammori JB, Abouassaly R. Predictors of surgical quality for retroperitoneal sarcoma: Volume matters. J Surg Oncol. 2017;116:766-74.
9. Gutierrez JC, Perez EA, Moffat FL, Livingstone AS, Franceschi D, Koniaris LG. Should soft tissue sarcomas be treated at high-volume centers? An analysis of 4205 patients. Ann Surg. 2007;245:952-8.
10. Abarca T, Gao Y, Monga V, Tanas MR, Milhem MM, Miller BJ. Improved survival for extremity soft tissue sarcoma treated in high-volume facilities. J Surg Oncol. 2018.
11. Blay JY, Soibinet P, Penel N, Bompas E, Duffaud F, Stoeckle E, et al. Improved survival using specialized multidisciplinary board in sarcoma patients. Ann Oncol. 2017;28:2852-9.
12. Bhangu AA, Beard JA, Grimer RJ. Should Soft Tissue Sarcomas be Treated at a Specialist Centre? Sarcoma. 2004;8:1-6.
13. Derbel O, Heudel PE, Cropet C, Meeus P, Vaz G, Biron P, et al. Survival impact of centralization and clinical guidelines for soft tissue sarcoma (A prospective and exhaustive population-based cohort). PLoS One. 2017;12:e0158406.
14. Blay JY, Stoeckle E, Italiano A, Rochwerger RA, Duffaud F, Bonvalot S, et al. Improved overall and progression free survival after surgery in expert sites for sarcoma patients: A nationwide study of FSG-GETO/NETSARC (1474O). Annals of Oncology. 2017;28:mdx387.001-mdx387.001.
15. Martin-Broto J, Hindi N, Cruz J, Martinez-Trufero J, Valverde C, De Sande LM, et al. Relevance of Reference Centers in Sarcoma Care and Quality Item Evaluation: Results from the Prospective Registry of the Spanish Group for Research in Sarcoma (GEIS). Oncologist. 2018.
16. Venigalla S, Nead KT, Sebro R, Guttmann DM, Sharma S, Simone CB, 2nd, et al. Association Between Treatment at High-Volume Facilities and Improved Overall Survival in Soft Tissue Sarcomas. Int J Radiat Oncol Biol Phys. 2018;100:1004-15.
17. Hoshi M, Ieguchi M, Takami M, Aono M, Taguchi S, Kuroda T, et al. Clinical problems after initial unplanned resection of sarcoma. Jpn J Clin Oncol. 2008;38:701-9.
18. Smolle MA, Andreou D, Tunn PU, Szkandera J, Liegl-Atzwanger B, Leithner A. Diagnosis and treatment of soft-tissue sarcomas of the extremities and trunk. EFORT Open Rev. 2017;2:421-31.

19. Koulaxouzidis G, Schwarzkopf E, Bannasch H, Stark GB. Is revisional surgery mandatory when an unexpected sarcoma diagnosis is made following primary surgery? World J Surg Oncol. 2015;13:306.
20. Noria S, Davis A, Kandel R, Levesque J, O'Sullivan B, Wunder J, et al. Residual disease following unplanned excision of soft-tissue sarcoma of an extremity. J Bone Joint Surg Am. 1996;78:650-5.
21. Styring E, Billing V, Hartman L, Nilbert M, Seinen JM, Veurink N, et al. Simple guidelines for efficient referral of soft-tissue sarcomas: a population-based evaluation of adherence to guidelines and referral patterns. J Bone Joint Surg Am. 2012;94:1291-6.
22. Stichting Oncologische Samenwerking (SONCOS). Standardisation of multidisciplinary cancer care in the Netherlands - SONCOS Standardisatrion Report 5 2017. SONCOS; 2017.

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.

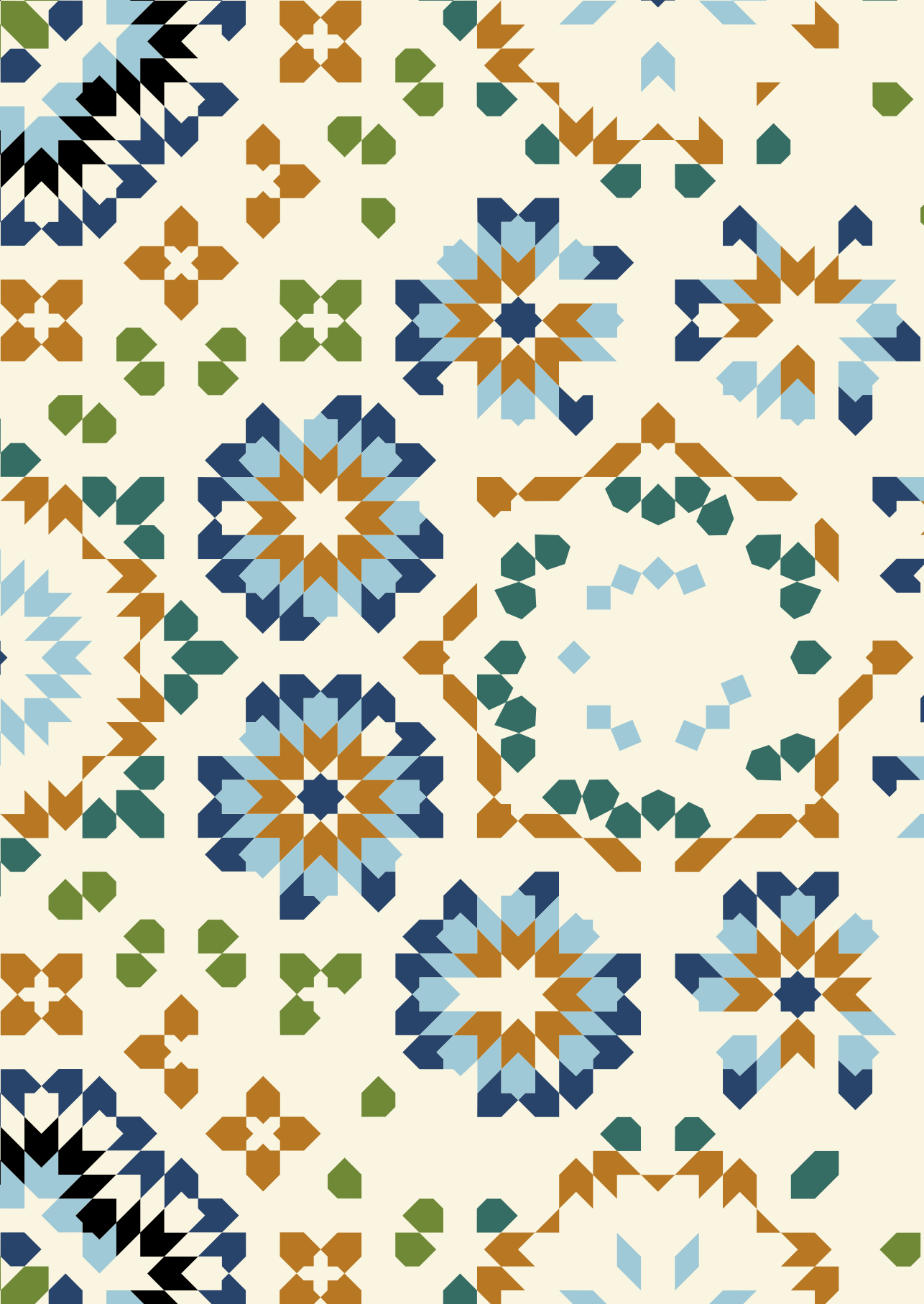
	Total	Grade			Size			Depth			Tumor site			
STS subtype		**Low**	**High**	**Unknown**	**≤5 cm**	**>5 cm**	**Unknown**	**Superficial**	**Deep**	**Unknown**	**H&N**	**Trunk**	**Extremity**	**(Retro) peritoneum**
Liposarcoma	1,087	415	634	38	163	873	51	417	610	60	28	349	501	209
Fibrosarcoma	653	160	343	150	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
Rhabdomyosar-coma	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.

Abstract

Introduction and aim: Timely recognition of soft tissue sarcomas (STS) remains challenging, potentially leading to unplanned resections (also known as 'whoops procedures'). This population-based study charted the occurrence of unplanned resections and identified associated patient, tumor, and hospital characteristics. Furthermore, it presents an overview of the outcomes and management following an unplanned resection.

Methods: From the Netherlands Cancer Registry (NCR) database, information was obtained on adult patients diagnosed with STS in 2016–2018 who underwent surgery. Tumors located in the mediastinum, heart or retroperitoneum were excluded. Differences between patients with planned and unplanned resections were assessed with chi-square tests and a multivariable logistic regression model.

Results: Unplanned resections occurred in 17.4% of operations for STS. Tumors not suspected of being STS prior to surgery were generally smaller (≤5 cm) and were more often located in the upper extremity. Preoperative imaging was omitted more frequently in these cases. Patients who had an unplanned resection outside of a sarcoma center were more often discussed at or referred to a sarcoma center, especially when there was residual tumor left in situ.

Discussion: The number of unplanned resections in this study was relatively low, and they occurred mostly with smaller tumors and those located in the upper extremity. Further improvement should be achieved by better compliance to preoperative imaging protocols as well as fostering better awareness of STS.

Introduction

Soft tissue sarcomas (STS) are a heterogeneous group of malignancies that account for less than 1% of all adult cancers, with an incidence rate of approximately 4/100,000 persons in the Netherlands [1]. At the same time, benign soft tissue tumors are much more common and are considered to outnumber STS with at least a factor 100 [2-4]. Therefore, it is not unusual for a STS to initially be considered a benign soft tissue tumor and excised without proper diagnostic workup – such as preoperative imaging and a biopsy – leaving inadequate surgical margins. These unplanned resections, also known as 'whoops procedures', may affect patient prognosis in terms of survival [5-7]. Furthermore, as these unplanned resections entail a higher risk of residual disease, possibly aggravated by contamination of adjacent tissue, they are associated with more extensive surgery (re-excision, amputation) to achieve adequate surgical margins [8].

The extent to which unplanned resections take place in daily clinical practice is unclear. Rates reported in the literature vary widely from one-fifth to over half of all STS resections [6, 9-11]. In addition, variation may depend on the clinical setting in which surgery took place, with dedicated sarcoma centers achieving lower rates. More insight is needed to recognize opportunities for reducing the number of unplanned resections and to optimize clinical management following these procedures, thereby improving STS care.

In the current study, the aim was to assess the overall incidence of unplanned resections of STS using the Netherlands Cancer Registry (NCR), and to identify patient, tumor, and treatment-related characteristics that are associated with these resections. Furthermore, it provides an overview of the clinical management and outcomes of patients following unplanned resections. In the context of the current discussions regarding centralization in the Netherlands, results were also evaluated with respect to care delivery by dedicated sarcoma centers.

Methods

Database

The NCR contains information on all newly diagnosed Dutch cancer patients since 1989. Case notification of STS is received from all pathology laboratories in the Netherlands, after which trained data managers subsequently acquire information on patient, tumor, and treatment-related characteristics from hospitals' electronic medical records. Tumor site and morphology were coded according to the International Classification of Diseases for Oncology (ICD-O, third edition) [12] and classified according to the World Health Organization (WHO) classification 2013 [4]. Tumor size was partly coded using the TNM classification system of the International Union against Cancer (UICC, eighth edition) [13]. Due to changes in the 8th

edition of the TNM classification, information on tumor depth was only available until 2017. The surgical margins were classified as R0 (negative margins), R1–R2 (microscopically or macroscopically positive margins) or Rx (unknown margins/margins not assessed). Sarcoma centers were characterized by their participation in the European Organization for Research and Treatment of Cancer (EORTC) sarcoma group, or designated as such by The Netherlands Federation of University Medical Centers (Nederlandse Federatie van Universitair Medische Centra; NFU).

As of 2016, the NCR extended its registry with additional data items specifically for STS. This included the intention of surgery, which enabled us to distinguish unplanned from planned resections. A resection was considered unplanned when, based on a search in patients' medical records, a soft tissue tumor was (partially) surgically removed in the absence of any suspicion of malignancy. Hence, excisional and incisional biopsies did not qualify as unplanned resections.

Patient selection

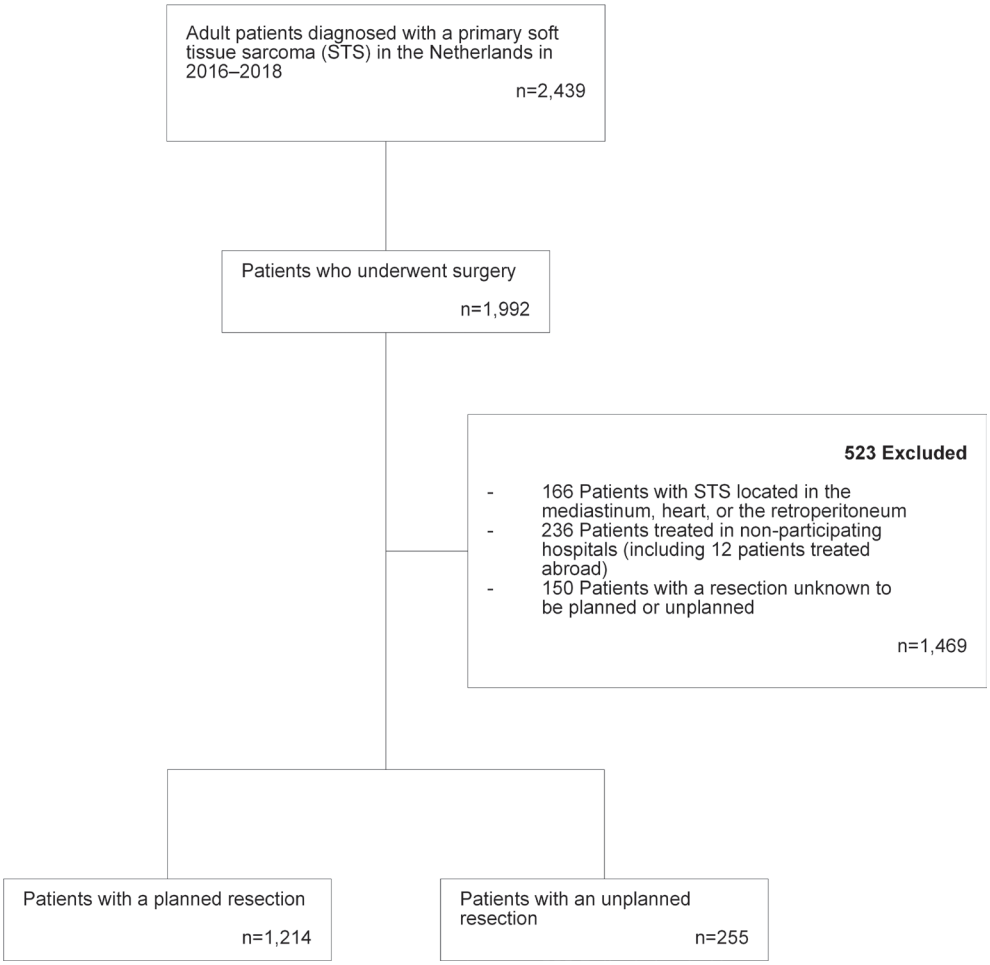
All adult patients (≥18 years) diagnosed with an STS (excluding Kaposi sarcoma) in 2016–2018 and who underwent surgery in a Dutch hospital as part of their primary treatment were retrieved from the NCR and included in the study. Patients with an STS located in the viscera, including those in the mediastinum, heart, retroperitoneum and/or peritoneum, were excluded. For one sarcoma center, no information regarding the intent of surgery was available, and with this reason all cases with a first surgery in this center were excluded. From another center, information was only available from 2017, and patients were included from that year on.

Statistical analyses

Differences in patient and tumor characteristics between patients with unplanned and patients with planned resections were evaluated using chi-square tests. The analyses focused on information that was available prior to surgery and included sex, age (at time of diagnosis), tumor site, clinical tumor size, tumor depth (for 2016), the use of preoperative imaging, and whether the first surgery took place in a sarcoma center.

Independent predictors for unplanned resections were identified using a multivariable logistic regression model, following their selection based on a p-value of <0.1 in the univariable analyses. Since sarcoma centers were likely to welcome a patient population already suspect of having a STS, the analyses were also performed for patients who had their first surgery outside one of these centers. The results of the logistic regression analyses were presented as odds ratios (OR) with their corresponding 95% confidence intervals (95% CI). Statistical tests with a p-value of <0.05 were considered statistically significant. All analyses were performed using Stata (version 16.1, StataCorp, College Station, Texas).

Additionally, the rate of unplanned resections determined in this study was compared to prior estimations made with the NCR database [14]. These prior estimations have been based on a proxy for 'potential unplanned resections' that was defined by equal dates of STS surgery and the tumors' first histopathological confirmation. Although the risk of overestimating the number of unplanned resections using this proxy was acknowledged, the extent of overestimation remained unknown.



▲**Figure 1.** CONSORT diagram of patient selection.

Results

Patient and tumor characteristics

In total, 1,469 adult patients diagnosed with STS in 2016–2018 who had undergone a resection were extracted from the NCR (Figure 1). The median age at diagnosis was 65 years (interquartile range 50–76), 60.4% of the patients was older than 60 years (Table 1). Most tumors were localized in the lower extremity (35.0%) and the trunk (29.0%).

Unplanned resections occurred in 255 (17.4%) of first surgeries. Compared to planned procedures, unplanned resections more often involved a tumor located in the upper extremity (20.8% versus 13.8%, with unplanned resections comprising 24.1% of procedures performed for STS), tumors that were small (≤5 cm; 47.8% versus 39.3%) and located superficially (81.8% versus 61.0%). Furthermore, preoperative imaging was omitted more often (47.1% versus 30.5%). Almost all first surgeries performed in a sarcoma center were planned procedures (97.3%), while unplanned procedures comprised almost a quarter of first surgeries in other hospitals (24.0%). Although a minority of procedures (4.4%) was performed by a general practitioner or in a private practice, almost two-thirds of these (64.6%) were identified as unplanned.

Unplanned resection

In the overall multivariable logistic regression, first surgery performed in a sarcoma center was the most predominant determinant (Table 2A), with unplanned resections being much less likely compared to first surgeries in other hospitals (OR 8.73; 95%CI: 5.06–15.06). Tumor depth was excluded from the model as it was not evaluated as a significant determinant in an analysis focusing on cases diagnosed in 2016 only (data not shown). The overall analysis excluding the sarcoma centers (Table 2B) identified tumor site in the upper extremity (OR 1.83; 95% CI: 1.13–2.95 compared to the trunk) and an unknown tumor size (OR 2.43; 95% CI: 1.68-3.52) as significant factor associated with a higher risk of an unplanned resection, whereas patients with tumors >5 cm were less likely to have undergone an unplanned resection (OR 0.25; 95% CI: 0.15–0.41 compared to those with ≤5 cm tumors). Omittance of preoperative imaging was significantly associated with an unplanned resection after adjustment for other factors (OR 1.74, 95% CI 1.21–2.50).

Table 1. Univariable analysis of determinants of planned and unplanned resections for adult patients with a primary soft tissue sarcoma (STS).

	Total (n=1,469)		Planned resection (n=1,214; 82.6%)		Unplanned resection (n=255; 17.4%)		
	n	%	n	%	n	%	p
Sex							0.87
Male	865	58.9%	716	82.8%	149	17.2%	
Female	604	41.1%	498	82.5%	106	17.5%	
Age at diagnosis, years							<0.01
18–49	355	24.2%	281	79.2%	74	20.8%	
50–64	354	24.1%	285	80.5%	69	19.5%	
65–74	321	21.9%	285	88.8%	36	11.2%	
≥75	439	29.9%	363	82.7%	76	17.3%	
median (interquartile range)	65 (50–76)		66 (51–76)		63 (47–76)		
Sarcoma site							<0.01
Head and neck	308	21.0%	247	80.2%	61	19.8%	
Trunk	427	29.0%	358	83.8%	69	16.2%	
Upper extremity	220	15.0%	167	75.9%	53	24.1%	
Lower extremity	514	35.0%	442	86.0%	72	14.0%	
Clinical tumor size							<0.01
≤5 cm	599	40.8%	477	79.6%	122	20.4%	
>5 cm	625	42.6%	594	95.0%	31	5.0%	
Unknown	245	16.7%	143	58.4%	102	41.6%	
Tumor depth (2016)							<0.01
Superficial	282	64.7%	219	77.7%	63	22.3%	
Deep	134	30.7%	125	93.3%	9	6.7%	
Unknown	20	4.6%	15	75.0%	5	25.0%	
Preoperative imaging							<0.01
No	490	33.4%	370	75.5%	120	24.5%	
Yes	979	66.6%	844	86.2%	135	13.8%	
Location of first surgery							<0.01
Sarcoma center	585	39.8%	569	97.3%	16	2.7%	
Other hospital	819	55.8%	622	76.0%	197	24.0%	
General practitioner/private practice	65	4.4%	23	35.4%	42	64.6%	

Table 2. Multivariable analysis of determinants of all unplanned resections (**A**) and of unplanned resections outside sarcoma centers (**B**).

(A)		**Odds ratio**	**95%CI**
Age at diagnosis (years)	18–49	1.00	(ref)
	50–64	1.06	0.69–1.63
	65–74	0.64	0.39–1.04
	≥75	0.77	0.50–1.19
Sarcoma site	Trunk	1.00	(ref)
	Head and neck	1.09	0.69–1.72
	Upper extremity	1.68*	1.05–2.68
	Lower extremity	1.35	0.89–2.05
Tumor size	≤5 cm	1.00	(ref)
	>5 cm	0.27*	0.17–0.43
	Unknown	2.51*	1.74–3.63
Preoperative imaging	No	1.00	(ref)
	Yes	1.88*	1.31–2.71
Location of first surgery	Sarcoma center	1.00	(ref)
	Other hospital	8.73*	5.06–15.06
	General practitioner/private practice	32.42*	15.11–69.55
(B)		**Odds ratio**	**95% CI**
Age at diagnosis (years)	18–49	1.00	(ref)
	50–64	1.25	0.80–1.94
	65–74	0.63*	0.38–1.06
	≥75	0.75	0.48–1.17
Sarcoma site	Trunk	1.00	(ref)
	Head and neck	1.02	0.64–1.63
	Upper extremity	1.83*	1.13–2.95
	Lower extremity	1.33	0.87–2.04
Tumor size	≤5 cm	1.00	(ref)
	>5 cm	0.25*	0.15–0.41
	unknown	2.43*	1.68–3.52
Preoperative imaging	No	1.00	(ref)
	Yes	1.74*	1.21–2.50

Postsurgical management

The majority of unplanned resections resulted in residual disease (62.8% compared to 30.2% for planned procedures, p<0.01; Table 3). As a result, more patients underwent a reresection following an unplanned primary resection (66.3%) than after a planned resection (19.4%; p<0.01) with the aim to obtain clear margins. Adjuvant radiotherapy was more often administered in patients after an unplanned resection (23.2% versus 10.6%, p<0.01). After initial unplanned resection outside a sarcoma center, the majority of patients (66.9%) were referred to or discussed with a sarcoma center. This was higher for patients left with residual disease following their first surgery (70.3%).

Table 3. Postsurgical management of planned and unplanned resections.

	Total		R0		R1/R2		Rx	
	n	%	n	%	n	%	n	%
Planned resection	**1,214**	**82.6%**	**756**	**62.3%**	**366**	**30.2%**	**92**	**7.6%**
Treatment following first surgery								
Reresection	212	17.4%	39	5.2%	153	41.8%	20	21.7%
Reresection+RTx	24	2.0%	2	0.3%	19	5.2%	3	3.3%
RTx	104	8.6%	53	7.0%	45	12.3%	6	6.5%
Other	874	72.0%	662	87.6%	149	40.7%	63	68.5%
Sarcoma center involvement								
First surgery in sarcoma center	569	46.9%	405	53.6%	134	36.6%	30	32.6%
Referral to/consultation with sarcoma center	265	21.8%	109	14.4%	128	35.0%	28	30.4%
Not referred to/consulted with sarcoma center	380	31.3%	242	32.0%	104	28.4%	34	37.0%
Unplanned resection	**255**	**17.4%**	**16**	**6.3%**	**160**	**62.8%**	**79**	**31.0%**
Treatment following first surgery								
Reresection	125	49.0%	14	25.9%	92	59.0%	19	42.2%
Reresection+RTx	44	17.3%	1	1.9%	34	21.8%	9	20.0%
RTx	15	5.9%	5	9.3%	9	5.8%	1	2.2%
Other	71	27.8%	34	63.0%	21	13.5%	16	35.6%
Sarcoma center involvement								
First surgery in sarcoma center	16	6.3%	8	14.8%	8	5.1%	0	0.0%
Referral to/consultation with sarcoma center	160	62.8%	22	40.7%	104	66.7%	34	75.6%
Not referred to/consulted with sarcoma center	79	31.0%	24	44.4%	44	28.2%	11	24.4%

R0: negative margins, R1/R2: microscopically or macroscopically positive margins, Rx: unknown margins/ margins not assessed, RTx: radiotherapy

After adjustment for potential confounders, patients with an unplanned resection were more likely to undergo a reresection (OR 3.78; 95% CI: 2.25–6.33) and had a significant higher risk of residual disease (OR 12.35; 95% CI: 7.55–20.22) (data not shown). Older patients (≥75 years, OR 0.50; 95% CI: 0.29–0.89), patients with a larger tumor (OR 0.34; 95% CI: 0.20–0.56) and patients treated in a sarcoma center (OR 0.31; 95% CI: 0.19–0.49) were less likely to undergo a reresection after an initial unplanned excision.

'Potential' versus real unplanned resections

Application of the proxy previously used by Vos et al. [14], based on coinciding dates of surgery and first histopathological confirmation to determine 'potential' unplanned resections, would have resulted in 611 unplanned resections. This would have accounted for 41.6% of the total number of performed resections, compared to 17.4% 'true' unplanned resections. This implies an overestimation of 24.2% when using the proxy.

Discussion

The overall incidence of unplanned resections is estimated at 17.4%, which is considerably lower than most rates reported in other studies, which range from 19% to 53% [6, 9-11]. To some extent, the difference may be attributed to the more recent study period. Aside from, for example, the advances in STS imaging, centralization of STS surgery has increased significantly over the last decade in the Netherlands [14, 15] as well as in other countries. The lower rate of unplanned resections achieved by sarcoma centers could partly be explained by the diagnostic expertise of dedicated multidisciplinary STS teams, but should also be interpreted in light of their specific patient population. Given the referral of cases suspected for STS, the a priori likelihood of malignancy is higher in sarcoma centers, and the ratio of benign and malignant soft tissue tumors is altered.

The low unplanned resection rate could also be the result of the way unplanned resections were determined. A major strength of this study, next to its population-based coverage, is found in the systematic and focused collection of information on the intent of STS surgery. This higher accuracy accounts for a more precise estimate compared to a previous study [14], which reported a rate of (potential) unplanned resections based on overlapping dates of surgery and first histopathological confirmation of STS diagnosis. If this proxy would have been applied to the current study cohort, it would have resulted in a two- to threefold increase of unplanned resections.

Some of the disadvantages of the study are inherent to studying rare diseases such as STS, and include the relative low number of cases. Among other issues, this unfortunately

makes most stratified analyses inapplicable, how worthwhile and valuable these may be. Examples include STS subtype-specific assessment of factors associated with unplanned resections, and an evaluation of to what extent unplanned resections result in different outcomes in different STS subtypes. Also, despite the dedicated efforts of the NCR to distinguish unplanned from planned resections, accurate and complete data registry largely depends on the information quality as found in hospital electronic systems, for example with respect to tumor size.

In general, lumps and bumps are very common in an average practice of general practitioners and general surgeons. The rule has always been that superficial, small lesions (<5 cm) without any suspicious signs of a malignancy can be removed without pre-operative imaging, while patients with larger and/or deep tumors or tumors with a form of suspicion should have pre-operative imaging and should be discussed with or referred to a sarcoma center. In this study, however, there is still a considerable number of patients with larger and/or deep tumors that were resected without pre-operative imaging or biopsy. Given this finding, the pivotal question remains whether and how unplanned resections of these tumors could be prevented. It is essential for sarcoma experts as well as patient organizations to focus on creating more awareness of STS, amongst physicians as well as the general population. Also, the relatively high number of patients not discussed with or referred to a sarcoma center (28% after R1/R2 resection) is still quite worrisome. Therefore, education may also be aimed at standardization of STS management, thereby stimulating better adherence to imaging and referral guidelines. Those efforts have already been undertaken, but this should be a continuous focus of the sarcoma community. In our study, the increased number of reresections represented an unfavorable outcome, potentially leading to spill of tumor cells in adjacent tissue, thereby increasing the risk of local recurrence [16]. On the other hand, as discussed above, unplanned resections of small, superficial STS are unlikely to be preventable at all times. Because of the high incidence of benign soft tissue tumors encountered in common clinical practice, these should perhaps be considered 'all-in-the-game'. Fortunately, these STS are more often low grade tumors, and unplanned excisional biopsies of such lesions do not necessarily seem to result in an unfavorable outcome for these patients.

Although this study could not assess potential unfavorable outcomes in terms of recurrence and survival, unplanned resections do affect patients' well-being. Patients with an unplanned resection more often underwent a subsequent reresection, which was also shown in other studies [2, 17].

A more detailed registration of STS, and longer follow-up of cases is required to monitor the impact of unplanned resections on the local recurrence rate, distant metastasis

rate (which might be higher due to tumor spill) and survival of these patients. This should enable in-depth analyses for specific subgroups of patients and STS subtypes, and will result in a more comprehensive assessment of the prognosis of STS patients after unplanned resection. Even though these may not prevent unplanned resections from occurring at all, it should contribute to more optimal management of STS following unplanned resections.

Conclusion

Unplanned resections were performed in approximately in 17% of all STS resections, mostly on small tumors. These patients more often had residual disease after surgery and subsequently underwent a reresection and adjuvant radiotherapy more often. Potential improvement may be achieved by better compliance to preoperative imaging and increasing the awareness of STS.

Declarations of interest

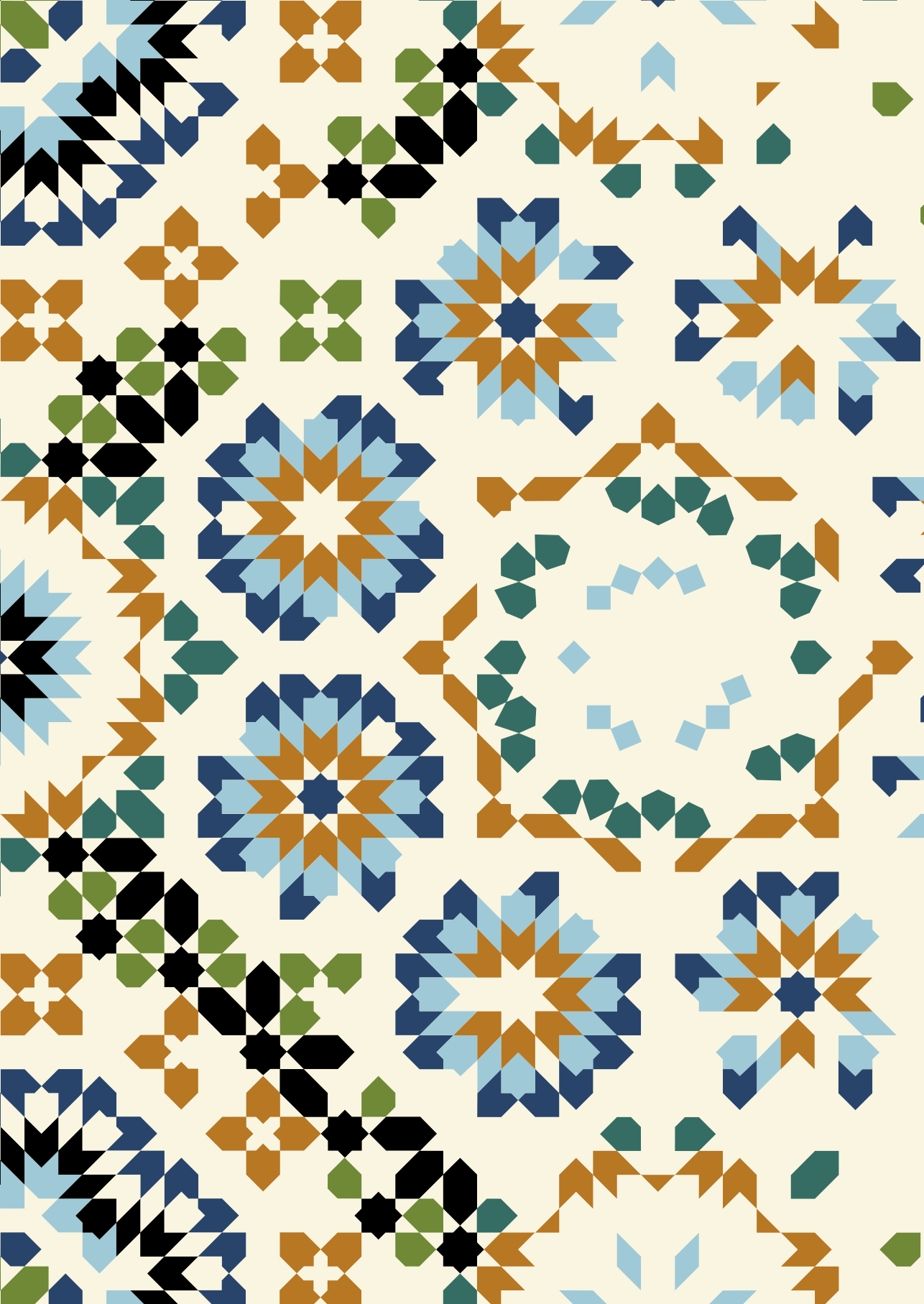
None.

Funding

None.

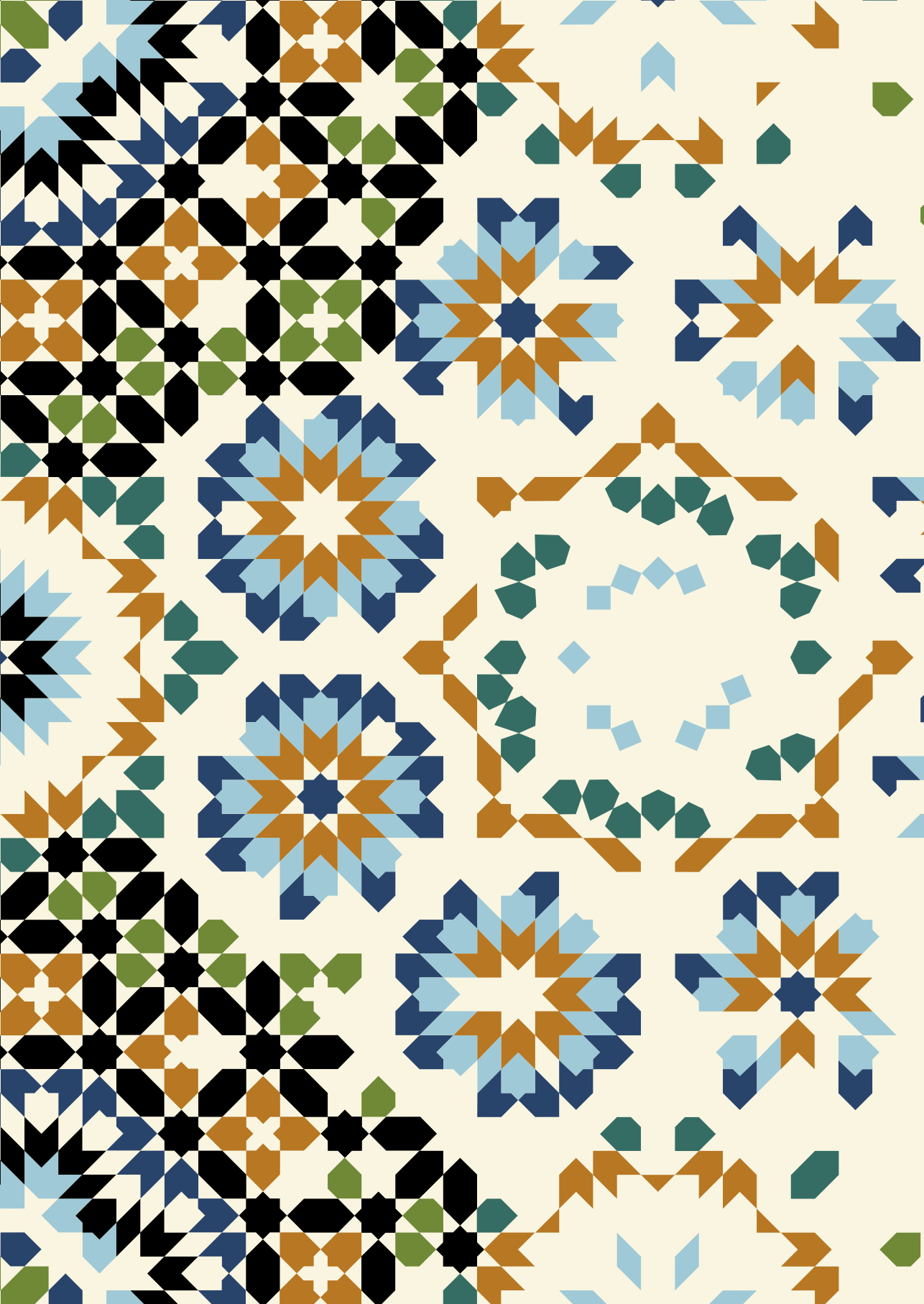
References

1. (IKNL) NCCO. 2019.
2. Umer HM, Umer M, Qadir I, Abbasi N, Masood N. Impact of unplanned excision on prognosis of patients with extremity soft tissue sarcoma. Sarcoma. 2013;2013:498604.
3. Fiore M, Casali PG, Miceli R, Mariani L, Bertulli R, Lozza L, et al. Prognostic effect of re-excision in adult soft tissue sarcoma of the extremity. Annals of surgical oncology. 2006;13:110-7.
4. Fletcher CD, Bridge JA, Hogendoorn PC, Mertens F. WHO Classification of tumours of soft tissue and bone. 2013. Lyon: IARC. 2003:305-10.
5. Arai E, Nishida Y, Tsukushi S, Wasa J, Ishiguro N. Clinical and treatment outcomes of planned and unplanned excisions of soft tissue sarcomas. Clinical Orthopaedics and Related Research®. 2010;468:3028-34.
6. Bianchi G, Sambri A, Cammelli S, Galuppi A, Cortesi A, Righi A, et al. Impact of residual disease after "unplanned excision" of primary localized adult soft tissue sarcoma of the extremities: evaluation of 452 cases at a single Institution. Musculoskeletal surgery. 2017;101:243-8.
7. Kepka L, Suit HD, Goldberg SI, Rosenberg AE, Gebhardt MC, Hornicek FJ, et al. Results of radiation therapy performed after unplanned surgery (without re-excision) for soft tissue sarcomas. Journal of surgical oncology. 2005;92:39-45.
8. Venkatesan M, Richards C, McCulloch T, Perks A, Raurell A, Ashford R, et al. Inadvertent surgical resection of soft tissue sarcomas. European Journal of Surgical Oncology (EJSO). 2012;38:346-51.
9. Traub F, Griffin AM, Wunder JS, Ferguson PC. Influence of unplanned excisions on the outcomes of patients with stage III extremity soft-tissue sarcoma. Cancer. 2018;124:3868-75.
10. Noria S, Davis A, Kandel R, Levesque J, O'Sullivan B, Wunder J, et al. Residual disease following unplanned excision of a soft-tissue sarcoma of an extremity. JBJS. 1996;78:650-5.
11. Trovik C, Bauer H, Alvegård T, Anderson H, Blomqvist C, Berlin Ö, et al. Surgical margins, local recurrence and metastasis in soft tissue sarcomas: 559 surgically-treated patients from the Scandinavian Sarcoma Group Register. European journal of cancer. 2000;36:710-6.
12. Fritz A, Percy C, Jack A, Shanmugaratnam K, Sobin LH, Parkin DM, et al. International classification of diseases for oncology. 2000.
13. Brierley J, Gospodarowicz M, Wittekind C. TNM Classification of Malignant Tumours Eighth Edition. Chichester, West Sussex, UK; Hoboken. NJ, John Wiley & Sons, Inc; 2017.
14. Vos M, Blaauwgeers HG, Ho VK, van Houdt WJ, van der Hage JA, Been LB, et al. 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. European Journal of Cancer. 2019;110:98-106.
15. Hoekstra HJ, Haas RL, Verhoef C, Suurmeijer AJ, van Rijswijk CS, Bongers BG, et al. Adherence to guidelines for adult (non-GIST) soft tissue sarcoma in the Netherlands: a plea for dedicated sarcoma centers. Annals of surgical oncology. 2017;24:3279-88.
16. Chandrasekar C, Wafa H, Grimer R, Carter S, Tillman R, Abudu A. The effect of an unplanned excision of a soft-tissue sarcoma on prognosis. The Journal of bone and joint surgery British volume. 2008;90:203-8.
17. Pretell-Mazzini J, Barton Jr MD, Conway SA, Temple HT. Unplanned excision of soft-tissue sarcomas: current concepts for management and prognosis. JBJS. 2015;97:597-603.



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-STSs. The latter group, adult-STSs, forms the topic of the current overview.

Most adult-STS 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 STSs 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.

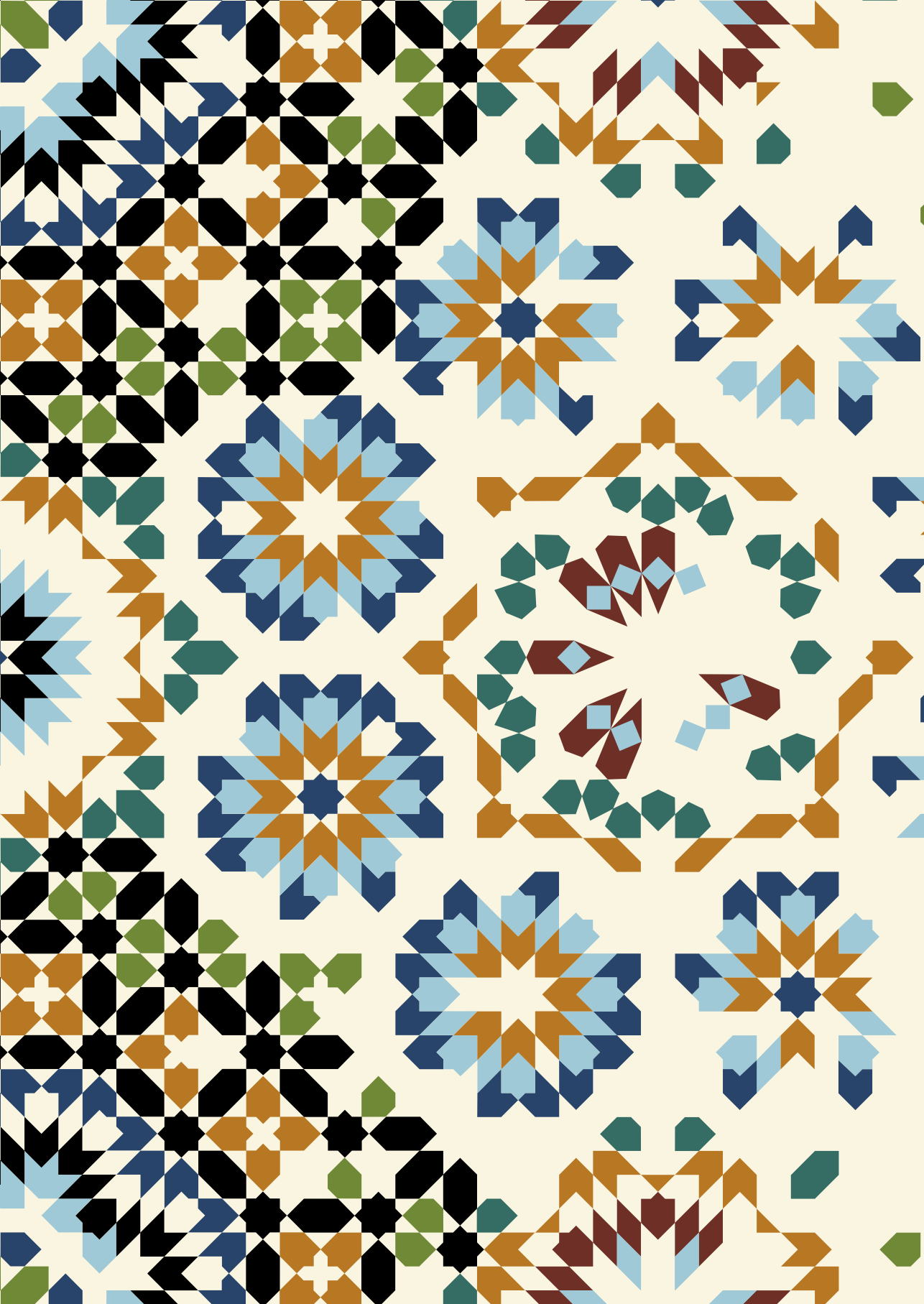
S. Sleijfer: advisory board EliLilly (all funding for institute).

References

1. Judson I, Verweij J, Gelderblom H, Hartmann JT, Schoffski P,Blay JY, et al. Doxorubicin alone versus intensified doxorubicin plus ifosfamide for first-line treatment of advanced or metastatic soft-tissue sarcoma: a randomised controlled phase 3 trial. Lancet Oncol 2014;15:415e23.
2. Tap WD, Jones RL, Van Tine BA, Chmielowski B, Elias AD, Adkins D, et al. Olaratumab and doxorubicin versus doxorubicin alone for treatment of soft-tissue sarcoma: an open-label phase 1b and randomised phase 2 trial. Lancet 2016;388:488e97.
3. Seddon B, Strauss SJ, Whelan J, Leahy M, Woll PJ, Cowie F,et al. Gemcitabine and docetaxel versus doxorubicin as first-linetreatment in previously untreated advanced unresectable or metastaticsoft-tissue sarcomas (GeDDiS): a randomised controlledphase 3 trial. Lancet Oncol 2017;18:1397e410.
4. Tap WD, Papai Z, Van Tine BA, Attia S, Ganjoo KN, Jones RL,et al. Doxorubicin plus evofosfamide versus doxorubicin alone inlocally advanced, unresectable or metastatic soft-tissue sarcoma(TH CR-406/SARC021): an international, multicentre, openlabel,randomised phase 3 trial. Lancet Oncol 2017;18:1089e103.
5. Ryan CW, Merimsky O, Agulnik M, Blay JY, Schuetze SM, VanTine BA, et al. PICASSO III: aphase III, placebo-controlled studyof doxorubicin with or without palifosfamide in patients withmetastatic soft tissue sarcoma. J Clin Oncol 2016.
6. Demetri GD, Chawla SP, von Mehren M, Ritch P, Baker LH,Blay JY, et al. Efficacy and safety of trabectedin in patients withadvanced or metastatic liposarcoma or leiomyosarcoma afterfailure of prior anthracyclines and ifosfamide: results of a randomized phase II study of two different schedules. J Clin Oncol 2009;27:4188e96.
7. Demetri GD, von Mehren M, Jones RL, Hensley ML, Schuetze SM, Staddon A, et al. Efficacy and safety of trabectedin or dacarbazine for metastatic liposarcoma or leiomyosarcoma after failure of conventional chemotherapy: results of a phase III randomized multicenter clinical trial. J Clin Oncol 2016;34: 786e93.
8. Van der Graaf WT, Blay JY, Chawla SP, Kim DW, Bui-Nguyen B, Casali PG, et al. Pazopanib for metastatic soft-tissue sarcoma (PALETTE): a randomised, double-blind, placebocontrolled phase 3 trial. Lancet 2012;379:1879e86.
9. Sleijfer S, Ray-Coquard I, Papai Z, Le Cesne A, Scurr M, Schoffski P, et al. Pazopanib, a multikinase angiogenesis inhibitor, in patients with relapsed or refractory advanced soft tissue sarcoma: a phase II study from the European organisation for research and treatment of cancer-soft tissue and bone sarcoma group (EORTC study 62043). J Clin Oncol 2009;27:3126e32.
10. Nakamura T, Matsumine A, Kawai A, Araki N, Goto T, Yonemoto T, et al. The clinical outcome of pazopanib treatment in Japanese patients with relapsed soft tissue sarcoma: a Japanese Musculoskeletal Oncology Group (JMOG) study. Cancer 2016; 122:1408e16.
11. Samuels BL, Chawla SP, Somaiah N, Staddon AP, Skubitz KM, Milhem MM, et al. Results of a prospective phase 2 study of pazopanib in patients with advanced intermediate-grade or highgrade liposarcoma. Cancer 2017.
12. Schoffski P, Chawla S, Maki RG, Italiano A, Gelderblom H, Choy E, et al. Eribulin versus dacarbazine in previously treated patients with advanced liposarcoma or leiomyosarcoma: a randomised, open-label, multicentre, phase 3 trial. Lancet 2016;387: 1629e37.
13. Maki RG, Wathen JK, Patel SR, Priebat DA, Okuno SH, Samuels B, et al. Randomized phase II study of gemcitabine and docetaxel compared with gemcitabine alone in patients with metastatic soft tissue sarcomas: results of sarcoma alliance for research through collaboration study 002 [corrected]. J Clin Oncol 2007;25:2755e63.

14. Garcia-Del-Muro X, Lopez-Pousa A, Maurel J, Martin J, Martinez-Trufero J, Casado A, et al. Randomized phase II study comparing gemcitabine plus dacarbazine versus dacarbazine alone in patients with previously treated soft tissue sarcoma: a Spanish Group for Research on Sarcomas study. J Clin Oncol 2011;29:2528e33.
15. Penel N, Bui BN, Bay JO, Cupissol D, Ray-Coquard I, Piperno- Neumann S, et al. Phase II trial of weekly paclitaxel for unresectable angiosarcoma: the ANGIOTAX Study. J Clin Oncol2008;26:5269e74.
16. Judson IR, Morden JP, Leahy MG, Bhadri V, Campbell- Hewson Q, Cubedo R, et al. Activity of cediranib in alveolar soft part sarcoma (ASPS) confirmed by CASPS (cediranib in ASPS), an international, randomised phase II trial (C2130/A12118). JClin Oncol 2017;35:11004.
17. Stacchiotti S, Negri T, Zaffaroni N, Palassini E, Morosi C, Brich S, et al. Sunitinib in advanced alveolar soft part sarcoma: evidence of a direct antitumor effect. Ann Oncol 2011;22: 1682e90.
18. Chawla SP, Papai Z, Mukhametshina G, Sankhala K, Vasylyev L, Fedenko A, et al. First-line aldoxorubicin vs doxorubicin in metastatic or locally advanced unresectable soft-tissue sarcoma: aphase 2b randomized clinical trial. JAMA Oncol 2015;1:1272e80.
19. Chawla SP, Ganjoo KN, Schuetze S, Papai Z, van Tine BA, Choy E, et al. Phase III study of aldoxorubicin vs investigators' choice as treatment for relapsed/refractory soft tissue sarcomas. J Clin Oncol 2017;35:11000.
20. Toulmonde M, Penel N, Adam J, Chevreau C, Blay J-Y, Le Cesne A, et al. Combination of pembrolizumab and metronomic cyclophosphamide in patients with advanced sarcomas and GIST: a French Sarcoma Group phase II trial. J Clin Oncol 2017;35: 11053.
21. Mir O, Domont J, Cioffi A, Bonvalot S, Boulet B, Le Pechoux C, et al. Feasibility of metronomic oral cyclophosphamide plus prednisolone in elderly patients with inoperable or metastatic soft tissue sarcoma. Eur J Cancer 2011;47:515e9.
22. Jones RL, Maki R, Patel S, Wang G, Shin CR, Knoblauch R, et al. Efficacy and safety of trabectedin in an elderly patient subgroup (_65 years) with advanced leiomyosarcoma (LMS) or liposarcoma (LPS) from the Expanded Access Program (EAP) (1489P). Ann Oncol 2017;28. mdx387.025-mdx387.025.
23. Mir O, Brodowicz T, Italiano A, Wallet J, Blay J-Y, Bertucci F, et al. Safety and efficacy of regorafenib in patients with advanced soft tissue sarcoma (REGOSARC): a randomised, double-blind, placebo-controlled, phase 2 trial. Lancet Oncol 2016;17:1732e42.
24. D'Angelo SP, Mahoney MR, van Tine BA, Atkins JN, Milhem MM, Tap WD, et al. A multi-center phase II study of nivolumab þ/_ ipilimumab for patients with metastatic sarcoma (Alliance A091401). J Clin Oncol 2017;35:11007.
25. Tawbi HA, Burgess M, Bolejack V, Van Tine BA, Schuetze SM, Hu J, et al. Pembrolizumab in advanced soft-tissue sarcoma and bone sarcoma (SARC028): a multicentre, two-cohort, single-arm, open-label, phase 2 trial. Lancet Oncol 2017.
26. Endo M, de Graaff MA, Ingram DR, Lim S, Lev DC, Briaire-de Bruijn IH, et al. NY-ESO-1 (CTAG1B) expression in mesenchymal tumors. Mod Pathol 2015;28:587e95.
27. Mackall C, Tap WD, Glod J, Druta M, Chow WA, Araujo DM, et al. Open label, non-randomized, multi-cohort pilot study of genetically engineered NY-ESO-1 specific NY-ESO-1c259t in HLA-A2þ patients with synovial sarcoma (NCT01343043). J Clin Oncol 2017;35:3000.
28. Somaiah N, Chawla SP, Block MS, Morris JC, Do KT, Kim JW, et al. Immune response, safety, and survival impact from CMB305 in NY-ESO-1þ recurrent soft tissue sarcomas (STS). J Clin Oncol 2017;35:11006.

29. Chawla S, Van Tine BA, Pollack S, Ganjoo K, Elias A, Riedel RF, et al. A phase 2 study of CMB305 and atezolizumab in NY-ESO-1þ soft tissue sarcoma: interim analysis of immunogenicity, tumor control and survival (1480PD). Ann Oncol 2017;28. mdx387.007-mdx387.007.
30. Wiemer EA, Wozniak A, Burger H, Smid M, Floris G, Nzokirantevye A, et al. Identification of microRNA biomarkers for response of advanced soft tissue sarcomas to eribulin: translational results of the EORTC 62052 trial. Eur J Cancer 2017;75: 33e40.
31. Burgess MA, Bolejack V, van Tine BA, Schuetze S, Hu J, D'Angelo SP, et al. Multicenter phase II study of pembrolizumab (P) in advanced soft tissue (STS) and bone sarcomas (BS): final results of SARC028 and biomarker analyses. J Clin Oncol 2017; 35:11008.



CHAPTER 12

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.

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

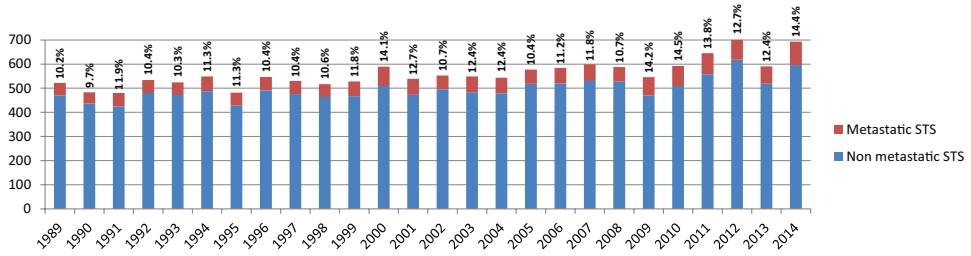
Median follow-up time with corresponding interquartile range (IQR) was calculated by the reversed Kaplan–Meier method [22]. Overall survival (OS) was defined as time in months between diagnosis (first pathological confirmation) and death or last follow-up. Patients alive at date of last linkage to the 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 ≤.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)	

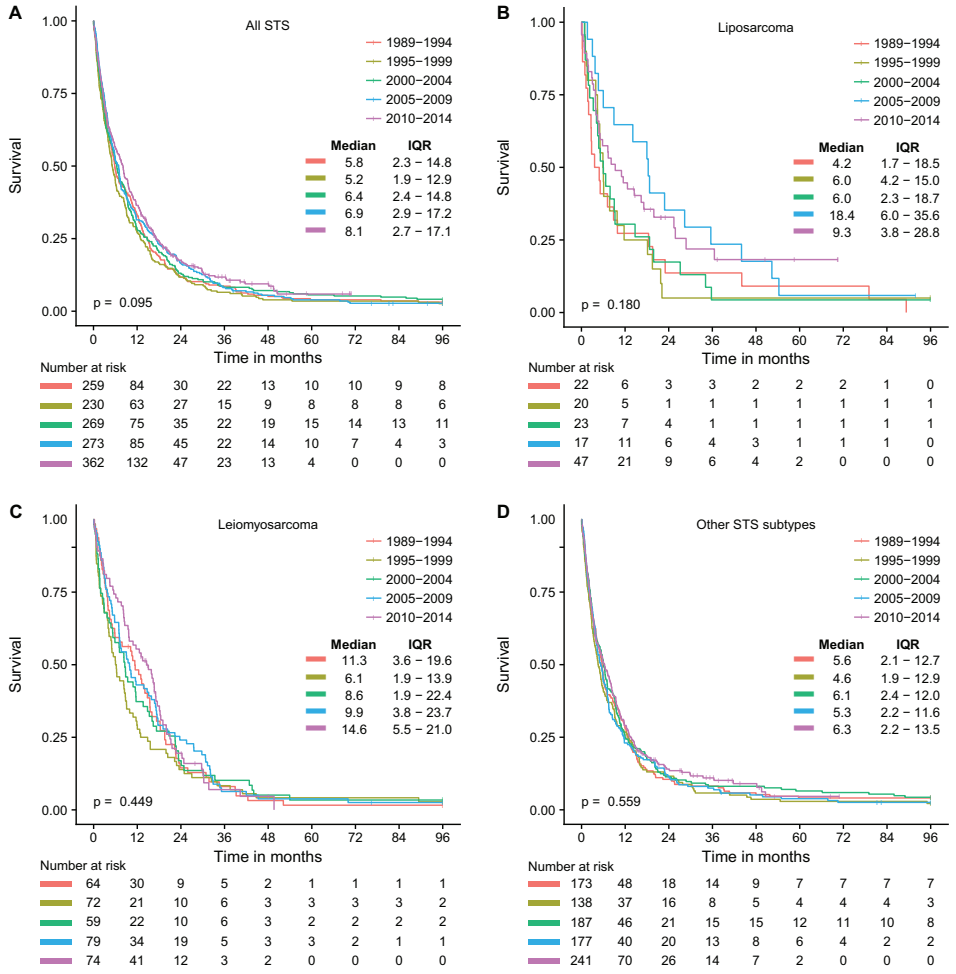
Abbreviations: CTx, chemotherapy; FU, follow-up; IQR, interquartile range; NA, not applicable; NOS, not otherwise specified; RTx, radiotherapy.

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

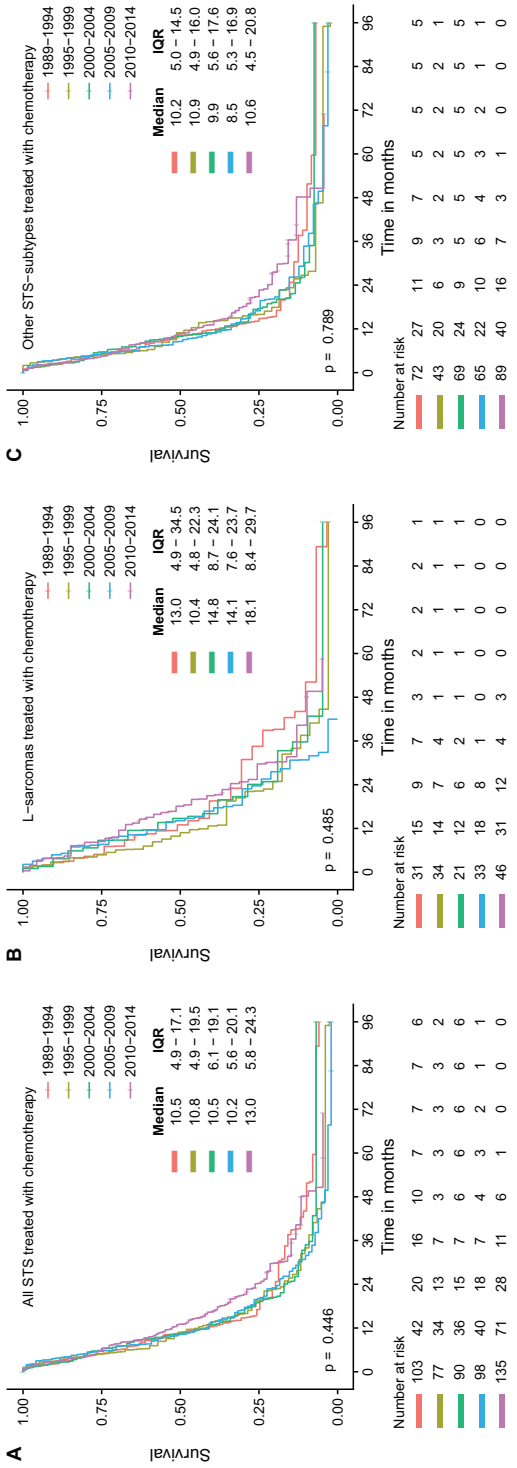
in the upper or lower extremity, any type of treatment (chemotherapy, radiotherapy, and/or surgery), and undergoing surgery in an academic center (compared with a nonacademic center) had a favorable effect on survival.

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
	Unknown (X)	532	1.556	1.303-1.857	<.001
Depth	Deep	571	1		
	Superficial	136	0.698	0.571-0.853	<.001
	Unknown	568	1.124	0.998-1.267	.054
Size (T-stadium)	≤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

[a]Variables with p value <.05 are included in multivariable analysis. [b]Treatment 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
	Lower extremity	393	0.782	0.685-0.894	<.001
Subtype	Leiomyosarcoma	348	1		
	Liposarcoma	129	1.159	0.926-1.450	.197
	Other	916	1.418	1.233-1.631	<.001
Grade	Low (I/II)	181	1		
	High (III/NA)	680	1.561	1.291-1.887	<.001
	Unknown (X)	532	1.347	1.115-1.628	.002
Nodal involvement	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

[a]Variables significant at an α-level of .05. [b]Treatment 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 PDGFRα-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.

Acknowledgments

The authors thank the registration team of the Netherlands Comprehensive Cancer Organisation (IKNL) for collecting data in the Netherlands Cancer Registry (NCR). This work was presented as a poster at the ASCO Annual Meeting, June 2–6, 2017, Chicago, IL.

Disclosures

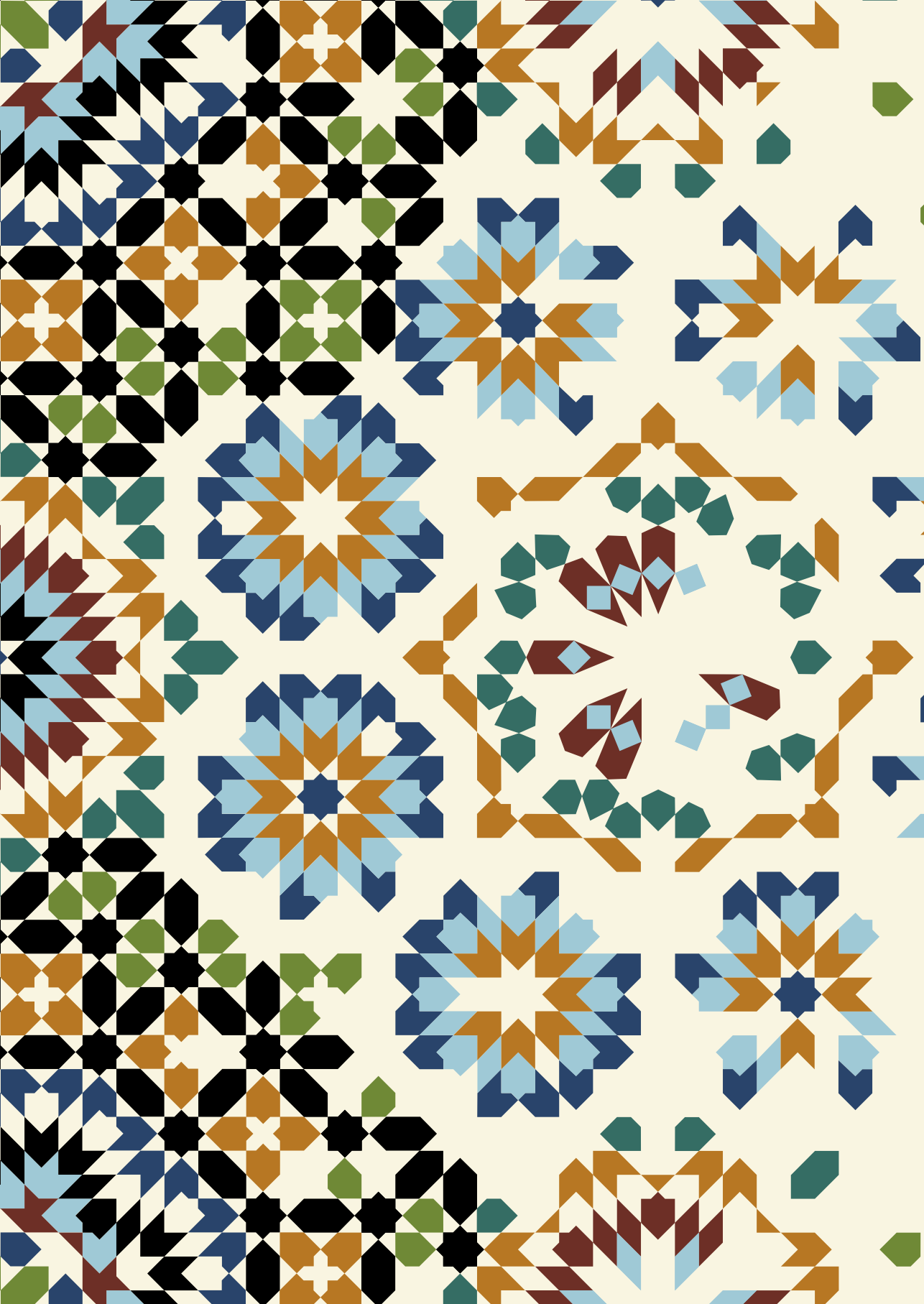
The authors indicated no financial relationships.

References

1. The Netherlands Comprehensive Cancer Registry. Bijlage D Deelrapportage voor wekedelensarcomen. The Netherlands Comprehensive Cancer Organisation (IKNL); 2014.
2. Fletcher CDM, Bridge JA, Hogendoorn PCW, Mertens F, World Health Organization, International Agency for Research on Cancer. WHO classification of tumours of soft tissue and bone. Lyon: IARC Press; 2013.
3. The Netherlands Cancer Registry. Dutch Cancer Figures (Cijfers over kanker). Netherlands Comprehensive Cancer Organisation (IKNL); 2011-2016.
4. Tsukushi S, Nishida Y, Wasa J, Urakawa H, Ishiguro N. Clinicopathological assessment of T1 soft tissue sarcomas. Arch Orthop Trauma Surg. 2011;131:695-9.
5. Pisters PW, Pollock RE, Lewis VO, Yasko AW, Cormier JN, Respondek PM, et al. Long-term results of prospective trial of surgery alone with selective use of radiation for patients with T1 extremity and trunk soft tissue sarcomas. Ann Surg. 2007;246:675-81; discussion 81-2.
6. Al-Refaie WB, Habermann EB, Jensen EH, Tuttle TM, Pisters PW, Virnig BA. Surgery alone is adequate treatment for early stage soft tissue sarcoma of the extremity. Br J Surg. 2010;97:707-13.
7. Nijhuis PH, Schaapveld M, Otter R, Molenaar WM, van der Graaf WT, Hoekstra HJ. Epidemiological aspects of soft tissue sarcomas (STS)- Consequences for the design of clinical STS trials. Eur J Cancer. 1999;35:1705-10.
8. Chudgar NP, Brennan MF, Munhoz RR, Bucciarelli PR, Tan KS, D'Angelo SP, et al. Pulmonary metastasectomy with therapeutic intent for soft-tissue sarcoma. J Thorac Cardiovasc Surg. 2017;154:319-30 e1.
9. Billingsley KG, Burt ME, Jara E, Ginsberg RJ, Woodruff JM, Leung DH, et al. Pulmonary metastases from soft tissue sarcoma: analysis of patterns of diseases and postmetastasis survival. Ann Surg. 1999;229:602-10; discussion 10-2.
10. van Geel AN, Pastorino U, Jauch KW, Judson IR, van Coevorden F, Buesa JM, et al. Surgical treatment of lung metastases: The European Organization for Research and Treatment of Cancer-Soft Tissue and Bone Sarcoma Group study of 255 patients. Cancer. 1996;77:675-82.
11. Choong PF, Pritchard DJ, Rock MG, Sim FH, Frassica FJ. Survival after pulmonary metastasectomy in soft tissue sarcoma. Prognostic factors in 214 patients. Acta Orthop Scand. 1995;66:561-8.
12. Pawlik TM, Vauthey JN, Abdalla EK, Pollock RE, Ellis LM, Curley SA. Results of a single-center experience with resection and ablation for sarcoma metastatic to the liver. Arch Surg. 2006;141:537-43; discussion 43-4.
13. Savina M, Le Cesne A, Blay JY, Ray-Coquard I, Mir O, Toulmonde M, et al. Patterns of care and outcomes of patients with METAstatic soft tissue SARComa in a real-life setting: the METASARC observational study. BMC Med. 2017;15:78.
14. Blay JY, Sleijfer S, Schoffski P, Kawai A, Brodowicz T, Demetri GD, et al. International expert opinion on patient-tailored management of soft tissue sarcomas. Eur J Cancer. 2014;50:679-89.
15. Judson I, Verweij J, Gelderblom H, Hartmann JT, Schoffski P, Blay JY, et al. Doxorubicin alone versus intensified doxorubicin plus ifosfamide for first-line treatment of advanced or metastatic soft-tissue sarcoma: a randomised controlled phase 3 trial. Lancet Oncol. 2014;15:415-23.
16. Demetri GD, Chawla SP, von Mehren M, Ritch P, Baker LH, Blay JY, et al. Efficacy and safety of trabectedin in patients with advanced or metastatic liposarcoma or leiomyosarcoma after failure of prior anthracyclines and ifosfamide: results of a randomized phase II study of two different schedules. J Clin Oncol. 2009;27:4188-96.

17. Demetri GD, von Mehren M, Jones RL, Hensley ML, Schuetze SM, Staddon A, et al. Efficacy and Safety of Trabectedin or Dacarbazine for Metastatic Liposarcoma or Leiomyosarcoma After Failure of Conventional Chemotherapy: Results of a Phase III Randomized Multicenter Clinical Trial. J Clin Oncol. 2016;34:786-93.
18. Sleijfer S, Ray-Coquard I, Papai Z, Le Cesne A, Scurr M, Schoffski P, et al. Pazopanib, a multikinase angiogenesis inhibitor, in patients with relapsed or refractory advanced soft tissue sarcoma: a phase II study from the European organisation for research and treatment of cancer-soft tissue and bone sarcoma group (EORTC study 62043). J Clin Oncol. 2009;27:3126-32.
19. van der Graaf WT, Blay JY, Chawla SP, Kim DW, Bui-Nguyen B, Casali PG, et al. Pazopanib for metastatic soft-tissue sarcoma (PALETTE): a randomised, double-blind, placebo-controlled phase 3 trial. Lancet. 2012;379:1879-86.
20. ESMO/European Sarcoma Network Working Group. Soft tissue and visceral sarcomas: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2014;25 Suppl 3:iii102-12.
21. Fletcher CDM, Unni KK, Mertens F, World Health O, International Agency for Research on C. Pathology and genetics of tumours of soft tissue and bone. Lyon: IARC Press; 2002.
22. Schemper M, Smith TL. A note on quantifying follow-up in studies of failure time. Controlled Clinical Trials. 1996;17:343-6.
23. Garcia-Carbonero R, Supko JG, Manola J, Seiden MV, Harmon D, Ryan DP, et al. Phase II and pharmacokinetic study of ecteinascidin 743 in patients with progressive sarcomas of soft tissues refractory to chemotherapy. J Clin Oncol. 2004;22:1480-90.
24. Le Cesne A, Blay JY, Judson I, Van Oosterom A, Verweij J, Radford J, et al. Phase II study of ET-743 in advanced soft tissue sarcomas: a European Organisation for the Research and Treatment of Cancer (EORTC) soft tissue and bone sarcoma group trial. J Clin Oncol. 2005;23:576-84.
25. Yovine A, Riofrio M, Blay JY, Brain E, Alexandre J, Kahatt C, et al. Phase II study of ecteinascidin-743 in advanced pretreated soft tissue sarcoma patients. J Clin Oncol. 2004;22:890-9.
26. Kasper B, Sleijfer S, Litiere S, Marreaud S, Verweij J, Hodge RA, et al. Long-term responders and survivors on pazopanib for advanced soft tissue sarcomas: subanalysis of two European Organisation for Research and Treatment of Cancer (EORTC) clinical trials 62043 and 62072. Ann Oncol. 2014;25:719-24.
27. van Glabbeke M, van Oosterom AT, Oosterhuis JW, Mouridsen H, Crowther D, Somers R, et al. Prognostic factors for the outcome of chemotherapy in advanced soft tissue sarcoma: an analysis of 2,185 patients treated with anthracycline-containing first-line regimens--a European Organization for Research and Treatment of Cancer Soft Tissue and Bone Sarcoma Group Study. J Clin Oncol. 1999;17:150-7.
28. Karavasilis V, Seddon BM, Ashley S, Al-Muderis O, Fisher C, Judson I. Significant clinical benefit of first-line palliative chemotherapy in advanced soft-tissue sarcoma: retrospective analysis and identification of prognostic factors in 488 patients. Cancer. 2008;112:1585-91.
29. Krishnan CK, Kim HS, Park JW, Han I. Outcome After Surgery for Extremity Soft Tissue Sarcoma in Patients Presenting With Metastasis at Diagnosis. Am J Clin Oncol. 2016.
30. Kane JM, Finley JW, Driscoll D, Kraybill WG, Gibbs JF. The treatment and outcome of patients with soft tissue sarcomas and synchronous metastases. Sarcoma. 2002;6:69-73.
31. King JJ, Fayssoux RS, Lackman RD, Ogilvie CM. Early outcomes of soft tissue sarcomas presenting with metastases and treated with chemotherapy. Am J Clin Oncol. 2009;32:308-13.
32. Ryan CW, Merimsky O, Agulnik M, Blay JY, Schuetze SM, Van Tine BA, et al. PICASSO III: A Phase III, Placebo-Controlled Study of Doxorubicin With or Without Palifosfamide in Patients With Metastatic Soft Tissue Sarcoma. J Clin Oncol. 2016;34:3898-905.

33. Tap WD, Papai Z, Van Tine BA, Attia S, Ganjoo KN, Jones RL, et al. Doxorubicin plus evofosfamide versus doxorubicin alone in locally advanced, unresectable or metastatic soft-tissue sarcoma (TH CR-406/SARC021): an international, multicentre, open-label, randomised phase 3 trial. Lancet Oncol. 2017;18:1089-103.
34. Seddon B, Strauss SJ, Whelan J, Leahy M, Woll PJ, Cowie F, et al. Gemcitabine and docetaxel versus doxorubicin as first-line treatment in previously untreated advanced unresectable or metastatic soft-tissue sarcomas (GeDDiS): a randomised controlled phase 3 trial. Lancet Oncol. 2017;18:1397-410.
35. Hensley ML, Miller A, O'Malley DM, Mannel RS, Behbakht K, Bakkum-Gamez JN, et al. Randomized phase III trial of gemcitabine plus docetaxel plus bevacizumab or placebo as first-line treatment for metastatic uterine leiomyosarcoma: an NRG Oncology/Gynecologic Oncology Group study. J Clin Oncol. 2015;33:1180-5.
36. Dossett LA, Toloza EM, Fontaine J, Robinson LA, Reed D, Druta M, et al. Outcomes and clinical predictors of improved survival in a patients undergoing pulmonary metastasectomy for sarcoma. J Surg Oncol. 2015;112:103-6.
37. Feinstein AR, Sosin DM, Wells CK. The Will Rogers phenomenon. Stage migration and new diagnostic techniques as a source of misleading statistics for survival in cancer. N Engl J Med. 1985;312:1604-8.
38. Sormani MP. The Will Rogers phenomenon: the effect of different diagnostic criteria. J Neurol Sci. 2009;287 Suppl 1:S46-9.
39. Kindblom LG, Remotti HE, Aldenborg F, Meis-Kindblom JM. Gastrointestinal pacemaker cell tumor (GIPACT): gastrointestinal stromal tumors show phenotypic characteristics of the interstitial cells of Cajal. Am J Pathol. 1998;152:1259-69.
40. Hirota S, Isozaki K, Moriyama Y, Hashimoto K, Nishida T, Ishiguro S, et al. Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. Science. 1998;279:577-80.
41. Demetri GD, von Mehren M, Blanke CD, Van den Abbeele AD, Eisenberg B, Roberts PJ, et al. Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. N Engl J Med. 2002;347:472-80.
42. Verweij J, Casali PG, Zalcberg J, LeCesne A, Reichardt P, Blay JY, et al. Progression-free survival in gastrointestinal stromal tumours with high-dose imatinib: randomised trial. Lancet. 2004;364:1127-34.
43. Schoffski P, Chawla S, Maki RG, Italiano A, Gelderblom H, Choy E, et al. Eribulin versus dacarbazine in previously treated patients with advanced liposarcoma or leiomyosarcoma: a randomised, open-label, multicentre, phase 3 trial. Lancet. 2016;387:1629-37.
44. Tap WD, Jones RL, Van Tine BA, Chmielowski B, Elias AD, Adkins D, et al. Olaratumab and doxorubicin versus doxorubicin alone for treatment of soft-tissue sarcoma: an open-label phase 1b and randomised phase 2 trial. Lancet. 2016;388:488-97.



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

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.

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≥6 months, 36%) and long-term survival (OS≥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 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.

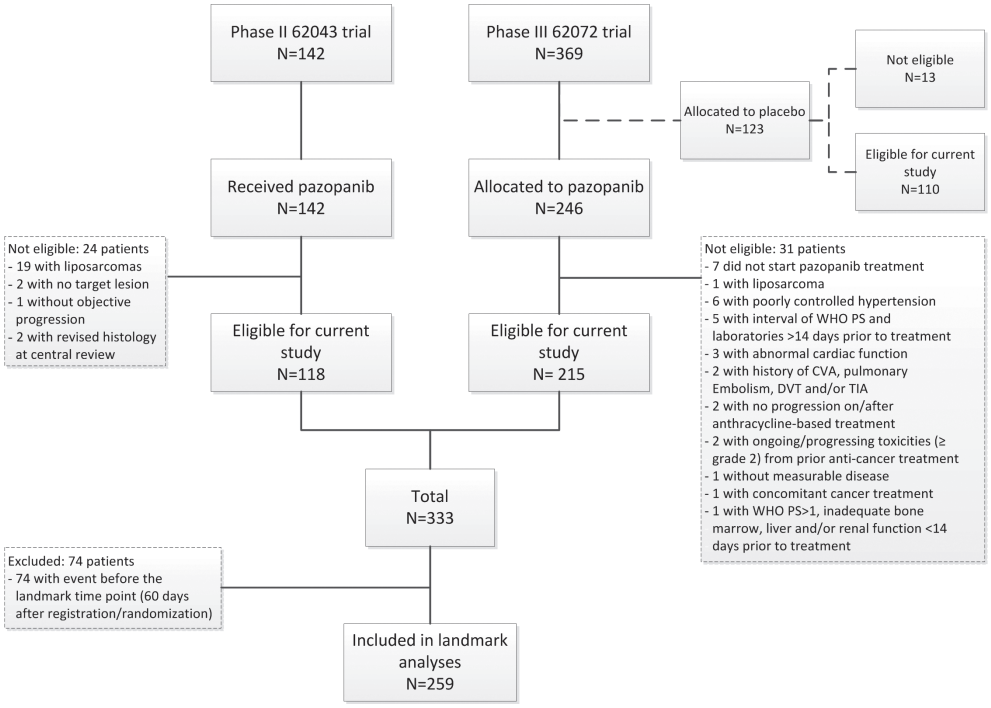
Results

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=194) N (%)	Yes (N=65) N (%)	No (N=237) N (%)	Yes (N=22) N (%)	No (N=202) N (%)	Yes (N=57) N (%)	No (N=244) N (%)	Yes (N=15) N (%)	No (N=151) N (%)	Yes (N=108) N (%)	(N=259) 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)

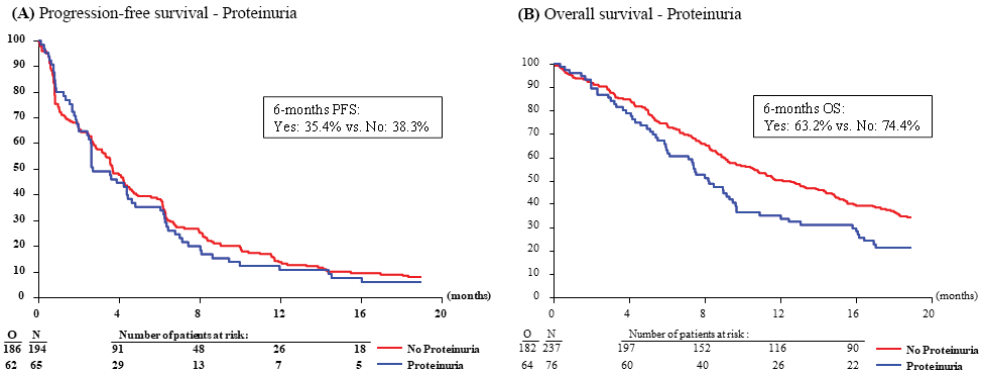
	Proteinuria		Persistent proteinuria		Hypothyroidism		Cardiotoxicity Grade 3-4		Cardiotoxicity Any grade		Total
Age at randomization											
≤50	87 (44.8)	15 (23.1)	98 (41.4)	4 (18.2)	76 (37.6)	26 (45.6)	96 (39.3)	6 (40.0)	69 (45.7)	33 (30.6)	102 (39.4)
>50	107 (55.2)	50 (76.9)	139 (58.6)	18 (81.8)	126 (62.4)	31 (54.4)	148 (60.7)	9 (60.0)	82 (54.3)	75 (69.4)	157 (60.6)
Cardiac history											
No							188 (77.0)	8 (53.3)	116 (76.8)	80 (74.1)	196 (75.7)
Yes							56 (23.0)	7 (46.7)	35 (23.2)	28 (25.9)	63 (24.3)
Type of cardiotoxicity*											
Arrhythmia							259 (100.0)	0 (0.0)	256 (98.8)	3 (1.2)	3 (1.2)
Hypertension							244 (94.2)	15 (5.8)	152 (58.7)	107(41.3)	107 (41.3)
Ischemia							259 (100.0)	0 (0.0)	258 (99.6)	1 (0.4)	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).

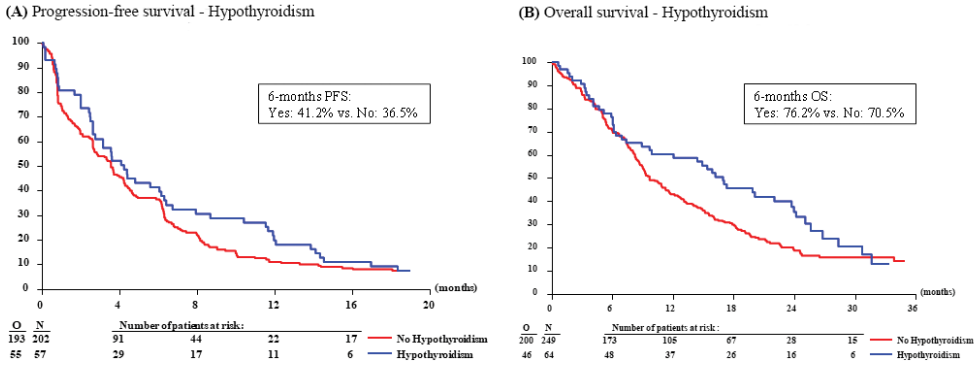


▲**Figure 2.** Kaplan-Meier curves showing the progression-free survival (**A**) and overall survival (**B**) of patients with and without pazopanib-induced proteinuria.

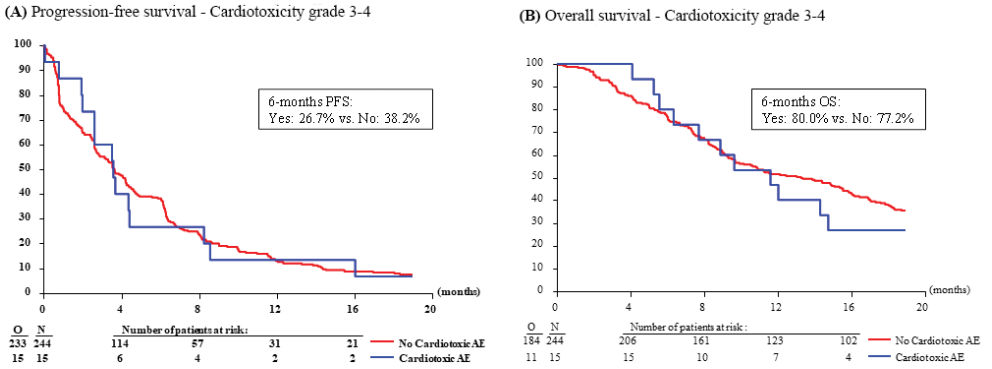
Table 2. Overview of the effect of the three pazopanib-induced toxicities on PFS and OS in patients receiving pazopanib at landmark time point of 60 days, univariate (6-months PFS/OS rates) and multivariable after adjustment for other prognostic factors in multivariable Cox regression analysis. The reported 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						
Noz	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.



▲**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.

Acknowledgments

The authors would like to thank all participants and the investigators of the EORTC 62043 and 62072 trials for their contribution to these studies.

Disclosure of interest

The authors report no conflicts of interest.

Funding

This publication was supported by the EORTC Cancer Research Fund. Nathan Touati's work as Fellow at the EORTC Headquarters was supported by a grant from the EORTC Soft Tissue and Bone Sarcoma Group.

References

1. Fletcher CDM, Bridge JA, Hogendoorn PCW, Mertens F, World Health Organization, International Agency for Research on Cancer. WHO classification of tumours of soft tissue and bone. Lyon: IARC Press; 2013.
2. Karavasilis V, Seddon BM, Ashley S, Al-Muderis O, Fisher C, Judson I. Significant clinical benefit of first-line palliative chemotherapy in advanced soft-tissue sarcoma: retrospective analysis and identification of prognostic factors in 488 patients. Cancer. 2008;112:1585-91.
3. Ryan CW, Merimsky O, Agulnik M, Blay JY, Schuetze SM, Van Tine BA, et al. PICASSO III: A Phase III, Placebo-Controlled Study of Doxorubicin With or Without Palifosfamide in Patients With Metastatic Soft Tissue Sarcoma. J Clin Oncol. 2016;34:3898-905.
4. Tap WD, Papai Z, Van Tine BA, Attia S, Ganjoo KN, Jones RL, et al. Doxorubicin plus evofosfamide versus doxorubicin alone in locally advanced, unresectable or metastatic soft-tissue sarcoma (TH CR-406/SARC021): an international, multicentre, open-label, randomised phase 3 trial. Lancet Oncol. 2017;18:1089-103.
5. Seddon B, Strauss SJ, Whelan J, Leahy M, Woll PJ, Cowie F, et al. Gemcitabine and docetaxel versus doxorubicin as first-line treatment in previously untreated advanced unresectable or metastatic soft-tissue sarcomas (GeDDiS): a randomised controlled phase 3 trial. Lancet Oncol. 2017;18:1397-410.
6. Hensley ML, Miller A, O'Malley DM, Mannel RS, Behbakht K, Bakkum-Gamez JN, et al. Randomized phase III trial of gemcitabine plus docetaxel plus bevacizumab or placebo as first-line treatment for metastatic uterine leiomyosarcoma: an NRG Oncology/Gynecologic Oncology Group study. J Clin Oncol. 2015;33:1180-5.
7. Judson I, Verweij J, Gelderblom H, Hartmann JT, Schoffski P, Blay JY, et al. Doxorubicin alone versus intensified doxorubicin plus ifosfamide for first-line treatment of advanced or metastatic soft-tissue sarcoma: a randomised controlled phase 3 trial. Lancet Oncol. 2014;15:415-23.
8. Tap WD, Jones RL, Van Tine BA, Chmielowski B, Elias AD, Adkins D, et al. Olaratumab and doxorubicin versus doxorubicin alone for treatment of soft-tissue sarcoma: an open-label phase 1b and randomised phase 2 trial. Lancet. 2016;388:488-97.
9. Sleijfer S, Ray-Coquard I, Papai Z, Le Cesne A, Scurr M, Schoffski P, et al. Pazopanib, a multikinase angiogenesis inhibitor, in patients with relapsed or refractory advanced soft tissue sarcoma: a phase II study from the European organisation for research and treatment of cancer-soft tissue and bone sarcoma group (EORTC study 62043). J Clin Oncol. 2009;27:3126-32.
10. van der Graaf WT, Blay JY, Chawla SP, Kim DW, Bui-Nguyen B, Casali PG, et al. Pazopanib for metastatic soft-tissue sarcoma (PALETTE): a randomised, double-blind, placebo-controlled phase 3 trial. Lancet. 2012;379:1879-86.
11. Kasper B, Sleijfer S, Litiere S, Marreaud S, Verweij J, Hodge RA, et al. Long-term responders and survivors on pazopanib for advanced soft tissue sarcomas: subanalysis of two European Organisation for Research and Treatment of Cancer (EORTC) clinical trials 62043 and 62072. Ann Oncol. 2014;25:719-24.
12. Rini BI, Cohen DP, Lu DR, Chen I, Hariharan S, Gore ME, et al. Hypertension as a biomarker of efficacy in patients with metastatic renal cell carcinoma treated with sunitinib. J Natl Cancer Inst. 2011;103:763-73.
13. Rixe O, Billemont B, Izzedine H. Hypertension as a predictive factor of Sunitinib activity. Ann Oncol. 2007;18:1117.
14. George S, Reichardt P, Lechner T, Li S, Cohen DP, Demetri GD. Hypertension as a potential biomarker of efficacy in patients with gastrointestinal stromal tumor treated with sunitinib. Ann Oncol. 2012;23:3180-7.

15. Rutkowski P, Bylina E, Klimczak A, Świtaj T, Falkowski S, Kroc J, et al. The outcome and predictive factors of sunitinib therapy in advanced gastrointestinal stromal tumors (GIST) after imatinib failure - one institution study. BMC Cancer. 2012;12:107.
16. Howell J, Pinato DJ, Ramaswami R, Bettinger D, Arizumi T, Ferrari C, et al. On-target sorafenib toxicity predicts improved survival in hepatocellular carcinoma: a multi-centre, prospective study. Aliment Pharmacol Ther. 2017;45:1146-55.
17. Cho JY, Paik YH, Lim HY, Kim YG, Lim HK, Min YW, et al. Clinical parameters predictive of outcomes in sorafenib-treated patients with advanced hepatocellular carcinoma. Liver Int. 2013;33:950-7.
18. Sorich MJ, Rowland A, Kichenadasse G, Woodman RJ, Mangoni AA. Risk factors of proteinuria in renal cell carcinoma patients treated with VEGF inhibitors: a secondary analysis of pooled clinical trial data. British Journal of Cancer. 2016;114:1313-7.
19. Bailey EB, Tantravahi SK, Poole A, Agarwal AM, Straubhar AM, Batten JA, et al. Correlation of degree of hypothyroidism with survival outcomes in patients with metastatic renal cell carcinoma receiving vascular endothelial growth factor receptor tyrosine kinase inhibitors. Clin Genitourin Cancer. 2015;13:e131-7.
20. Sleijfer S, Rizzo E, Litiere S, Mathijssen RHJ, Judson IR, Gelderblom H, et al. Predictors for doxorubicin-induced hematological toxicity and its association with outcome in advanced soft tissue sarcoma patients; a retrospective analysis of the EORTC-soft tissue and bone sarcoma group database. Acta Oncol. 2018;57:1117-26.
21. Duffaud F, Sleijfer S, Litiere S, Ray-Coquard I, Le Cesne A, Papai Z, et al. Hypertension (HTN) as a potential biomarker of efficacy in pazopanib-treated patients with advanced non-adipocytic soft tissue sarcoma. A retrospective study based on European Organisation for Research and Treatment of Cancer (EORTC) 62043 and 62072 trials. Eur J Cancer. 2015;51:2615-23.
22. Garber JR, Cobin RH, Gharib H, Hennessey JV, Klein I, Mechanick JI, et al. Clinical practice guidelines for hypothyroidism in adults: cosponsored by the American Association of Clinical Endocrinologists and the American Thyroid Association. Endocr Pract. 2012;18:988-1028.
23. Cancer Therapy Evaluation Program. Common Terminology Criteria for Adverse Events v3.0 (CTCAE) National Cancer Institute Division of Cancer Treatment and Diagnosis; 2006.
24. Motzer RJ, Hutson TE, Cella D, Reeves J, Hawkins R, Guo J, et al. Pazopanib versus Sunitinib in Metastatic Renal-Cell Carcinoma. New England Journal of Medicine. 2013;369:722-31.
25. Sternberg CN, Davis ID, Mardiak J, Szczylik C, Lee E, Wagstaff J, et al. Pazopanib in locally advanced or metastatic renal cell carcinoma: results of a randomized phase III trial. J Clin Oncol. 2010;28:1061-8.
26. Matrana MR, Duran C, Shetty A, Xiao L, Atkinson BJ, Corn P, et al. Outcomes of patients with metastatic clear-cell renal cell carcinoma treated with pazopanib after disease progression with other targeted therapies. Eur J Cancer. 2013;49:3169-75.
27. Hall PS, Harshman LC, Srinivas S, Witteles RM. The frequency and severity of cardiovascular toxicity from targeted therapy in advanced renal cell carcinoma patients. JACC Heart Fail. 2013;1:72-8.
28. Pinkhas D, Ho T, Smith S. Assessment of pazopanib-related hypertension, cardiac dysfunction and identification of clinical risk factors for their development. Cardiooncology. 2017;3.
29. Maurea N, Coppola C, Piscopo G, Galletta F, Riccio G, Esposito E, et al. Pathophysiology of cardiotoxicity from target therapy and angiogenesis inhibitors. Journal of Cardiovascular Medicine. 2016;17:e19-e26.
30. Touyz RM, Herrmann J. Cardiotoxicity with vascular endothelial growth factor inhibitor therapy. npj Precision Oncology. 2018;2:13.

31. Feliu J, Salud A, Safont MJ, García-Girón C, Aparicio J, Losa F, et al. Correlation of Hypertension and Proteinuria with Outcome in Elderly Bevacizumab-Treated Patients with Metastatic Colorectal Cancer. PLoS ONE. 2015;10:e0116527.
32. Schmidinger M, Vogl UM, Bojic M, Lamm W, Heinzl H, Haitel A, et al. Hypothyroidism in patients with renal cell carcinoma: blessing or curse? Cancer. 2011;117:534-44.
33. Baldazzi V, Tassi R, Lapini A, Santomaggio C, Carini M, Mazzanti R. The impact of sunitinib-induced hypothyroidism on progression-free survival of metastatic renal cancer patients: a prospective single-center study. Urol Oncol. 2012;30:704-10.
34. Reig M, Torres F, Rodriguez-Lope C, Forner A, N LL, Rimola J, et al. Early dermatologic adverse events predict better outcome in HCC patients treated with sorafenib. J Hepatol. 2014;61:318-24.
35. Otsuka T, Eguchi Y, Kawazoe S, Yanagita K, Ario K, Kitahara K, et al. Skin toxicities and survival in advanced hepatocellular carcinoma patients treated with sorafenib. Hepatol Res. 2012;42:879-86.

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 Ineligible to chemotherapy or failure to no more than two prior cytotoxic agents (sequentially as single agents or one combination regimen)	Metastatic non-adipocytic STS 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**
		No	Yes	(N=333)
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	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**
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.

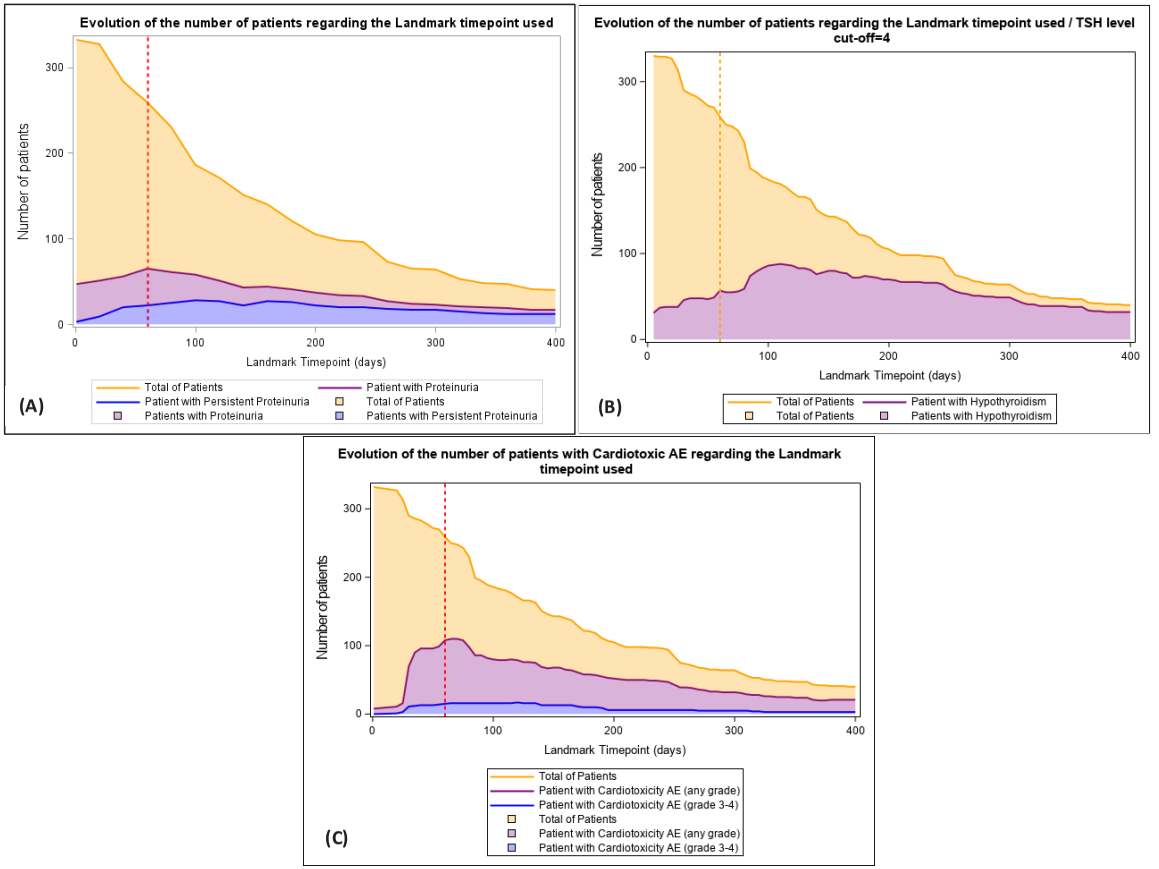
Landmark Analysis at t=60days	Multivariable Cox model of progression-free survival				Multivariable Cox model of overall survival			
Covariates	**Patients (N=257)**	**Observed Events (O=246)**	**Hazard Ratio (95%CI)**	**Wald p-value**	**Patients (N=311)**	**Observed Events (O=244)**	**Hazard Ratio (95%CI)**	**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.

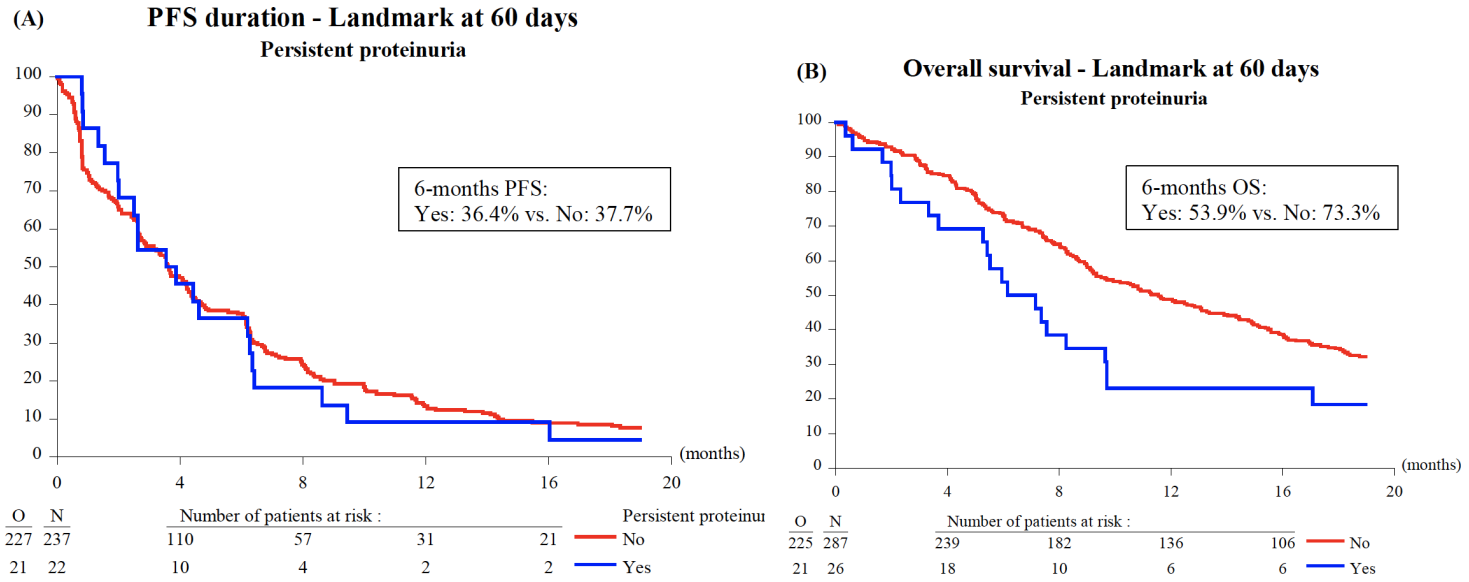
Landmark Analysis at t=60days	Multivariable Cox model of progression-free survival				Multivariable Cox model of overall survival			
Covariates	**Patients (N=257)**	**Observed Events (O=246)**	**Hazard Ratio (95%CI)**	**Wald p-value**	**Patients (N=311)**	**Observed Events (O=244)**	**Hazard Ratio (95%CI)**	**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.

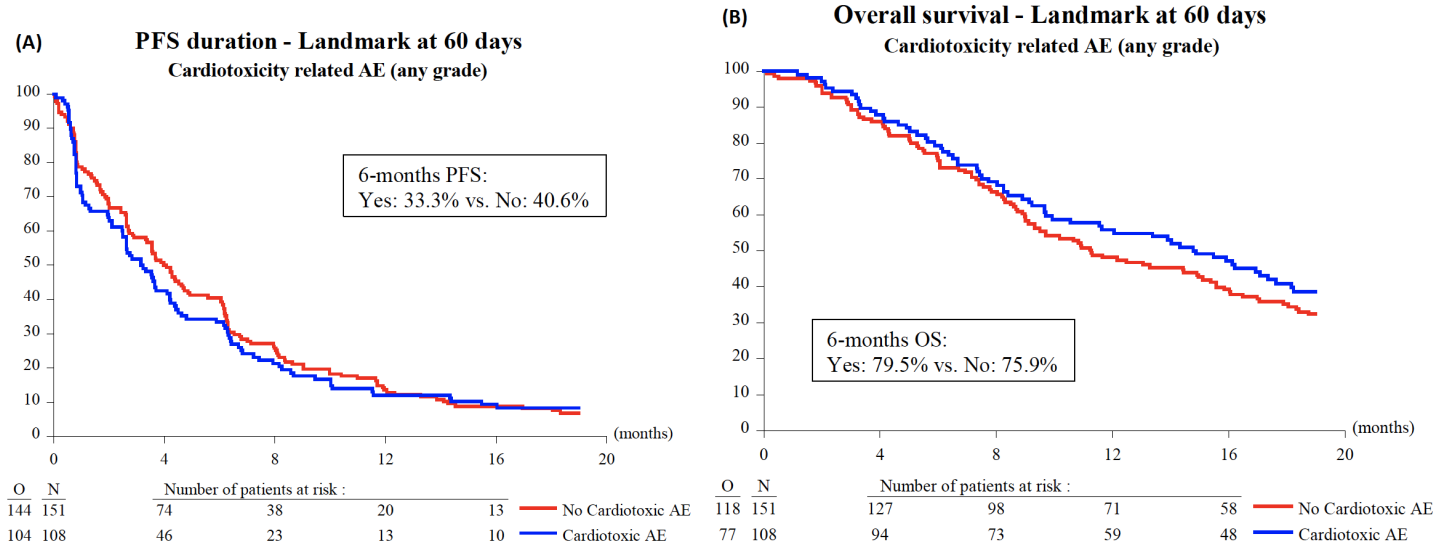
Landmark Analysis at t=60days	Multivariable Cox model of progression-free survival				Multivariable Cox model of overall survival			
Covariates	**Patients (N=41)**	**Observed Events (O=41)**	**Hazard Ratio (95%CI)**	**Wald p-value**	**Patients (N=101)**	**Observed Events (O=75)**	**Hazard Ratio (95%CI)**	**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)	



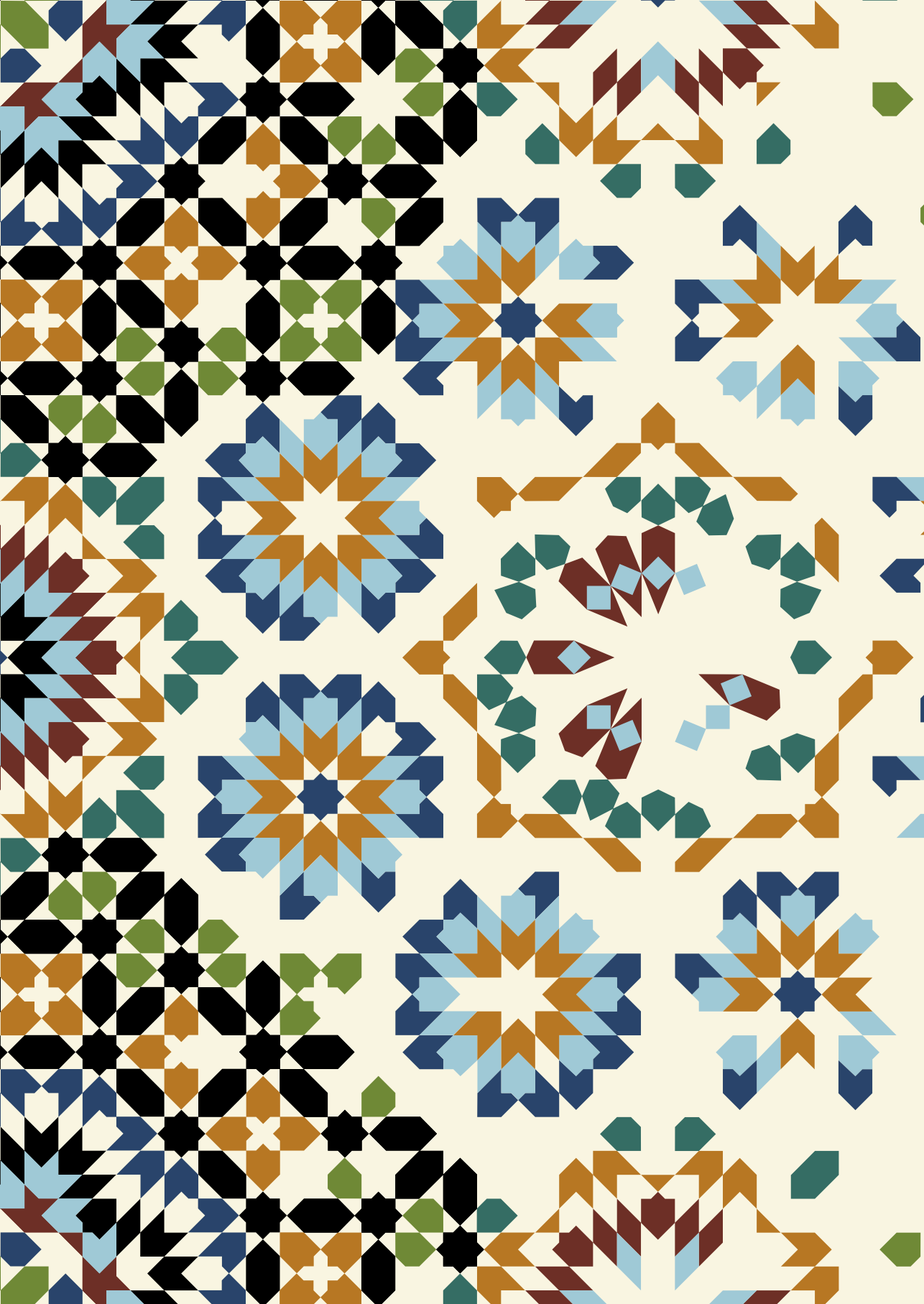
▲**Supporting Figure S1.** Incidences and landmark choice of the three pazopanib-induced toxicities. (**A**) Number of patients with proteinuria while on treatment with pazopanib. (**B**) Number of patients with hypothyroidism (TSH cut-off point 4mU/L) while on treatment with pazopanib. (**C**) Number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

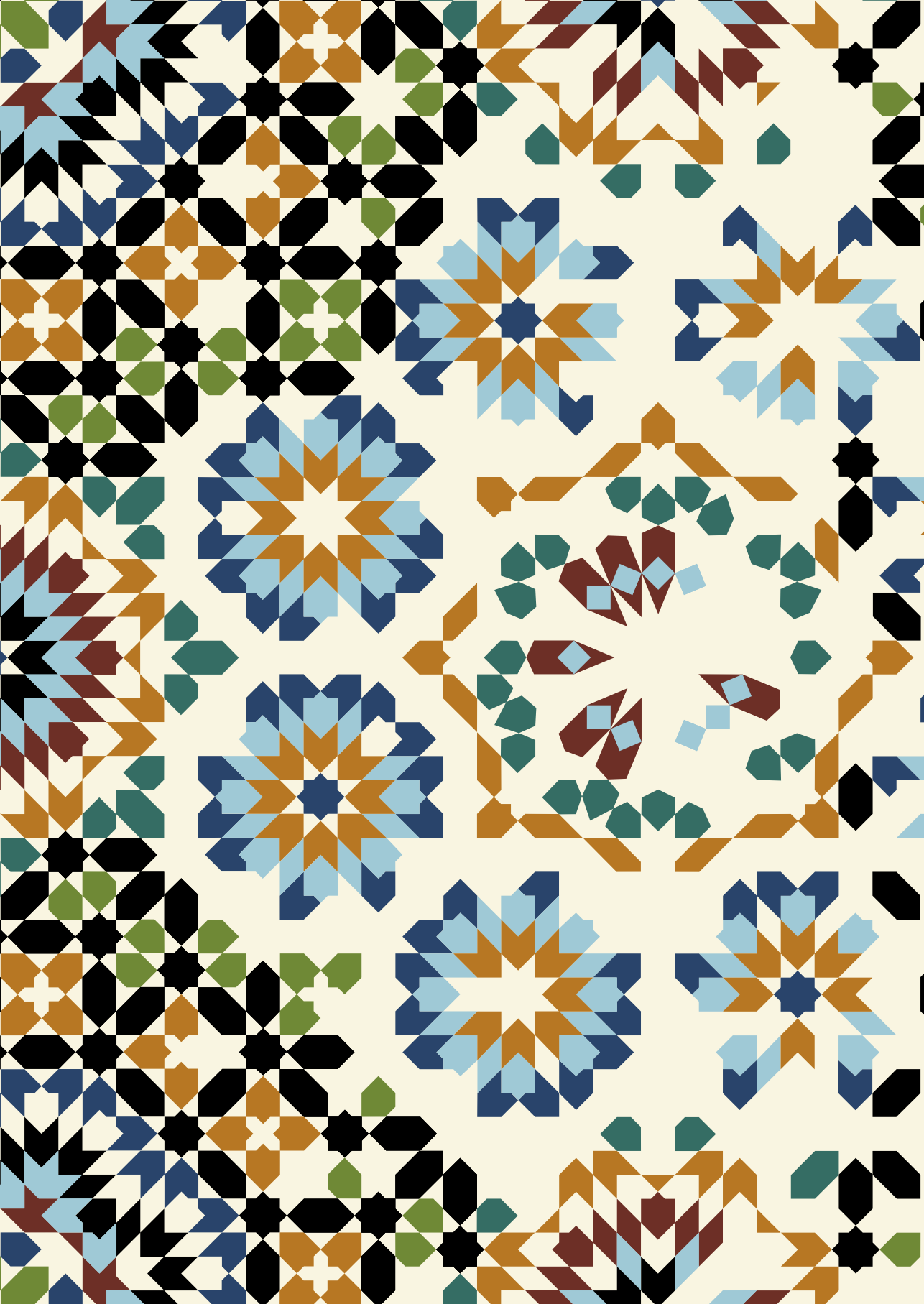


▲**Supporting Figure S3.** Kaplan-Meier curves showing the progression-free survival (**A**) and overall survival (**B**) of patients with and without cardiotoxicity of any grade.



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 an 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.

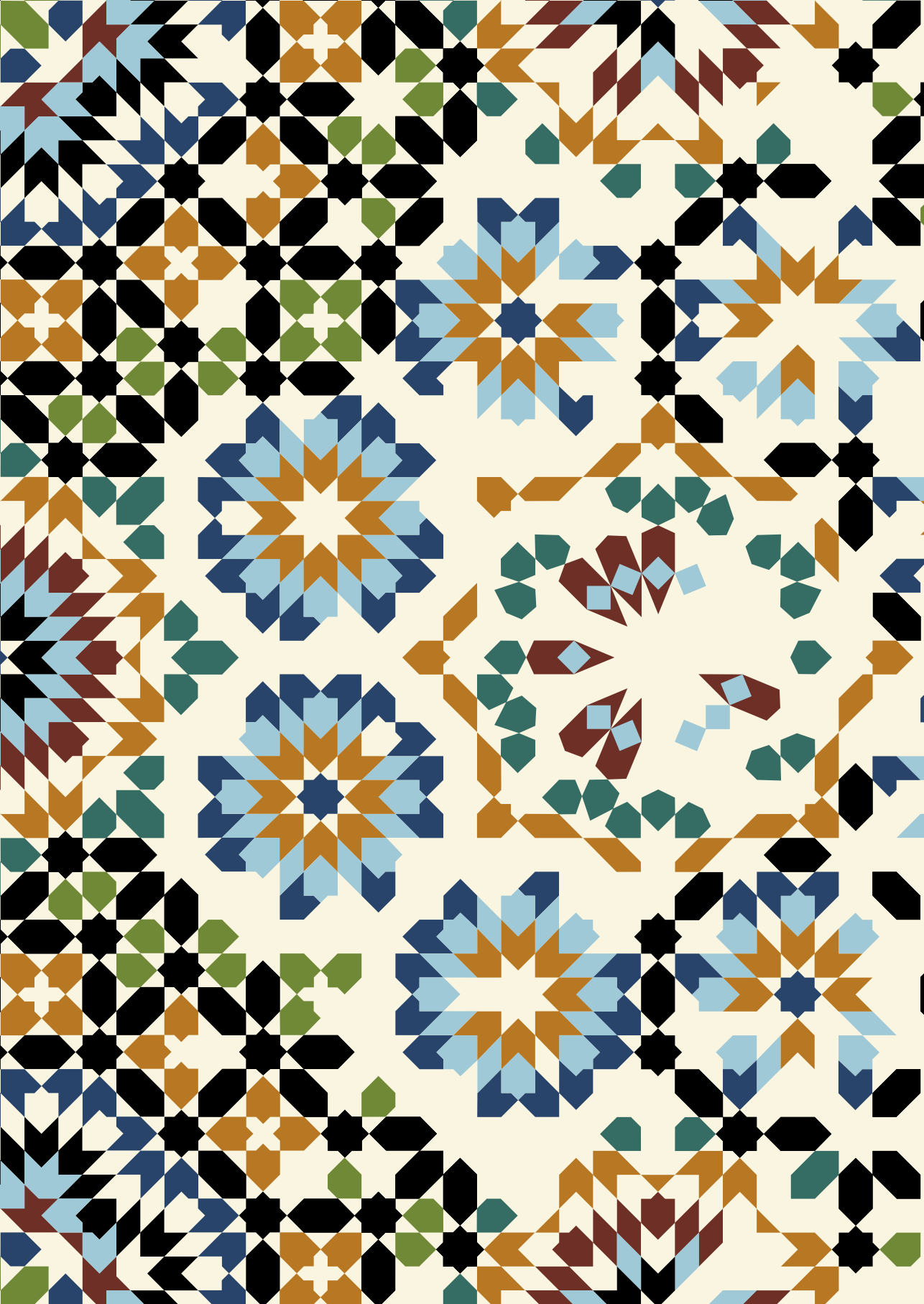
Overall, it can be concluded that there is still a lot to gain in the knowledge of soft tissue sarcomas and that the gaps of the mosaic are not all filled in.

References

1. Fletcher CDM, Bridge JA, Hogendoorn PCW, Mertens F, World Health Organization, International Agency for Research on Cancer. WHO classification of tumours of soft tissue and bone. Lyon: IARC Press; 2013.
2. Demetri GD, Chawla SP, von Mehren M, Ritch P, Baker LH, Blay JY, et al. Efficacy and safety of trabectedin in patients with advanced or metastatic liposarcoma or leiomyosarcoma after failure of prior anthracyclines and ifosfamide: results of a randomized phase II study of two different schedules. J Clin Oncol. 2009;27:4188-96.
3. Demetri GD, von Mehren M, Jones RL, Hensley ML, Schuetze SM, Staddon A, et al. Efficacy and Safety of Trabectedin or Dacarbazine for Metastatic Liposarcoma or Leiomyosarcoma After Failure of Conventional Chemotherapy: Results of a Phase III Randomized Multicenter Clinical Trial. J Clin Oncol. 2016;34:786-93.
4. Schoffski P, Chawla S, Maki RG, Italiano A, Gelderblom H, Choy E, et al. Eribulin versus dacarbazine in previously treated patients with advanced liposarcoma or leiomyosarcoma: a randomised, open-label, multicentre, phase 3 trial. Lancet. 2016;387:1629-37.
5. Penel N, Bui BN, Bay JO, Cupissol D, Ray-Coquard I, Piperno-Neumann S, et al. Phase II trial of weekly paclitaxel for unresectable angiosarcoma: the ANGIOTAX Study. J Clin Oncol. 2008;26:5269-74.
6. Seddon B, Strauss SJ, Whelan J, Leahy M, Woll PJ, Cowie F, et al. Gemcitabine and docetaxel versus doxorubicin as first-line treatment in previously untreated advanced unresectable or metastatic soft-tissue sarcomas (GeDDiS): a randomised controlled phase 3 trial. Lancet Oncol. 2017;18:1397-410.
7. Tap WD, Papai Z, Van Tine BA, Attia S, Ganjoo KN, Jones RL, et al. Doxorubicin plus evofosfamide versus doxorubicin alone in locally advanced, unresectable or metastatic soft-tissue sarcoma (TH CR-406/SARC021): an international, multicentre, open-label, randomised phase 3 trial. Lancet Oncol. 2017;18:1089-103.
8. Gronchi A, Ferrari S, Quagliuolo V, Broto JM, Pousa AL, Grignani G, et al. Histotype-tailored neoadjuvant chemotherapy versus standard chemotherapy in patients with high-risk soft-tissue sarcomas (ISG-STS 1001): an international, open-label, randomised, controlled, phase 3, multicentre trial. Lancet Oncol. 2017;18:812-22.
9. van der Graaf WT, Blay JY, Chawla SP, Kim DW, Bui-Nguyen B, Casali PG, et al. Pazopanib for metastatic soft-tissue sarcoma (PALETTE): a randomised, double-blind, placebo-controlled phase 3 trial. Lancet. 2012;379:1879-86.
10. Tap WD, Wagner AJ, Schöffski P, Martin-Broto J, Krarup-Hansen A, Ganjoo KN, et al. Effect of Doxorubicin Plus Olaratumab vs Doxorubicin Plus Placebo on Survival in Patients With Advanced Soft Tissue Sarcomas: The ANNOUNCE Randomized Clinical Trial. Jama. 2020;323:1266-76.
11. Joensuu H, Wardelmann E, Sihto H, Eriksson M, Sundby Hall K, Reichardt A, et al. Effect of KIT and PDGFRA Mutations on Survival in Patients With Gastrointestinal Stromal Tumors Treated With Adjuvant Imatinib: An Exploratory Analysis of a Randomized Clinical Trial. JAMA Oncol. 2017;3:602-9.
12. Gastrointestinal Stromal Tumor Meta-Analysis G. Comparison of two doses of imatinib for the treatment of unresectable or metastatic gastrointestinal stromal tumors: a meta-analysis of 1,640 patients. J Clin Oncol. 2010;28:1247-53.
13. Farag S, Somaiah N, Choi H, Heeres B, Wang WL, van Boven H, et al. Clinical characteristics and treatment outcome in a large multicentre observational cohort of PDGFRA exon 18 mutated gastrointestinal stromal tumour patients. Eur J Cancer. 2017;76:76-83.

14. Corless CL, Ballman KV, Antonescu CR, Kolesnikova V, Maki RG, Pisters PW, et al. Pathologic and molecular features correlate with long-term outcome after adjuvant therapy of resected primary GI stromal tumor: the ACOSOG Z9001 trial. J Clin Oncol. 2014;32:1563-70.
15. Tap WD, Jones RL, Van Tine BA, Chmielowski B, Elias AD, Adkins D, et al. Olaratumab and doxorubicin versus doxorubicin alone for treatment of soft-tissue sarcoma: an open-label phase 1b and randomised phase 2 trial. Lancet. 2016;388:488-97.
16. Tawbi HA, Burgess M, Bolejack V, Van Tine BA, Schuetze SM, Hu J, et al. Pembrolizumab in advanced soft-tissue sarcoma and bone sarcoma (SARC028): a multicentre, two-cohort, single-arm, open-label, phase 2 trial. Lancet Oncol. 2017.
17. Novelli M, Rossi S, Rodriguez-Justo M, Taniere P, Seddon B, Toffolatti L, et al. DOG1 and CD117 are the antibodies of choice in the diagnosis of gastrointestinal stromal tumours. Histopathology. 2010;57:259-70.
18. Zhao EY, Shen Y, Pleasance E, Kasaian K, Leelakumari S, Jones M, et al. Homologous Recombination Deficiency and Platinum-Based Therapy Outcomes in Advanced Breast Cancer. Clin Cancer Res. 2017;23:7521-30.
19. Hauschild A, Grob J-J, Demidov LV, Jouary T, Gutzmer R, Millward M, et al. Dabrafenib in BRAF-mutated metastatic melanoma: a multicentre, open-label, phase 3 randomised controlled trial. Lancet (London, England). 2012;380:358-65.
20. Fribbens C, O'Leary B, Kilburn L, Hrebien S, Garcia-Murillas I, Beaney M, et al. Plasma ESR1 Mutations and the Treatment of Estrogen Receptor-Positive Advanced Breast Cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2016;34:2961-8.
21. Dalal KM, Kattan MW, Antonescu CR, Brennan MF, Singer S. Subtype specific prognostic nomogram for patients with primary liposarcoma of the retroperitoneum, extremity, or trunk. Ann Surg. 2006;244:381-91.
22. Bonvalot S, Gronchi A, Le Pechoux C, Swallow CJ, Strauss DC, Meeus P, et al. STRASS (EORTC 62092): A phase III randomized study of preoperative radiotherapy plus surgery versus surgery alone for patients with retroperitoneal sarcoma. Journal of Clinical Oncology. 2019;37:11001-.
23. Vamathevan J, Clark D, Czodrowski P, Dunham I, Ferran E, Lee G, et al. Applications of machine learning in drug discovery and development. Nature Reviews Drug Discovery. 2019;18:463-77.
24. Cain EH, Saha A, Harowicz MR, Marks JR, Marcom PK, Mazurowski MA. Multivariate machine learning models for prediction of pathologic response to neoadjuvant therapy in breast cancer using MRI features: a study using an independent validation set. Breast Cancer Res Treat. 2019;173:455-63.
25. Shayesteh SP, Alikhassi A, Farhan F, Gahletaki R, Soltanabadi M, Haddad P, et al. Prediction of Response to Neoadjuvant Chemoradiotherapy by MRI-Based Machine Learning Texture Analysis in Rectal Cancer Patients. J Gastrointest Cancer. 2019.
26. Abdollahi H, Mofid B, Shiri I, Razzaghdoust A, Saadipoor A, Mahdavi A, et al. Machine learning-based radiomic models to predict intensity-modulated radiation therapy response, Gleason score and stage in prostate cancer. Radiol Med. 2019;124:555-67.
27. Maurice MJ, Yih JM, Ammori JB, Abouassaly R. Predictors of surgical quality for retroperitoneal sarcoma: Volume matters. J Surg Oncol. 2017;116:766-74.
28. Gutierrez JC, Perez EA, Moffat FL, Livingstone AS, Franceschi D, Koniaris LG. Should soft tissue sarcomas be treated at high-volume centers? An analysis of 4205 patients. Ann Surg. 2007;245:952-8.
29. Abarca T, Gao Y, Monga V, Tanas MR, Milhem MM, Miller BJ. Improved survival for extremity soft tissue sarcoma treated in high-volume facilities. J Surg Oncol. 2018;117:1479-86.

30. Blay JY, Soibinet P, Penel N, Bompas E, Duffaud F, Stoeckle E, et al. Improved survival using specialized multidisciplinary board in sarcoma patients. Ann Oncol. 2017;28:2852-9.
31. Bhangu AA, Beard JA, Grimer RJ. Should Soft Tissue Sarcomas be Treated at a Specialist Centre? Sarcoma. 2004;8:1-6.
32. Derbel O, Heudel PE, Cropet C, Meeus P, Vaz G, Biron P, et al. Survival impact of centralization and clinical guidelines for soft tissue sarcoma (A prospective and exhaustive population-based cohort). PLoS One. 2017;12:e0158406.
33. Martin-Broto J, Hindi N, Cruz J, Martinez-Trufero J, Valverde C, De Sande LM, et al. Relevance of Reference Centers in Sarcoma Care and Quality Item Evaluation: Results from the Prospective Registry of the Spanish Group for Research in Sarcoma (GEIS). Oncologist. 2018.
34. Venigalla S, Nead KT, Sebro R, Guttmann DM, Sharma S, Simone CB, 2nd, et al. Association Between Treatment at High-Volume Facilities and Improved Overall Survival in Soft Tissue Sarcomas. Int J Radiat Oncol Biol Phys. 2018;100:1004-15.
35. Stichting Oncologische Samenwerking (SONCOS). Standardisation of multidisciplinary cancer care in the Netherlands - SONCOS Standardisatrion Report 5 2017. SONCOS; 2017.
36. Hoekstra HJ, Haas RLM, Verhoef C, Suurmeijer AJH, van Rijswijk CSP, Bongers BGH, et al. Adherence to Guidelines for Adult (Non-GIST) Soft Tissue Sarcoma in the Netherlands: A Plea for Dedicated Sarcoma Centers. Ann Surg Oncol. 2017;24:3279-88.
37. Judson I, Verweij J, Gelderblom H, Hartmann JT, Schoffski P, Blay JY, et al. Doxorubicin alone versus intensified doxorubicin plus ifosfamide for first-line treatment of advanced or metastatic soft-tissue sarcoma: a randomised controlled phase 3 trial. Lancet Oncol. 2014;15:415-23.
38. Ryan CW, Merimsky O, Agulnik M, Blay JY, Schuetze SM, Van Tine BA, et al. PICASSO III: A Phase III, Placebo-Controlled Study of Doxorubicin With or Without Palifosfamide in Patients With Metastatic Soft Tissue Sarcoma. J Clin Oncol. 2016;34:3898-905.
39. Maki RG, Wathen JK, Patel SR, Priebat DA, Okuno SH, Samuels B, et al. Randomized phase II study of gemcitabine and docetaxel compared with gemcitabine alone in patients with metastatic soft tissue sarcomas: results of sarcoma alliance for research through collaboration study 002 [corrected]. J Clin Oncol. 2007;25:2755-63.
40. Garcia-Del-Muro X, Lopez-Pousa A, Maurel J, Martin J, Martinez-Trufero J, Casado A, et al. Randomized phase II study comparing gemcitabine plus dacarbazine versus dacarbazine alone in patients with previously treated soft tissue sarcoma: a Spanish Group for Research on Sarcomas study. J Clin Oncol. 2011;29:2528-33.
41. Chakravarty D, Gao J, Phillips S, Kundra R, Zhang H, Wang J, et al. OncoKB: A Precision Oncology Knowledge Base. JCO Precision Oncology. 2017:1-16.
42. Wiemer EA, Wozniak A, Burger H, Smid M, Floris G, Nzokirantevye A, et al. Identification of microRNA biomarkers for response of advanced soft tissue sarcomas to eribulin: Translational results of the EORTC 62052 trial. Eur J Cancer. 2017;75:33-40.
43. Patel SP, Kurzrock R. PD-L1 Expression as a Predictive Biomarker in Cancer Immunotherapy. Mol Cancer Ther. 2015;14:847-56.
44. Sleijfer S, Rizzo E, Litiere S, Mathijssen RHJ, Judson IR, Gelderblom H, et al. Predictors for doxorubicin-induced hematological toxicity and its association with outcome in advanced soft tissue sarcoma patients; a retrospective analysis of the EORTC-soft tissue and bone sarcoma group database. Acta Oncol. 2018;57:1117-26.
45. Duffaud F, Sleijfer S, Litiere S, Ray-Coquard I, Le Cesne A, Papai Z, et al. Hypertension (HTN) as a potential biomarker of efficacy in pazopanib-treated patients with advanced non-adipocytic soft tissue sarcoma. A retrospective study based on European Organisation for Research and Treatment of Cancer (EORTC) 62043 and 62072 trials. Eur J Cancer. 2015;51:2615-23.



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.

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 (≤5cm), 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'.

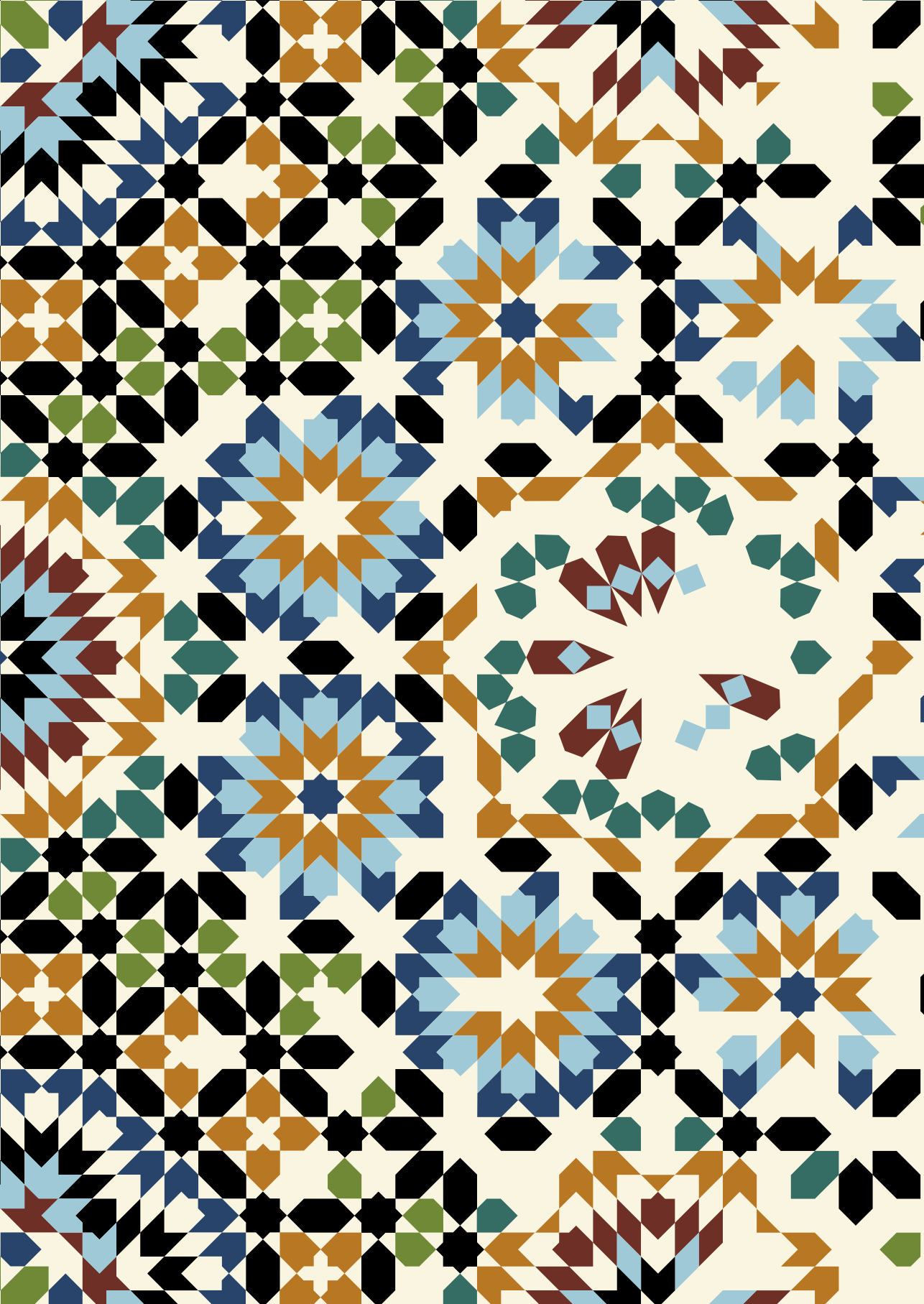
Part IV – Evaluation of the systemic treatment of metastatic soft tissue sarcoma

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 de 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, unfitness 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.

Hoofstuk 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 gededifferentieerde 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 gededifferentieerde 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 gededifferentieerde 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.

16

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 perifeer 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 (≤5cm), 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'.

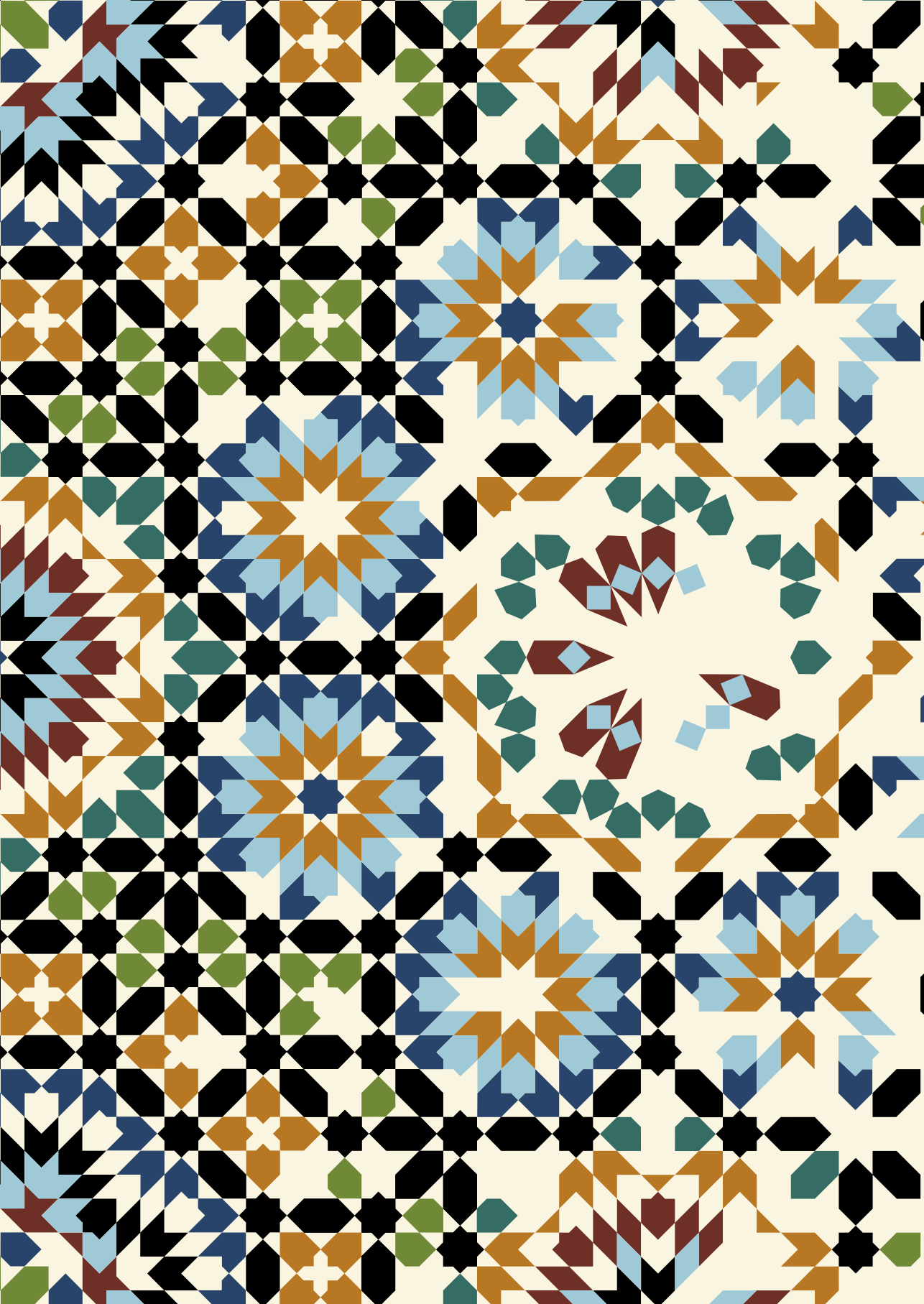
Deel IV – Systemische behandeling van het gemetastaseerde wekedelen sarcoom

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 eerstelijnstherapie, 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 Specialismen (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) + vervolgtraining '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. Koseła-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. Koseła-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

*Contributed equally to this work

List of contributing authors

Dr. L.B. (Lukas) Been
Department of Surgical Oncology, University Medical Center Groningen, Groningen, the Netherlands

Dr. M.H.A. (Marc) Bemelmans
Department of Surgical Oncology, Maastricht University Medical Center+, Maastricht, the Netherlands

Dr. H.G.T. (Harriët) Blaauwgeers
Netherlands Comprehensive Cancer Organization (IKNL), Utrecht, the Netherlands

J. (Joachim) Boers MSc
Department of Developmental Biology, Erasmus University Medical Center, Rotterdam, the Netherlands

Drs. R. (Ruben) Boers
Department of Developmental Biology, Erasmus University Medical Center, Rotterdam, the Netherlands

W.C. (Williene) Boeve
Department of Surgical Oncology, Erasmus MC Cancer Institute, Rotterdam, the Netherlands

Dr. J.J. (Han) Bonenkamp
Department of Surgical Oncology, Radboud University Medical Center, Nijmegen, the Netherlands

Prof. dr. ir. E. (Edwin) Cuppen
Center for Molecular Medicine and Oncode Institute, University Medical Center Utrecht, Utrecht, The Netherlands, and Hartwig Medical Foundation, Amsterdam, the Netherlands

Dr. T. (Thijs) van Dalen
Department of Surgical Oncology, University Medical Center Utrecht, Utrecht, the Netherlands

Dr. I.M.E. (Ingrid) Desar
Departement of Medical Oncology, Radboud University Medical Center, Nijmegen, the Netherlands

Prof. dr. F. (Florence) Duffaud
Department of Medical Oncology, La Timone University Hospital & Aix-Marseille University (AMU), Marseille, France

Prof. dr. A.J. (Hans) Gelderblom
Department of Medical Oncology, Leiden University Medical Center, Leiden, the Netherlands

Dr. T.M. (Tessa) van Ginhoven
Department of Surgical Oncology, Erasmus MC Cancer Institute, Rotterdam, the Netherlands

Prof. dr. W.T. (Winette) van der Graaf
Department of Medical Oncology, Netherlands Cancer Institute-Antoni van Leeuwenhoek, Amsterdam, the Netherlands & Department of Medical Oncology, Radboud University Medical Center, Nijmegen, the Netherlands

Prof. dr. J. (Joost) Gribnau
Department of Developmental Biology, Erasmus University Medical Center, Rotterdam, the Netherlands

Dr. D.J. (Dirk) Grünhagen
Department of Surgical Oncology, Erasmus MC Cancer Institute, Rotterdam, the Netherlands

Dr. J.J. (Jacco) de Haan
Department of Medical Oncology, University Medical Center Groningen, Groningen, the Netherlands

Dr. R.L. (Rick) Haas
Department of Radiotherapy, Netherlands Cancer Institute-Antoni van Leeuwenhoek, Amsterdam, the Netherlands

Prof. dr. J.A. (Jos) van der Hage
Department of Surgical Oncology, Leiden University Medical Center, Leiden, the Netherlands

V.K.Y. (Vincent) Ho MSc
Netherlands Comprehensive Cancer Organization (IKNL), Utrecht, the Netherlands

Dr. W.J. (Winan) van Houdt
Department of Surgical Oncology, Netherlands Cancer Institute-Antoni van Leeuwenhoek, Amsterdam, the Netherlands

W. (Wouter) Kessels BSc
Departments of Radiology and Nuclear Medicine & Medical Informatics, Erasmus University Medical Center, Rotterdam, the Netherlands and Faculty of Applied Sciences, Delft University of Technology, Delft, the Netherlands

Dr. ir. S. (Stefan) Klein
Departments of Radiology and Nuclear Medicine & Medical Informatics, Erasmus University Medical Center, Rotterdam, the Netherlands

Dr. H. (Hanna) Koseła-Paterczyk
Department of Soft Tissue/Bone Sarcoma and Melanoma, Maria Skłodowska-Curie Institute-Oncology Center, Warsaw, Poland

P.F. (Patricia) van Kuijk BSc
Department of Medical Oncology, Erasmus MC Cancer Institute, Rotterdam, the Netherlands

A. (Adrian) Lecyk
First Faculty of Medicine, Medical University of Warsaw, Warsaw, Poland

Dr. G.J.L.H. (Arno) van Leenders
Department of Pathology, Erasmus University Medical Center, Rotterdam, the Netherlands

Dr. M.P.J.K. (Martijn) Lolkema
Center for Personalized Cancer Treatment and department of Medical Oncology, Erasmus MC Cancer Institute, Rotterdam, the Netherlands

Dr. S. (Saskia) Litière
Department of Biostatistics, European Organisation for Research and Treatment of Cancer (EORTC Headquarters, Brussels, Belgium

A.S. (Annemarie) Melis
Department of Research & Development, Netherlands Comprehensive Cancer Organisation (IKNL), Utrecht, the Netherlands

Prof. W.J. (Wiro) Niessen
Departments of Radiology and Nuclear Medicine & Medical Informatics, Erasmus University Medical Center, Rotterdam, the Netherlands and Faculty of Applied Sciences, Delft University of Technology, Delft, the Netherlands

Dr. J.W.M. (Johanna) Nin
Department of Medical Oncology, Maastricht University Medical Center+, Maastricht, the Netherlands

M. (Monika) Normantowicz
First Faculty of Medicine, Medical University of Warsaw, Warsaw, Poland

Dr. A.W. (Astrid) Oosten
Department of Medical Oncology, Erasmus MC Cancer Institute, Rotterdam, the Netherlands

Drs. G.A. (Anthony) Padmos
Department of Radiology and Nuclear Medicine, Erasmus University Medical Center, Rotterdam, the Netherlands

J. (Job) van Riet PhD
Cancer Computational Biology Center and department of Urology, Erasmus University Medical Center, Rotterdam, the Netherlands

Prof. dr. P. (Piotr) Rutkowski
Department of Soft Tissue/Bone Sarcoma and Melanoma, Maria Skłodowska-Curie Institute-Oncology Center, Warsaw, Poland

M.S. (Melinda) Schuurman MSc
Department of Research & Development, Netherlands Comprehensive Cancer Organisation (IKNL), Utrecht, the Netherlands

Prof. dr. S. (Stefan) Sleijfer
Department of Medical Oncology, Erasmus MC Cancer Institute, Rotterdam, the Netherlands

M.P.A. (Martijn) Starmans MSc
Departments of Radiology and Nuclear Medicine & Medical Informatics, Erasmus University Medical Center, Rotterdam, the Netherlands

Dr. N. (Neeltje) Steeghs
Department of Medical Oncology, Antoni van Leeuwenhoek-Netherlands Cancer Institute, Amsterdam, the Netherlands

Dr. M.J.M. (Milea) Timbergen
Departments of Surgical Oncology and Medical Oncology, Erasmus MC Cancer Institute, Rotterdam, the Netherlands

N. (Nathan) Touati MSc
Department of Biostatistics, European Organisation for Research and Treatment of Cancer (EORTC) Headquarters, Brussels, Belgium

Prof. dr. C. (Kees) Verhoef
Department of Surgical Oncology, Erasmus MC Cancer Institute, the Netherlands

Dr. J.J. (Jan-Jaap) Visser
Department of Radiology and Nuclear Medicine, Erasmus University Medical Center, Rotterdam, the Netherlands

S.R. (Sebastian) van der Voort MSc
Departments of Radiology and Nuclear Medicine & Medical Informatics, Erasmus University Medical Center, Rotterdam, the Netherlands

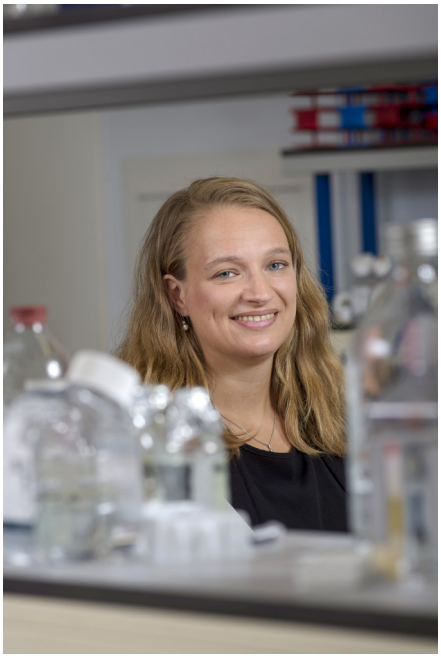
A.L.M. (Anne) Vriends MSc
Department of Medical Oncology, Erasmus MC Cancer Institute, Rotterdam, the Netherlands

Dr. M. (Michal) Wagrodzki
Department of Pathology, Maria Skłodowska-Curie Institute-Oncology Center, Warsaw, Poland

H.J.G. (Harmen) van de Werken PhD
Cancer Computational Biology Center and department of Urology, Erasmus University Medical Center, Rotterdam, the Netherlands

Dr. E.A.C. (Erik) Wiemer
Department of Medical Oncology, Erasmus MC Cancer Institute, Rotterdam, the Netherlands

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.

Dankwoord

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.

Hooggeachte **dr. D.J. Grünhagen** en **dr. E.A.C. Wiemer**, beste Dirk en Erik, ook voor jullie was deze manier van samenwerken nieuw en wellicht even wennen. Dirk, ik kon altijd bij jou terecht met mijn vragen en om te sparren over mijn projecten. Ook jij liet mij heel erg vrij in het ontdekken en ontwikkelen van mijn eigen onderzoekstijl. Heel erg bedankt voor al je hulp, je enthousiasme, begeleiding en supervisie. Ik hoop dat de MINIMALIST snel uitgebreid kan worden naar andere centra en de inclusie gestaag verloopt! Erik, al liep het in het lab met de experimenten niet altijd van een leien dakje, uiteindelijk kwam het allemaal goed met een aantal mooie publicaties (in wording) als resultaat. Heel erg bedankt voor al je geduld om mij als clinicus toch wat biologische en experimentele kennis bij te brengen.

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.

Daarnaast wil ik **alle coauteurs** bedanken voor de samenwerking aan de projecten in dit proefschrift. In particular, I would like to thank prof. **dr. Piotr Rutkowski** and **dr.**

Hanna Koseła-Paterczyk. In the first year of my PhD, I was 'sent' to Warsaw to start a collaboration on soft tissue sarcomas between the Erasmus MC Cancer Institute and the Maria Skłodowska-Curie Institute-Oncology Center, which has led to three joint publications so far. Drogi profesorze Rutkowski i Hanna, dziękuję bardzo za ciepłe przyjęcie w Warszawie i miłą współpracę w ostatnich latach. Verder wil ik graag **Vincent Ho** van het Integraal Kankercentrum Nederland (IKNL) bedanken. Ook onze samenwerking is in mijn eerste jaar begonnen, en ook deze samenwerking heeft tot drie mooie artikelen geleid, waarvoor dank.

Beste collega's en mede-onderzoekers van de Interne Oncologie en Oncologische Chirurgie, jullie hebben de tijd laten vliegen. Jullie hebben van mijn onderzoeksjaren een fantastische tijd gemaakt, ik ga jullie zeker missen in de kliniek. Vanuit 'de Daniël': **Mirelle**, **Charlotte**, **Florian**, **Maarten**, **Boris**, **Daniëlle**, **Milea**, **Pien**, **Jan**, **Nadine**, **Ivona**, **Evalyn**, **Janine**, **Diederik**, **Job**, **Wills**, en na de samensmelting team 'Na-21 flex OGC' met toevoeging van alle andere GE- en onco-onderzoekers. Het teamgevoel en de saamhorigheid die er heerst is uniek. Bedankt voor de leerzame en waardevolle discussies, maar vooral ontzettend veel dank voor alle leuke momenten. Het zijn er teveel om op te noemen, maar toch een aantal highlights: de lunches (met pindasoep!) aan Lago di Daniel, de curling winteruitjes, de borrels/barbecues op het dakterras van de Daniël, de pecannotenbroodjes/oliebollen op vrijdagochtend, de koffies (excuses, overleg in het D-gebouw) en natuurlijk de ESSO 2018 in Boedapest. En sorry mannen, maar de 'girls dinners' en het concert van Justin Timberlake mogen niet ontbreken in dit lijstje. Meiden, ik hoop dat er nog vele etentjes en borrels zullen volgen! Vanuit het lab: **Azi**, **Patricia** en **Anne**, bedankt voor al jullie hulp in het lab, zonder jullie waren mijn experimenten nooit van de grond gekomen. Ik heb ontzettend veel van jullie geleerd. Azi, despite the failure of Dutch-speaking Wednesdays, it was really fun to have you as a colleague on the lab. Patricia, bedankt voor al je hulp en gezelligheid in mijn eerste jaren op het lab. Anne, ik ga de Brabantse gezelligheid met de verhalen over het bloemencorso, de harmonie en verbouwing missen. Ook de promovendi vanuit de andere Interne Onco-groepen wil ik graag bedanken: **Lindsay**, **Lisanne**, **Marjolein**, **Inge**, **Pauline**, **Femke**, **Bodine**, **Daan**, **Koen**, **Ruben**, **Louwrens** en **Florence.** Bedankt dat jullie mij in het begin op sleeptouw hebben genomen, bedankt voor alle gezellige lunches, borrels, feestjes en congressen. Florence, ik zal onze trip naar de ASCO 2017 in Chicago nooit meer vergeten. Tenslotte, **Anne-Rose**, bedankt voor het overnemen van het stokje, ik weet zeker dat de MINIMALIST bij jou in goede handen is en een succes wordt!

Beste secretaresses van de Interne Oncologie en Oncologische Chirurgie, en in het bijzonder beste **Corine**, **Sandra** en **Eline**, dank voor al jullie goede zorgen, hulp en inspanningen. Zonder jullie zou onderzoek doen binnen deze afdelingen een stuk lastiger worden. Ook was het telkens weer een uitdaging om alle agenda's te combineren, maar het lukte jullie altijd!

Beste collega's en kinderartsen van de **Kindergeneeskunde uit het Haaglanden Medisch Centrum**, bedankt voor de kans om mij uit te laten zoeken of de Kindergeneeskunde inderdaad echt mijn passie is. Mede door de fijne samenwerking en de goede sfeer heeft het alleen maar bevestigd dat dit de weg is die ik wil bewandelen. Ik hoop jullie in de toekomst weer tegen te komen.

Beste collega's en kinderartsen van de **Kindergeneeskunde uit het Maasstad Ziekenhuis**, bedankt voor het warme welkom ondanks deze wat gekke tijden. Ik kijk uit naar de komende tijd met veel leerzame maar ook gezellige momenten.

Lieve **paranimfen**, bedankt dat jullie hier vandaag naast mij staan en mij voorzien van mentale support. Lieve **Milea**, mijn mede-wekedelen onderzoeker en wekedelen maatje. Het bleek ontzettend moeilijk voor sommige mensen (met name een bepaalde 2-meter lange professor, maar ik zal geen namen noemen) om onze namen uit elkaar te houden en zijn we daarom maar omgedoopt tot M&M. Ook het aanmeten van onze 'ghetto' namen, Shaniqua en Shay'Nay'Nay, mocht niet baten. Vaak deelden we dezelfde frustraties en liepen we tegen dezelfde problemen aan, bedankt voor je steun en toeverlaat de afgelopen jaren. Trots ben ik dan ook op jou dat jij hier vlak voor mij stond! Lieve **Noor**, van 1e-jaars studiegroep-genoten tot nog steeds goede vriendinnen jaren later en beide promovendi. Ook jij bedankt voor al je steun, voor alle koffies en lunches/etentjes waarbij we ons onderzoeksleed konden delen. Hopelijk blijven we dit voortzetten tijdens onze assistententijd. Ik kijk uit naar de dag dat jij hier staat!

Lieve vriendinnen, **Noor**, **Lindsay**, **Linsey**, **Roxanna**, **Luciënne** en **Eva**, ook jullie bedankt voor alle gezelligheid, afleiding en ontspanning de afgelopen jaren. Bedankt voor jullie vriendschap. Lindsay, van huisgenoot tot goede vriendin en mede-promovendus bij dezelfde professor, ook jij heel erg bedankt voor alle steun en toeverlaat de afgelopen jaren. Ik kijk uit naar jouw promotie!

Lieve **tennismaatjes**, met name **Fred** en **Caroline**, ook bij jullie heb ik de afgelopen jaren regelmatig even mijn hoofd leeg kunnen maken en heb ik met veel plezier met jullie op de baan gestaan, waarvoor ik jullie wil bedanken.

Lieve **familie**, opa & oma's, Shirley, maar in het bijzonder lieve **papa** en **mama**, zonder jullie had ik hier niet gestaan. Woorden kunnen niet omschrijven hoe dankbaar ik jullie ben. Jullie hebben mij altijd onvoorwaardelijk gesteund en altijd in mij geloofd. Jullie hebben mij altijd aangemoedigd om mijn eigen weg te kiezen en ik kan altijd bij jullie terecht. Ik weet niet hoe ik jullie hier ooit genoeg voor kan bedanken, ik hou van jullie.

Lieve **Roderik**, mijn laatste dankwoord is voor jou. Ook al werd het aangaan van een relatie tijdens mijn promotietijd mij ten strengste verboden door beide professoren en houd ik mij normaal netjes aan de regels, deze 'regel' heb ik maar al te graag aan mijn laars gelapt. Ook jij hebt mij vanaf begin af aan altijd gesteund en me alle vrijheid gegeven om dit te kunnen bereiken, waardoor ik mijzelf nu dan toch echt slimmer dan jij mag noemen. De

afgelopen jaren hebben we al een aantal mooie reizen mogen maken, ik hoop dat we dat we de komende jaren blijven doen. Ik had deze tijd nooit met iemand anders willen delen, bedankt voor je onvoorwaardelijke steun en liefde. Ik ben gek op je.