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Molecular Heterogeneity in Leiomyosarcoma and Implications for Personalised Medicine

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Opinion statement

Leiomyosarcoma (LMS) is one of the more common subtypes of soft tissue sarcomas (STS), accounting for about 20% of cases. Differences in anatomical location, risk of recurrence and histomorphological variants contribute to the substantial clinical heterogeneity in survival outcomes and therapy responses observed in patients. There is therefore a need to move away from the current one-size-fits-all treatment approach towards a personalised strategy tailored for individual patients. Over the past decade, tissue profiling studies have revealed key genomic features and an additional layer of molecular heterogeneity among patients, with potential utility for optimal risk stratification and biomarker-matched therapies. Furthermore, recent studies investigating intratumour heterogeneity and tumour evolution patterns in LMS suggest some key features that may need to be taken into consideration when designing treatment strategies and clinical trials. Moving forward, national and international collaborative efforts to aggregate expertise, data, resources and tools are needed to achieve a step change in improving patient survival outcomes in this disease of unmet need.

Introduction

Leiomyosarcoma (LMS) is one of the most common soft tissue sarcoma (STS) subtypes, accounting for 10–20% of cases [1]. The disease arises from the smooth muscle cell lineage and therefore can affect various anatomical sites. However, LMS commonly develops in the uterus, the abdomen, retroperitoneum and extremities [1]. LMS can also arise from the smooth muscle layer of the vasculature, mainly affecting the inferior vena cava [2]. Due to the distinct clinical features of uterine LMS, the disease is currently classified as uterine LMS and non-uterine LMS. Along with anatomical site heterogeneity, LMS displays a range of histomorphological variants. While conventional LMS displays a spindle cell histology resembling smooth muscle tissue, other variants exhibit epithelioid or myxoid appearance [3]. In addition, dedifferentiated LMS which is characterised by reduced expression or loss of smooth muscle markers has been described and is associated with worse prognosis [3–5]. For localised LMS, the standard clinical management relies on wide surgical excision with clear margins. In certain situations, (neo)adjuvant radiation and chemotherapy can be considered. However, there is a high risk of recurrence in LMS [6, 7], and the treatment options for advanced/ metastatic LMS are limited and rely on chemotherapy with doxorubicin in combination with either ifosfamide or dacarbazine as a first-line treatment. Retrospective data suggest that ifosfamide may not be as active in LMS compared to other STS subtypes. Patients who experience disease progression on first-line therapy can be considered for other systemic agents including trabectedin [8] or pazopanib [8, 9]. The combination of gemcitabine and docetaxel, although not recommended as first-line due to increased toxicity [10], is considered for patients with disease progression on doxorubicin-based first-line treatment [8]. More recently, doxorubicin combined with trabectedin in first-line treatment of LMS has shown promising results in the LMS-04 phase 3 trial (NCT02997358), as the combination treatment significantly prolonged progression-free and overall survival of patients compared to doxorubicin monotherapy [11]. However, the clinical benefit of current treatment options is still very limited, particularly for patients with advanced disease [12]. Studies on the molecular biology of LMS have reported key genomic and proteomic features, revealing significant heterogeneity in disease biology and identifying potential new therapeutic avenues. Here, we review the current molecular understanding of key genetic features and inter- and intra-patient heterogeneity in LMS and their implications for clinical management.

Molecular heterogeneity in LMS

Molecular biology of LMS and inter-patient heterogeneity

Common genetic alterations

Both uterine and non-uterine LMS are considered to be sarcomas with complex karyotypes [13, 14]. Large-scale genome sequencing studies have identified *TP53*, *RB1* and *PTEN* as the most altered genes in LMS [15, 16••, 17, 18, 19•, 20•]. While the exact frequencies of these alterations vary across study cohorts, there is a high level of concordance between paired primary and recurrent LMS samples indicating that these genetic alterations are likely to be early initiating events in disease development [15, 21••]. In addition, loss of function mutations in *ATRX* are common (16–24% of all LMS reported to have deleterious mutations in ATRX [16••, 20•, 22, 23]) and enriched in uterine LMS cases [16••, 17]. In addition to alterations in specific genes, widespread somatic copy number alterations and whole genome doubling events

have been reported in LMS [16••, 21••]. Consistent with most STS subtypes which have an overall low tumour mutational burden (TMB) compared to other solid tumours such as lung cancer or melanoma [24], TMB in the majority of LMS cases is low with a median of < 5 mutations/megabase pairs (Mbp) compared to median of 10 mutations/Mbp in other solid tumours [19•]. However, there is also some TMB heterogeneity within LMS where a subset of uterine LMS tumours (15%) have been reported to harbour increased levels of tumour mutational burden (TMB > 5 mutations/Mbp) [19•].

Molecular heterogeneity and LMS subtypes

Various transcriptomic studies have shown that there are several molecular subtypes within LMS that harbour distinct biology and clinicopathological features [19•, 20•, 21••, 25•, 26•, 27–30]. These molecular subtypes appear to be conserved in paired primary and relapse patient specimens indicating that they are likely to be an intrinsic feature of the disease [21••, 25•, 28]. Anderson et al. showed that although genomic structural rearrangements including kataegis and chromothripsis varied considerably between primary and metastatic specimens, recurrent tumours shared the transcriptional subtype and > 60% of clonal substitutions and indel mutations with its primary tumours [21••]. However, there is currently no consensus definition of LMS molecular subtypes as the results from the different studies are not always consistent [27]. That said, transcriptomic studies describing molecular subtypes in LMS consistently showed three molecular subtypes [20•, 25•, 26•, 28]. Although there are some discrepancies in the molecular and clinicopathological factors defining these LMS subtypes among different studies, there appear to be some molecular features, namely anatomical site distribution, immune cell composition and smooth muscle differentiation that are associated with the three molecular subtypes. The anatomical site of the disease seems to contribute to the molecular stratification of LMS. This is particularly apparent in uterine LMS as various studies have identified a distinct LMS molecular subtype enriched in uterine cases [21••, 26•, 28]. Additionally, transcriptomics and proteomics studies have consistently identified a subset of dedifferentiated LMS with significantly reduced smooth muscle differentiation markers and myogenic-related signalling [21••, 26•, 31••].

In addition to anatomical site and myogenic markers, studies have reported an LMS subtype that is enriched in immune-related signalling and immune cell infiltration [19•, 20•, 21••, 28]. This immune-enriched LMS molecular subtype has been described to have increased natural killer (NK) and mast cell infiltration [19•], higher macrophage infiltration [21••, 28] and T cells [28]. In other research using immune deconvolution methods on transcriptomic data, Petitprez et al. identified five sarcoma immune clusters (SIC A-E) in two independent cohorts of STS including LMS patients [32•]. The authors showed that although the majority of LMS patients have lower scores for immune signatures (SIC A and B), some LMS cases indeed displayed an "immune hot" phenotype and were grouped with the SIC E cluster characterised by highest immune scores. Further, a study investigating the

immune heterogeneity in LMS identified three different immune consensus clusters based on immune deconvolution of transcriptomics data [33••]. The study showed that about 15% of LMS samples form an immune hot subtype. The authors also reported that these LMS immune clusters are associated with SICs defined by Petitprez et al. as well as other immune features including higher CD8 + immune cell infiltration. However, many of the studies described above rely on single institutional series which are retrospective in nature and susceptible to selection bias. In order for molecular subtyping to have clinical utility moving forward, it is important that findings need to be validated in independent cohorts using similar data acquisition and analytical approaches.

Intra-tumour heterogeneity and clinical implications

Intra-tumour heterogeneity (ITH) is an important consideration in tumour progression and treatment response. ITH is a general term that reflects the genetic and phenotypic heterogeneity of cancer cell populations within the tumour as well as the different microenvironmental elements and their spatial and temporal distribution across the tumour over the course of its evolution [34]. Various studies on common cancers have reported an association between genomic and transcriptional ITH and inferior clinical outcomes [35–37]. However, the landscape of ITH and its clinical relevance is less well-characterised in STS including LMS. This section summarises the current evidence of ITH in LMS and its prognostic and therapeutic relevance. Anderson et al. undertook multi-regional sampling of LMS tumours and performed genomic and transcriptomic profiling to investigate evolutionary patterns in LMS [21••]. The authors used phylogenetic reconstruction of multiple regions taken from the same tumour and showed that later widespread chromosomal rearrangements and kataegis events resulted in distinct tumour subpopulations in tumour regions that are only a few centimetres apart. In addition, bulk sequencing of paired primary and metastatic regions showed early origins of metastasis and seeding of metastatic clones 10–30 years before diagnosis [21••], consistent with previous reports from more common cancer types [38, 39].

In addition to genetic heterogeneity of tumour cells, stromal components including distribution of tumour infiltrating immune cells also contribute to ITH. The immune cell infiltrate has been of particular interest in sarcomas as this is a potential predictive indicator for response to immunotherapeutic agents [40]. However, the spatial distribution of immune cells has been reported to display considerable heterogeneity. In a study investigating the use of tumour microarrays in LMS, Lee et al. showed heterogeneous distribution of tumour infiltrating lymphocytes across tissue microarrays from the same tumour sample in some LMS cases [41]. A study by Feng et al. using different immune deconvolution methods and multiple LMS tumour regions showed distinct immune signatures of samples taken from the same tumour. Some LMS patient samples displayed both “immune hot” and “immune cold” phenotype when multiple regions were profiled [33••]. Other studies using multiplex immunohistochemistry also showed similar findings for different

immune cell populations. For example, Manzoni et al. showed heterogeneity in spatial distribution of tumour infiltrating lymphocytes as well as myeloid cells including macrophages in uterine LMS cases [42]. However, more work is needed to further understand the landscape of ITH and evolutionary patterns in LMS as intra-tumour genetic diversification and heterogeneity in spatial distribution of immune cell populations have important clinical implications in terms of biopsy sampling and the implementation of personalised medicine.

Personalised medicine avenues in LMS

The use of a personalised treatment strategy can help improve clinical outcomes for cancer patients [43]. The application of patient stratification based on molecular subclassification has shown prognostic and predictive clinical value in more common cancer types such as lung and breast cancer [44]. Developing a similar personalised treatment paradigm for LMS is attractive, and over the past decade, several distinct biomarker-matched molecular vulnerabilities have been identified. Here, we discuss some of the promising therapeutic avenues available for LMS patients based on the different molecular features of the disease. Key response predictive biomarkers described in LMS are summarised in Table 1.

Tyrosine kinase inhibitors

Pazopanib is a multitarget tyrosine kinase inhibitor (TKI) and is thought to mediate its anticancer effect through the inhibition of a range of TKI involved in angiogenesis and oncogenic signalling [58]. Pazopanib has been approved by the Food and Drug Administration (FDA) for selected advanced STS patients including LMS following results from the multicentre phase III PALLETTE study which showed improved progression-free survival in advanced non-adipocytic STS patients treated with pazopanib compared to placebo control [9]. There was, however, no improvement in overall survival, and responses vary considerably among patients, indicating the need for a predictive biomarker to help select patients for pazopanib treatment. One example of such biomarker development was undertaken by Heilig et al. who defined a pazopanib efficacy predictor (PEP) score using genomics and transcriptomics profiling on tumour tissue samples prior to pazopanib treatment [45••]. The PEP score was developed based on the mRNA expression of three tyrosine kinase genes (*NTRK3*, *IGF1R* and *KDR*) which was significantly associated with progression-free survival in a training dataset ($n = 62$) as well as a validation cohort ($n = 43$). Furthermore, the score was not associated with clinical outcome in pazopanib-naïve comparison cohorts suggesting its predictive, rather than prognostic value. Other efforts investigating biomarkers of pazopanib response in STS have shown that mutations in *TP53*, *PD-L1* expression, *PDGFRA* expression, and *FGFR1* expression also significantly correlate with

Table 1. Putative predictive biomarkers in leiomyosarcoma

Biomarker	Therapy	Reference	Method	Predictive value
Pazopanib efficacy score (PEP)	Pazopanib	[45••]	NGS	Score using expression of *NTRK3*, *IGF1R* and *KDR*, independent predictor of PFS in training and validation datasets
TP53	Pazopanib	[46]	NGS	Mutant *TP53* associated with better PFS
PD-L1	Pazopanib, ICI	[47]	IHC	PD-L1 protein expression associated with improved ORR
PARP1	PARP inhibitors	[48•, 49•]	IHC, preclinical	Increased expression significantly associated with survival benefit in TOMAS phase 1b trial
BRCA1/2	PARP inhibitors	[50•, 51, 52•]	NGS	Mutant *BRCA1/2* genes associated with improved response to olaparib
HRD-related signatures	PARP inhibitors	[53, 54]	NGS	Alteration in HRD-related genes associated with improved response to olaparib
RAD51 foci	PARP inhibitors	[55]	RAD51 assay	Loss of RAD51 foci associated with prolonged median PFS
ATRX mutations	ATR inhibitors	[56]	Preclinical	*ATRX* mutations associated with increased sensitivity to ATR inhibitors
TLS	ICI	[32•, 57••]	Fluorescence multiplexed IHC	Presence of TLS associated with improved response to ICIs

HRD homologous recombination deficiency, *ICI* immune checkpoint inhibitors, *IHC* immunohistochemistry, *NGS* next-generation sequencing, *PFS* progression-free survival, *TLS* tertiary lymphoid structures

progression-free survival outcome in pazopanib-treated patients [46, 47, 59, 60]. However, many of these findings are limited to small cohort sizes and often lack external validation.

Targeting homologous recombination deficiency in LMS

Genomics and transcriptomics studies using LMS patient cohorts have demonstrated frequent alterations in crucial components of homologous recombination repair (HRR) of DNA double-strand breaks. These include deleterious single base substitutions or genomic alterations affecting key genes in this pathway such as *BRCA1*, *BRCA2*, *RAD51*, *ATM*, *CHECK1*, *CHECK2*, *XRCC1*, *XRCC3*, *PTEN* and *FANCA1* and *FANCA2* [17, 20•, 21••, 61], suggesting homologous recombination deficiency (HRD) and a "BRCAness" phenotype. HRD is associated with increased sensitivity to DNA double-strand break-inducing agents such as poly (ADP-ribose) polymerase (PARP) inhibitors [62]. Olaparib, an FDA-approved PARP inhibitor for some ovarian, breast, pancreatic and prostate cancers, has been assessed in various clinical studies including LMS patients, particularly in the uterine LMS setting [50•, 51, 52•, 63]. Recent clinical trials have utilised the combination of olaparib together with other chemotherapeutic agents [48•, 55, 64] to further impair the ability of cancer cells to repair DNA damage and ultimately lead to apoptosis [65]. Ingham et al. assessed the combination of olaparib and temozolomide in a phase 2 clinical trial comprised of 22 advanced uterine LMS patients (NCT03880019). The study showed an overall objective response rate of 27% [55]. However, the authors reported considerable myelosuppression leading to dose reduction and toxicity. On the other hand, a phase 1b clinical trial (TOMAS) investigated the combination of olaparib with trabectedin, which binds to minor groove of DNA causing single- and double-strand breaks [48•]. The trial included 55 bone and soft tissue sarcoma patients and showed that the combination was safe and tolerable. The phase 2 multicentre TOMAS2 study compared the combination of olaparib and trabectedin to single agent trabectedin (NCT03838744) [64] and reported potential benefit of the combination treatment with 20% of patients in the combination arm showing durable response for over a year.

Due to heterogeneity in responses to PARP inhibition, there is a need to identify response-predictive biomarkers to enhance patient stratification. Increased PARP1 basal expression was associated with improved response to PARP inhibition in the TOMAS trial [48•]. In a preclinical study that preceded TOMAS, a synergistic effect of combining trabectedin and olaparib in sarcoma cell lines and mouse models was associated with PARP1 basal expression. Further evaluation using gene silencing and overexpression experiments confirmed a functional relevance of PARP1 expression in predicting treatment response [49•]. Additionally, prolonged clinical responses to olaparib have been reported in advanced LMS patients with *BRCA1/2* mutations [50•, 51, 52•]. However, the correlation between *BRCA1/2* mutational status and response is unclear as some studies reported no correlation with outcome [48•]. Other putative biomarkers to predict response to PARP

inhibition include RAD51. In the clinical trial conducted by Ingham et al. (NCT03880019) discussed above, the absence of RAD51 foci in patient samples was assessed as a biomarker for HRD. Patients with absent RAD51 foci had prolonged median progression-free survival compared to homologous recombination proficient patients [55]. Other studies assessed the use of HRD scores and polygenic mutational signatures as a putative predictive biomarker for PARP inhibitor response [53, 54]. However, it should be noted that all these biomarkers need to be further validated in independent cohorts and assessed prospectively to better understand their predictive relevance.

Alternative lengthening of telomeres (ALT)

Alternative lengthening of telomeres (ALT) is a mechanism utilised by ~ 10% of cancers to maintain telomere length and therefore achieve replicative immortality in a telomerase-independent fashion [66]. Using different methods to assess ALT status, various studies have reported a high proportion of ALT-positive LMS tumours [20•, 67–69]. In a study by Chudasama et al., DNA C-circles, an ALT-specific biomarker, were detected in 78% of LMS patients ($n = 49$) [20•]. ALT is commonly associated with *ATRX* alterations which have been associated with shorter progression-free survival in uterine LMS patients [61]. However, the high frequency of ALT in LMS cannot be explained by *ATRX* alterations alone as some ALT-positive tumours—assessed by DNA C-circles—did not have *ATRX* alterations, and alterations of other telomere maintenance genes including *RBL2* and *SP100* showed significant association with positive ALT status [20•]. Targeting the molecular players utilised in ALT may be a promising therapeutic strategy in LMS. For example, targeting the ATR kinase is thought to lead to synthetic lethality by inducing DNA double-strand breaks at telomeres. Cells with *ATRX* mutations demonstrated increased sensitivity to ATR inhibition [56]. A study using a panel of soft tissue sarcoma cell lines including 3 LMS cell lines showed that targeting ATR with the ATR inhibitor VE-822 resulted in a synergistic effect when combined with gemcitabine [70]. However, this effect was shown to be ALT independent, and thus, more studies and future clinical trials are needed to better evaluate the therapeutic vulnerabilities of ALT-positive tumours.

Targeting the LMS immune microenvironment

Results from clinical trials and retrospective cohort studies assessing the use of immune checkpoint inhibitors (ICIs) in LMS have thus far been disappointing, with no to modest efficacy observed in patients. This remains true when using multiple ICIs or ICIs in combination with chemotherapy or targeted therapies (immunotherapy trials in LMS reviewed in ref [71]). Recent studies exploring the immune biology of LMS have identified certain immune features that may help select patients for immunotherapeutic strategies [32•, 33••, 57••]. For example, in the study by Petitprez et al. discussed

earlier, the authors evaluated specimens from the SARC028 clinical trial and demonstrated that cases with a SIC E signature had an improved objective response rate (ORR) to pembrolizumab compared to patients with the other SIC signatures [32•]. In addition, using fluorescent multiplexed immunohistochemistry, the same study showed enrichment in tertiary lymphoid structures (TLS) in SIC E patients as 82% of these patients were identified to have one or more TLS. Although the LMS cases assessed in this study cohort were very limited ($n = 6$) and none were SIC E tumours, the study nonetheless demonstrates the potential utility of TLS as an immune biomarker of SIC status and response to pembrolizumab in STS. These results are also consistent with analysis from the PEMBROSARC trial which showed the presence of TLS as a potential predictor of response to pembrolizumab [57••]. The study reported a 6-month non-progression rate of 40% in TLS-positive patients ($n = 30$ including 4 LMS cases), compared to 4.9% in TLS-negative patients in the TLS-unselected cohort ($n = 41$ including 13 LMS cases).

Programmed death-ligand 1 (PD-L1) is an immune checkpoint molecule expressed on a range of normal, tumour and immune cells. PD-L1 expression has been associated with poor prognosis in STS including LMS [72], but its role as a response predictive biomarker to anti-PD-1 and anti-PD-L1 ICI in LMS is unclear. PD-L1 expression has been shown to be associated with response to pembrolizumab in some STS histological subtypes [73], but the predictive value of PD-L1 has not been robustly evaluated in STS, and more research in this area is required.

Novel immune targets: targeting the macrophages

Most clinical trials assessing the efficacy of immunotherapy in STS have focused on targeting lymphocyte-based immune checkpoint inhibitors, mainly targeting programmed cell death 1 (PD-1) or its ligand (PD-L1) and cytotoxic T-lymphocyte associated protein 4 (CTLA-4) molecules. On the other hand, studies have shown increased macrophage infiltration, particularly CD163 + macrophages in the immune microenvironment of STS and in particular LMS [74, 75]. Consistent with the immunosuppressive nature of CD163 + macrophages, some reports have shown an association between increased CD163 + infiltration in LMS and worse prognosis [74] which is in line with studies in other cancers [76, 77]. Thus, macrophage-directed therapeutics may form a promising strategy in the treatment of LMS. Targeting the immune checkpoint protein CD47 and its receptor SIRPa has shown exciting results in a range of solid and haematological malignancies [78]. In LMS cell lines co-cultured with peripheral blood mononuclear cells (PBMC)-derived macrophages, treatment with anti-CD47 monoclonal antibodies resulted in increased phagocytic capacity [79]. Results from a phase 1/2 clinical trial investigating the combination of doxorubicin together with the recombinant protein TTI-621 which acts as a decoy receptor for SIRPa are being evaluated in metastatic and high-grade LMS (NCT04996004) [80].

In addition to CD47, recent studies investigated more novel macrophage-directed therapeutic strategies including the CD40/CD40L as

well as CSF1/CSF1R axes. CD40 is a surface molecule expressed by macrophages, and its activation leads to enhanced antigen presentation and indirect activation of T cells. A phase II clinical trial assessing the safety and efficacy of targeting CD40 with the CD40 agonist APX005M in advanced STS patients including LMS, is under evaluation (NCT03719430) [81]. CSF1R is predominantly expressed by monocytes including macrophages, and macrophages with active CSF1R-mediated signalling are associated with pro-tumoural phenotype. In other cancer types, the inhibition of this pathway through targeting CSF1R increases macrophage polarisation towards proinflammatory phenotype and therefore increases antitumour activity [82]. CSF1R inhibition led to reprogramming of tumour-associated macrophages and boosted antitumour T cell responses in cancers with high macrophage infiltration in pancreatic cancer models [83]. An ongoing phase 1b trial is assessing the safety and efficacy of the combination of CSF1R inhibition and PD-1 inhibition in high-grade sarcomas including 7 LMS cases (NCT04242238) [84]. Results from these trials combined with more in-depth study of the immune landscape of LMS will provide exciting future therapeutic opportunities.

Future perspectives and role of international collaborations

The implementation of personalised medicine strategies in LMS is still in its infancy. More work is needed to evaluate predictive biomarkers prospectively in clinical trials and to understand their functional and mechanistic role in preclinical studies. Due to the rare and heterogeneous nature of LMS, national and international collaborations are needed to address key biological and clinical questions. These include efforts to achieve consensus molecular definitions of LMS molecular subtypes and establish molecular biomarkers of therapeutic relevance [27]. International consortia such as the LMS SPORE (https://www.rogelcancercenter.org/leiomyosarcoma-spore) and the Sarcoma Accelerator Consortium (https://sarcomaaccelerator.org.uk/) can help to facilitate curation and sharing of clinical and molecular datasets as well as to develop new research studies using cutting edge methods such as single-cell sequencing, spatial profiling and liquid biopsies. Working together with patient-partnered initiatives in LMS such as the Leiomyosarcoma Project (https://lmsproject.org/) and the Count Me In initiative provides an added opportunity to address patient-led research priorities which will ultimately advance our biological understanding of this rare and aggressive disease.

Declarations

Author Contributions

Conceptualization: SA and PHH; writing—original draft: SA, PHH; writing—review and editing: all authors; supervision: PHH; funding acquisition: RLJ and PHH.

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Compliance with Ethical Standards

Conflict of interest
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Human and Animal Rights and Informed Consent
This article does not contain any studies with human or animal subjects performed by any of the authors.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1. Kasper B, Achee A, Schuster K, Wilson R, Van Oortmerssen G, Gladdy RA, et al. Unmet medical needs and future perspectives for leiomyosarcoma patients—a position paper from the National LeioMyoSarcoma Foundation (NLMSF) and Sarcoma Patients EuroNet (SPAEN). Cancers. 2021;13:886.
2. Roland CL, Boland GM, Demicco EG, Lusby K, Ingram D, May CD, et al. Clinical observations and molecular variables of primary vascular leiomyosarcoma. JAMA Surg. 2016;151:347.
3. Demicco EG, Boland GM, Brewer Savannah KJ, Lusby K, Young ED, Ingram D, et al. Progressive loss of myogenic differentiation in leiomyosarcoma has prognostic value. Histopathology. 2015;66:627–38.
4. Chen E, O'Connell F, Fletcher CDM. Dedifferentiated leiomyosarcoma: clinicopathological analysis of 18 cases: dedifferentiated leiomyosarcoma. Histopathology. 2011;59:1135–43.
5. Thway K, Fisher C. Undifferentiated and dedifferentiated soft tissue neoplasms: immunohistochemical surrogates for differential diagnosis. Semin Diagn Pathol. 2021;38:170–86.
6. Gladdy RA, Qin L-X, Moraco N, Agaram NP, Brennan MF, Singer S. Predictors of survival and recurrence in primary leiomyosarcoma. Ann Surg Oncol. 2013;20:1851–7.
7. Korets SB, Curtin JP. Surgical options for recurrent uterine sarcomas. Am Soc Clin Oncol Educ Book. 2012;32:362–6.
8. Casali PG, Abecassis N, Bauer S, Biagini R, Bielack S, Bonvalot S, et al. Soft tissue and visceral sarcomas: ESMO–EURACAN Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2018;29:iv51-67.
9. Van Der Graaf WT, Blay J-Y, Chawla SP, Kim D-W, 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. 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.
11. Pautier P, Italiano A, Piperno-Neumann S, Chevreau C, Penel N, Firmin N, et al. Doxorubicin alone versus doxorubicin with trabectedin followed by trabectedin alone as first-line therapy for metastatic or unresectable leiomyosarcoma (LMS-04): a randomised, multicentre, open-label phase 3 trial. Lancet Oncol. 2022;23:1044–54.
12. Wang Z, Shi N, Naing A, Janku F, Subbiah V, Araujo DM, et al. Survival of patients with metastatic leiomyosarcoma: the MD Anderson Clinical Center for targeted therapy experience. Cancer Med. 2016;5:3437–44.
13. Svarvar C, Larramendy ML, Blomqvist C, Gentile M, Koivisto-Korander R, Leminen A, et al. Do DNA copy number changes differentiate uterine from non-uterine leiomyosarcomas and predict metastasis? Mod Pathol. 2006;19:1068–82.
14. Larramendy ML, Gentile M, Soloneski S, Knuutila S, Böhling T. Does comparative genomic hybridization reveal distinct differences in DNA copy number sequence patterns between leiomyosarcoma and malignant fibrous histiocytoma? Cancer Genet Cytogenet. 2008;187:1–11.
15. Schaefer I, Lundberg MZ, Demicco EG, Przybyl J, Matusiak M, Chibon F, et al. Relationships between highly recurrent tumor suppressor alterations in 489 leiomyosarcomas. Cancer. 2021;127:2666–73.
16.•• Nacev BA, Sanchez-Vega F, Smith SA, Antonescu CR, Rosenbaum E, Shi H, et al. Clinical sequencing of soft tissue and bone sarcomas delineates diverse genomic landscapes and potential therapeutic targets. Nat Commun. 2022;13:3405.
Next generation sequencing of 2138 sarcoma samples including 165 uterine LMS and 125 non-uterine LMS. Findings highlighted key genetic alterations commonly found in LMS.
17. Choi J, Manzano A, Dong W, Bellone S, Bonazzoli E, Zammataro L, et al. Integrated mutational landscape analysis of uterine leiomyosarcomas. Proc Natl Acad Sci U S A. 2021;118: e2025182118.
18. Liu W, Tong H, Zhang C, Zhuang R, Guo H, Lv C, et al. Integrated genomic and transcriptomic analysis revealed mutation patterns of de-differentiated liposarcoma and leiomyosarcoma. BMC Cancer. 2020;20:1035.
19.• Abeshouse A, Adebamowo C, Adebamowo SN, Akbani R, Akeredolu T, Ally A, et al. Comprehensive and integrated genomic characterization of adult soft tissue sarcomas. Cell. 2017;171:950-965.e28.
Integrated genomic and transcriptomic analysis from the Cancer Genome Atlas research network, showing molecular differences between uterine and non-uterine LMS.
20.• Chudasama P, Mughal SS, Sanders MA, Hübschmann D, Chung I, Deeg KI, et al. Integrative genomic and transcriptomic analysis of leiomyosarcoma. Nat Commun. 2018;9:144.
Genomic and transcriptomic landscape of LMS, highlighting its molecular landscape, features of BRCAness and high frequency of ALT in LMS.
21.•• Anderson ND, Babichev Y, Fuligni F, Comitani F, Layeghifard M, Venier RE, et al. Lineage-defined leiomyosarcoma subtypes emerge years before diagnosis and determine patient survival. Nat Commun. 2021;12:4496.
Genomic and transcriptomic profiling of LMS, identifying molecular subtypes. The authors also use phylogenetic reconstruction of paired primary and recurrence samples to show some LMS evolutionary patterns including the early seeding of metastasis in LMS.
22. Lee PJ, Yoo NS, Hagemann IS, Pfeifer JD, Cottrell CE, Abel HJ, et al. Spectrum of mutations in leiomyosarcomas identified by clinical targeted next-generation sequencing. Exp Mol Pathol. 2017;102:156–61.
23. Yang C-Y, Liau J-Y, Huang W-J, Chang Y-T, Chang M-C, Lee J-C, et al. Targeted next-generation sequencing of cancer genes identified frequent TP53 and ATRX mutations in leiomyosarcoma. Am J Transl Res. 2015;7:2072–81.
24. Sha D, Jin Z, Budczies J, Kluck K, Stenzinger A, Sinicrope FA. Tumor mutational burden as a predictive biomarker in solid tumors. Cancer Discov. 2020;10:1808–25.
25.• Beck AH, Lee C-H, Witten DM, Gleason BC, Edris B, Espinosa I, et al. Discovery of molecular subtypes in leiomyosarcoma through integrative molecular profiling. Oncogene. 2010;29:845–54.
Transcriptomic profiling of 51 LMS samples identifying three distinct molecular subtypes in LMS.
26.• Guo X, Jo VY, Mills AM, Zhu SX, Lee C-H, Espinosa I, et al. Clinically relevant molecular subtypes in leiomyosarcoma. Clin Cancer Res. 2015;21:3501–11.
Transcriptomic analysis of two independent cohorts shows three molecular subtypes in LMS.
27. Burns J, Jones RL, Huang PH. Molecular subtypes of leiomyosarcoma: moving toward a consensus. Clin Transl Discov. 2022;2: e149.
28. Hemming ML, Fan C, Raut CP, Demetri GD, Armstrong SA, Sicinska E, et al. Oncogenic gene-expression programs in leiomyosarcoma and characterization of conventional, inflammatory, and uterogenic subtypes. Mol Cancer Res. 2020;18:1302–14.
29. Italiano A, Lagarde P, Brulard C, Terrier P, Laë M, Marques B, et al. Genetic profiling identifies

two classes of soft-tissue leiomyosarcomas with distinct clinical characteristics. Clin Cancer Res. 2013;19:1190–6.
30. Lee Y-F, Roe T, Mangham DC, Fisher C, Grimer RJ, Judson I. Gene expression profiling identifies distinct molecular subgroups of leiomyosarcoma with clinical relevance. Br J Cancer. 2016;115:1000–7.
31.•• Burns J, Wilding CP, Krasny L, Zhu X, Chadha M, Tam YB, et al. The proteomic landscape of soft tissue sarcomas. Nat Commun. 2023;14:3834.
Mass spectrometry-based proteomics analysis of 80 LMS samples identifying three molecular subtypes.
32.• Petitprez F, De Reyniès A, Keung EZ, Chen TW-W, Sun C-M, Calderaro J, et al. B cells are associated with survival and immunotherapy response in sarcoma. Nature. 2020;577:556–60.
Authors describe five Sarcoma Immune Clusters (SIC A-E) and potential use in response prediction to the immune checkpoint inhibitor pembrolizumab.
33.•• Feng X, Tonon L, Li H, Darbo E, Pleasance E, Macagno N, et al. Comprehensive immune profiling unveils a subset of leiomyosarcoma with "hot" tumor immune microenvironment. Cancers. 2023;15:3705.
Immune profiling using immune deconvolution of gene expression data to show LMS subtypes with distinct immune features including an immune "hot" LMS subtype.
34. Ramón Y, Cajal S, Sesé M, Capdevila C, Aasen T, De Mattos-Arruda L, Diaz-Cano SJ, et al. Clinical implications of intratumor heterogeneity: challenges and opportunities. J Mol Med. 2020;98:161–77.
35. Schmelz K, Toedling J, Huska M, Cwikla MC, Kruetzfeldt L-M, Proba J, et al. Spatial and temporal intratumour heterogeneity has potential consequences for single biopsy-based neuroblastoma treatment decisions. Nat Commun. 2021;12:6804.
36. Martínez-Ruiz C, Black JRM, Puttick C, Hill MS, Demeulemeester J, Larose Cadieux E, et al. Genomic–transcriptomic evolution in lung cancer and metastasis. Nature. 2023;616:543–52.
37. Frankell AM, Dietzen M, Al Bakir M, Lim EL, Karasaki T, Ward S, et al. The evolution of lung cancer and impact of subclonal selection in TRACERx. Nature. 2023;616:525–33.
38. Al Bakir M, Huebner A, Martínez-Ruiz C, Grigoriadis K, Watkins TBK, Pich O, et al. The evolution of non-small cell lung cancer metastases in TRACERx. Nature. 2023;616:534–42.
39. Hu Z, Ding J, Ma Z, Sun R, Seoane JA, Scott Shaffer J, et al. Quantitative evidence for early metastatic seeding in colorectal cancer. Nat Genet. 2019;51:1113–22.
40. Bruni D, Angell HK, Galon J. The immune contexture and immunoscore in cancer prognosis and therapeutic efficacy. Nat Rev Cancer. 2020;20:662–80.
41. Lee ATJ, Chew W, Wilding CP, Guljar N, Smith MJ, Strauss DC, et al. The adequacy of tissue microarrays in the assessment of inter- and intra-tumoural heterogeneity of infiltrating lymphocyte burden in leiomyosarcoma. Sci Rep. 2019;9:14602.
42. Manzoni M, Bolognesi MM, Antoranz A, Mancari R, Carinelli S, Faretta M, et al. The adaptive and innate immune cell landscape of uterine leiomyosarcomas. Sci Rep. 2020;10:702.
43. Schwaederle M, Zhao M, Lee JJ, Eggermont AM, Schilsky RL, Mendelsohn J, et al. Impact of precision medicine in diverse cancers: a meta-analysis of phase II clinical trials. J Clin Oncol. 2015;33:3817–25.
44. Vuong D, Simpson PT, Green B, Cummings MC, Lakhani SR. Molecular classification of breast cancer. Virchows Arch. 2014;465:1–14.
45.•• Heilig CE, Laßmann A, Mughal SS, Mock A, Pirmann S, Teleanu V, et al. Gene expression-based prediction of pazopanib efficacy in sarcoma. Eur J Cancer. 2022;172:107–18.
Authors describe Pazopanib efficacy Predictor (PEP) score based on gene expression of *NTRK3*, *IGF1R* and *KDR* which independently associates with pazopanib response.
46. Koehler K, Liebner D, Chen JL. TP53 mutational status is predictive of pazopanib response in advanced sarcomas. Ann Oncol. 2016;27:539–43.
47. Kim SK, Kim JH, Kim SH, Lee YH, Han JW, Baek W, et al. PD-L1 tumour expression is predictive of pazopanib response in soft tissue sarcoma. BMC Cancer. 2021;21:336.
48.• Grignani G, D'Ambrosio L, Pignochino Y, Palmerini E, Zucchetti M, Boccone P, et al. Trabectedin and olaparib in patients with advanced and non-resectable bone and soft-tissue sarcomas (TOMAS): an open-label, phase 1b study from the Italian Sarcoma Group. Lancet Oncol. 2018;19:1360–71.
Phase 1b clinical trial showing safety and promising clinical activity of trabectedin and olaparib in advanced STS patients.
49.• Pignochino Y, Capozzi F, D'Ambrosio L, Dell'Aglio C, Basiricò M, Canta M, et al. PARP1 expression drives the synergistic antitumor activity of trabectedin and PARP1 inhibitors in sarcoma preclinical models. Mol Cancer. 2017;16:86.
Preclinical evidence showing relationship between PARP1 expression and response and olaparib and trabectedin synergy.
50.• Shammas N, Yang T, Abidi A, Amneus M, Hodeib M. Clinical use of PARP inhibitor in recurrent uterine leiomyosarcoma with presence of a somatic BRCA2 mutation. Gynecol Oncol Rep. 2022;42: 101044.
Case report showing prolonged response to olaparib in a patient harbouring *BRCA2* mutation.
51. Hensley ML, Chavan SS, Solit DB, Murali R, Soslow R, Chiang S, et al. Genomic landscape of uterine sarcomas defined through prospective clinical sequencing. Clin Cancer Res. 2020;26:3881–8.

52.• 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.
Authors show clinical benefit of olaparib in LMS patients with *BRCA1/2* mutations.
53. Merlini A, Centomo ML, Ferrero G, Chiabotto G, Miglio U, Berrino E, et al. DNA damage response and repair genes in advanced bone and soft tissue sarcomas: an 8-gene signature as a candidate predictive biomarker of response to trabectedin and olaparib combination. Front Oncol. 2022;12:844250.
54. Rosenbaum E, Jonsson P, Seier K, Qin LX, Chi P, Dickson M, et al. Clinical outcome of leiomyosarcomas with somatic alteration in homologous recombination pathway genes. JCO Precis Oncol. 2020;4:PO.20.00122. https://doi.org/10.1200/PO.20.00122.
55. Ingham M, Allred JB, Chen L, Das B, Kochupurakkal B, Gano K, et al. Phase II study of olaparib and temozolomide for advanced uterine leiomyosarcoma (NCI Protocol 10250). J Clin Oncol. 2023;41:4154–63.
56. Hu Y, Shi G, Zhang L, Li F, Jiang Y, Jiang S, et al. Switch telomerase to ALT mechanism by inducing telomeric DNA damages and dysfunction of ATRX and DAXX. Sci Rep. 2016;6:32280.
57.•• Italiano A, Bessede A, Pulido M, Bompas E, Piperno-Neumann S, Chevreau C, et al. Pembrolizumab in soft-tissue sarcomas with tertiary lymphoid structures: a phase 2 PEMBROSARC trial cohort. Nat Med. 2022;28:1199–206.
Analysis of samples from the PEMBROSARC trial demonstrating that tertiary lymphoid structures have potential predictive value for response to pembrolizumab.
58. Lee ATJ, Jones RL, Huang PH. Pazopanib in advanced soft tissue sarcomas. Signal Transduct Target Ther. 2019;4:16.
59. Huang P, Lee A, McCarthy F, Thway K, Morden J, Messiou C, et al. A molecular signature predictive of clinical outcome following pazopanib therapy in advanced soft tissue sarcoma. Ann Oncol. 2017;28: x149.
60. Sai S, Imamura Y, Kiyota N, Jimbo N, Toyoda M, Funakoshi Y, et al. Relationship between PDGFR expression and the response to pazopanib in intimal sarcoma of the pulmonary artery: a case report. Mol Clin Oncol. 2020;14:1–1.
61. Wozniak A, Boeckx B, Modave E, Weaver A, Lambrechts D, Littlefield BA, et al. Molecular biomarkers of response to eribulin in patients with leiomyosarcoma. Clin Cancer Res. 2021;27:3106–15.
62. Lord CJ, Ashworth A. BRCAness revisited. Nat Rev Cancer. 2016;16:110–20.
63. Dall G, Vandenberg CJ, Nesic K, Ratnayake G, Zhu W, Vissers JHA, et al. Targeting homologous recombination deficiency in uterine leiomyosarcoma. J Exp Clin Cancer Res. 2023;42:112.
64. D'Ambrosio L, Merlini A, Brunello A, Ferraresi V, Paioli A, Vincenzi B, et al. LBA91 TOMAS2: A randomized phase II study from the Italian Sarcoma Group (ISG) of trabectedin plus olaparib (T+O) or trabectedin (T) in advanced, metastatic, or unresectable soft tissue sarcomas (STS) after failure of standard treatments. Ann Oncol. 2023;34:S1332.
65. Lord CJ, Ashworth A. PARP inhibitors: synthetic lethality in the clinic. Science. 2017;355:1152–8.
66. Dilley RL, Greenberg RA. ALTernative telomere maintenance and cancer. Trends Cancer. 2015;1:145–56.
67. Henson JD, Hannay JA, McCarthy SW, Royds JA, Yeager TR, Robinson RA, et al. A robust assay for alternative lengthening of telomeres in tumors shows the significance of alternative lengthening of telomeres in sarcomas and astrocytomas. Clin Cancer Res. 2005;11:217–25.
68. Liau J-Y, Tsai J-H, Jeng Y-M, Lee J-C, Hsu H-H, Yang C-Y. Leiomyosarcoma with alternative lengthening of telomeres is associated with aggressive histologic features, loss of ATRX expression, and poor clinical outcome. Am J Surg Pathol. 2015;39:236–44.
69. Sharaf R, Jin DX, Grady J, Napier C, Ebot E, Frampton GM, et al. A pan-sarcoma landscape of telomeric content shows that alterations in RAD51B and GID4 are associated with higher telomeric content. npj Genom Med. 2023;8:26.
70. Laroche-Clary A, Chaire V, Verbeke S, Algéo M-P, Malykh A, Le Loarer F, et al. ATR inhibition broadly sensitizes soft-tissue sarcoma cells to chemotherapy independent of alternative lengthening telomere (ALT) status. Sci Rep. 2020;10:7488.
71. Cope BM, Traweek RS, Lazcano R, Keung EZ, Lazar AJ, Roland CL, et al. Targeting the molecular and immunologic features of leiomyosarcoma. Cancers. 2023;15:2099.
72. Bertucci F, Finetti P, Perrot D, Leroux A, Collin F, Le Cesne A, et al. PDL1 expression is a poor-prognosis factor in soft-tissue sarcomas. Oncoimmunology. 2017;6: e1278100.
73. Keung EZ, Burgess M, Salazar R, Parra ER, Rodrigues-Canales J, Bolejack V, et al. Correlative analyses of the SARC028 trial reveal an association between sarcoma-associated immune infiltrate and response to pembrolizumab. Clin Cancer Res. 2020;26:1258–66.
74. Kostine M, Briaire-de Bruijn IH, Cleven AHG, Vervat C, Corver WE, Schilham MW, et al. Increased infiltration of M2-macrophages, T-cells and PD-L1 expression in high grade leiomyosarcomas supports immunotherapeutic strategies. OncoImmunology. 2018;7: e1386828.
75. Dancsok AR, Gao D, Lee AF, Steigen SE, Blay J-Y, Thomas DM, et al. Tumor-associated macrophages and macrophage-related immune checkpoint expression in sarcomas. OncoImmunology. 2020;9:1747340.

76. Krijgsman D, De Vries NL, Andersen MN, Skovbo A, Tollenaar RAEM, Møller HJ, et al. CD163 as a biomarker in colorectal cancer: the expression on circulating monocytes and tumor-associated macrophages, and the soluble form in the blood. Int J Mol Sci. 2020;21:5925.
77. Shabo I, Stål O, Olsson H, Doré S, Svanvik J. Breast cancer expression of CD163, a macrophage scavenger receptor, is related to early distant recurrence and reduced patient survival. Intl J Cancer. 2008;123:780–6.
78. Jiang Z, Sun H, Yu J, Tian W, Song Y. Targeting CD47 for cancer immunotherapy. J Hematol Oncol. 2021;14:180.
79. Edris B, Weiskopf K, Volkmer AK, Volkmer J-P, Willingham SB, Contreras-Trujillo H, et al. Antibody therapy targeting the CD47 protein is effective in a model of aggressive metastatic leiomyosarcoma. Proc Natl Acad Sci U S A. 2012;109:6656–61.
80. Chawla SP, Kelly CM, Gordon EM, Quon DV, Moradkhani A, Chua-Alcala VS, et al. TTI-621–03: a phase I/II study of TTI-621 in combination with doxorubicin in patients with unresectable or metastatic high-grade leiomyosarcoma (LMS). J Clin Oncol. 2022;40:TPS11593–TPS11593.
81. Ingham M, Lee SM, Doshi S, Hernandez S, Singh-Kandah SV, Singer Z, et al. A phase II trial with safety lead-in to evaluate the addition of APX005M, a CD40 agonistic monoclonal antibody, to standard-of-care doxorubicin chemotherapy for the treatment of advanced soft tissue sarcoma. J Clin Oncol. 2020;38:TPS85–TPS85.
82. Wen J, Wang S, Guo R, Liu D. CSF1R inhibitors are emerging immunotherapeutic drugs for cancer treatment. Eur J Med Chem. 2023;245: 114884.
83. Zhu Y, Knolhoff BL, Meyer MA, Nywening TM, West BL, Luo J, et al. CSF1/CSF1R blockade reprograms tumor-infiltrating macrophages and improves response to T-cell checkpoint immunotherapy in pancreatic cancer models. Cancer Res. 2014;74:5057–69.
84. Rosenbaum E, Movva S, Kelly CM, Dickson MA, Keohan ML, Gounder MM, et al. A phase 1b study of avelumab plus DCC-3014, a potent and selective inhibitor of colony stimulating factor 1 receptor (CSF1R), in patients with advanced high-grade sarcoma. J Clin Oncol. 2021;39:11549–11549.

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