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Review Article

Molecular carcinogenesis and genetic insights in leiomyosarcoma: involvement of PI3K/AKT/mTOR/MAPK/ERK pathway

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Abstract: One of the most common soft-tissue sarcomas is LMS, a highly aggressive mesenchymal malignancy. LMS is characterized by significant molecular and clinical heterogeneity, arising from diverse anatomical sites and exhibiting distinct genomic alterations across subtypes. Among the key oncogenic drivers, the PI3K/AKT/mTOR signaling pathway plays a central role in regulating cell growth, proliferation, and survival. Dysregulation of this pathway, often through PTEN loss or upstream receptor activation, has been increasingly implicated in the pathogenesis of LMS, making it a critical therapeutic target. The MAPK pathway is among other intracellular signaling pathways and is significant in processes such as cell proliferation, differentiation, apoptosis, angiogenesis, and tumor metastasis. The important MAPK cascades found in eukaryotic cells include ERK, JNK/stress-activated protein kinase, p38 MAPK, and ERK5 signaling pathways. As these pathways are critical, they are promising areas for cancer therapy. In short, attention is on translating PI3K/AKT inhibitors into clinical practice to provide patients with new targeted therapy. This review summarizes the molecular mechanisms underlying LMS pathogenesis and discusses emerging therapeutic strategies to improve clinical outcomes.

Keywords: LMS, PI3K/AKT/mTOR pathway, MAPK/ERK pathway, targeted cancer therapy, tumorigenesis, carcinogenesis

Introduction

Leiomyosarcoma (LMS) is a typical example of a soft tissue sarcoma (STS), which accounts for 10-20 percent of all sarcomas. It develops from smooth muscle cells or their mesenchymal progenitors and most frequently occurs in the retroperitoneum, uterus, and extremities, in that order of prevalence. LMS genetics is a complex field, and there is much to learn about the underlying molecular drivers of the disease [1]. LMS may occur anywhere in the body, which is why its symptoms, signs, and clinical presentation can vary. The factors that determine the prognosis include the size, location, and grade of the tumor, among them being tumor grade, which plays a major role in determining the survival outcomes. Increased grade of tumors likely predetermines a greater probability of distal metastasis, which makes prognosis more difficult [2]. LMS is a highly individualized disease with a high likelihood of multidisciplinary management. Surgery is generally used as a tool of treatment for resectable tumors, with the application of chemotherapy and radiation treatment in particular instances. High-volume sarcoma centers have been shown to provide better patient outcomes [3, 4].

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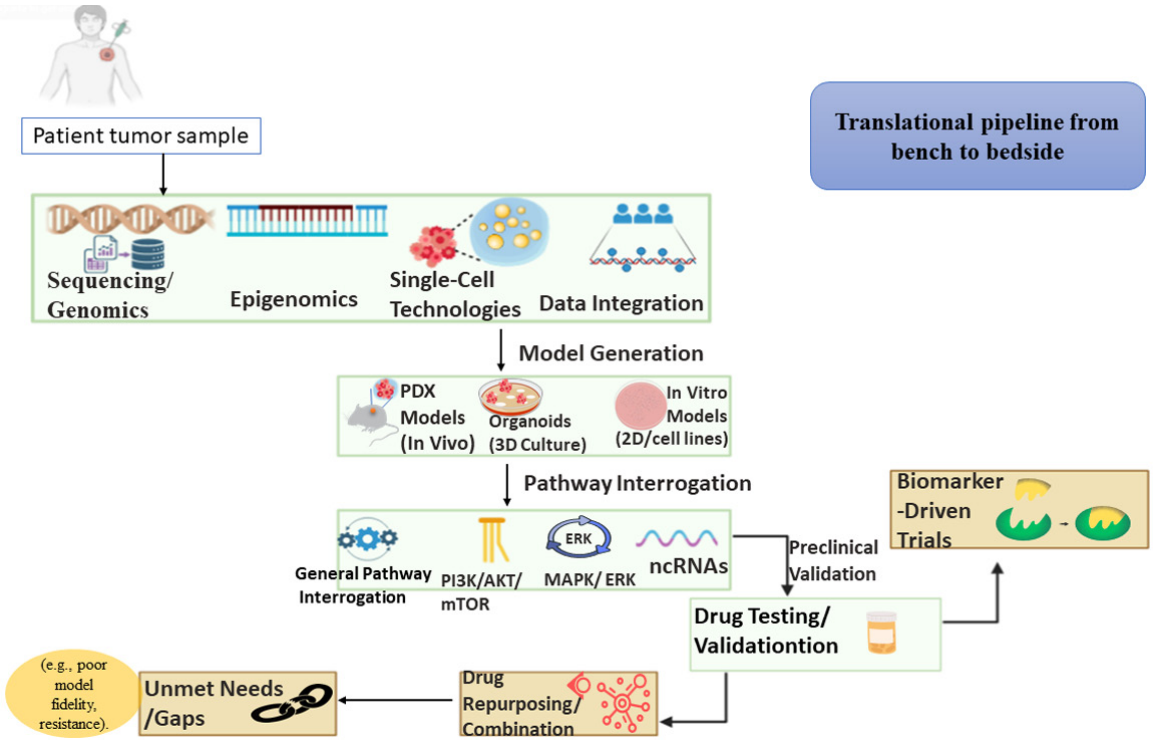

Figure 1. Integrated workflow for pathway-driven cancer modeling and therapeutic validation.

LMS is a malignant tumor arising from smooth muscle cells. These cells are found in various organs, contributing to the disease's diverse clinical presentation. There are two types of muscles in the body: voluntary and involuntary. Smooth muscles are also considered involuntary and therefore do not require conscious control by the brain. These muscles act on the automatic level in response to several stimuli. An example is the smooth muscle in the walls of the digestive tract, which forms wave-like contractions (peristalsis) that assist in digestion and the movement of food. Similarly, smooth muscle in the salivary glands stimulates the release of saliva whenever one eats, and at the same time, the goose pimples are caused by the same smooth muscle in the skin due to cold temperatures [5]. LMS is a form of cancer, a disease that involves unregulated cell proliferation, which infiltrates surrounding tissues and may even spread (metastasize) to other areas of the body by blood, lymphatic system, or other routes. Certain forms of cancer, such as LMS, are classified by the type of cells involved, the nature of the malignancy, and the stage of the disease. LMS either spreads via the blood or the lymphatic system, which is very rare [6, 7]. **Figure 1** describes an overview of the integrated pathway-driven modeling and validation strategy.

The molecular subtyping of LMS has clinical importance because the aforementioned subtypes exhibit distinct gene expression profiles, which may inform the development of targeted therapeutics [8, 9]. Previously reported the molecular subtypes of LMS in 2009, based on gene expression microarray data from 51 samples. Subsequently, other publications that have applied transcriptomics have continually identified three distinct molecular subtypes [10-12]. **Table 1** summarizes these molecular subtypes characteristics as described in different research. Although the relationship between these subtypes across various studies has not yet been formally analyzed, the identified subtypes are generally believed to be very close. Some of the common features that have been observed in these studies are the disparity in distribution of anatomical sites, activation of myogenic markers, immune responses, and their correlation with clinical outcome [13, 14].

Table 1. Clinicopathologic and molecular features of LMS subtypes

Site	Typical age/sex	Key histology	Frequent genomic alterations	Dominant pathway alterations	Prognostic notes
Uterine	Postmenopausal women	Spindle cells with pleomorphic nuclei and high mitosis [80]	TP53, RB1, PTEN loss, ATRX [29]	PI3K/AKT/mTOR [19]	Stage-dependent, 5-year survival rate [81]
Soft tissue	Middle-aged/older adults	Malignant smooth muscle cells	Chromosomal gains, TP53, RB1 [82]	PI3K/AKT/mTOR, MAPK [19]	Gain in chromosomal aberrations predicts reduced survival [82]
Vascular	Adults (vein)			mTOR [19]	5-year survival, recurrence in 2-3 years [83]
Others	Variable adults	Smooth muscle-like with atypia [5]	TP53, RB1 common [63]	PI3K/AKT/mTOR [19]	5-year disease-specific survival [84]

The intended goals of this review are to identify actionable targets and biomarkers across the various LMS subtypes that could be incorporated into future clinical trial designs and subgroup analyses. It may also be possible to identify preferential responses to certain LMS subtypes that would otherwise go undetected by relying solely on molecular data to enrol patients and select their treatment. Collectively, these clinical and molecular characteristics underscore the need for a deeper understanding of the biological mechanisms underlying LMS, which are discussed in the following sections.

Pathophysiology and molecular basis of LMS

The LMS pathophysiology is mostly associated with mutations in the tumor suppressor genes RB1 and PTEN. Approximately 90% of the patients have RB1 mutations. The cytogenetic and molecular changes in LMS are not fixed, which also allows the tumor to be very heterogeneous. The most recent evidence from The Cancer Genomic Atlas has indicated that LMS, like other STS, experiences a lower tumor mutational burden in comparison with other cancer types [8].

Cellular origin and histopathological features

LMS arise from smooth muscle cells or their mesenchymal derivatives. These tumors are typically solitary on gross appearance, well-circumscribed, and commonly have areas of cystic degeneration and necrosis [15]. Classic LMS is defined by the presence of the intersecting fascicles of cells that are in the shape of spindles, and this resembles the smooth muscle tissues. The nuclei are hyperchromatic, usually elongated, and have excess eosinophilic cytoplasm. Depending on the extent of differentiation, pleomorphism may vary, and in cases of low differentiation, LMS may appear as undifferentiated STS. There are other histologic forms of LMS, including pleomorphic, myxoid, and undifferentiated [2].

The occurrence of intramural and solitary cases of uterine LMS is usually typical, and rare cases arise from the cervix of the hymen. Unlike leiomyomas, which have distinct, non-infiltrating boundaries, LMS have irregular, infiltrative boundaries. On coarse examination, the majority of the uterine LMS appear as massive, single tumours with abnormal and penetrating irregularity. Microscopic features such as nuclear atypia, more than 5-10 mitoses per high-power field (HPF), and tumor necrosis are characteristic of LMS. Tumor necrosis is usually coagulative, and viable tumor cells are sharply contrasted with the necrotic ones. This phenomenon is not seen with infarctive necrosis, which is observed in benign tumor lesions such as the leiomyoma, which contains an adjacent regenerative region [7, 16]. A variety of uterine LMS subtypes is also possible, ranging from the classic spindle cell to the epithelioid (in which some epithelial cells make up more than 50% of the cells), through to the myxoid (hypocellular, basophilic), and other rare entities. Lesions arising from smooth muscle cells that lack clear features of LMS are classified as smooth muscle tumors of uncertain malignant potential (STUMP) [7].

Heavy metals in LMS are often associated with smooth muscle-specific antibodies, such as muscle-specific actin, desmin, and h-caldesmon. Diagnostic confirmation is usually by immunochemical staining, particularly in indeterminate tumors for which the origin is unknown. The subtypes of LMS may require additional staining methods. For example, epitheli-

oid LMS may resemble carcinoma because it often stains for epithelial markers, reflecting its characteristic staining pattern. In this situation, the diagnosis may be confirmed using histone deacetylase-8 and myocardin stains. Other common immunohistochemical markers used to distinguish LMS from leiomyoma include p16, p53, and a high Ki-67 proliferation index, which are highly sensitive and specific. As well, LMS also shows a lower level of estrogen (40% in LMS compared to 70% in leiomyoma) and progesterone receptors (38% in LMS versus 88% in leiomyoma) [17]. Building on the molecular complexity described above, several key intracellular signaling pathways have been identified as major contributors to tumorigenesis in LMS.

Key signaling pathways involved in tumorigenesis

PI3K/AKT/mTOR pathway: The PI3K/AKT/mTOR pathway has been a key driver of LMS progression; an imbalance in this signaling cascade is often associated with LMS. Destruction of PTEN protein expression is a common event that normally helps regulate the pathway. Without the PTEN protein, the PI3K/AKT/mTOR pathways become overactive, leading to uncontrolled sarcoma cell growth, making the cells resistant to programmed cell death, and allowing the sarcoma to progress unchecked. This impaired PTEN protein function removes a crucial inhibitory control on the pathway, thereby contributing to the sustainability of detrimental sarcoma development progression [18]. In LMS cells, targeted drugs that block PI3K, AKT, or mTOR at any level reduce cell division and trigger cell death. Previous studies have shown that it has antitumor effects in laboratory models and in early clinical studies. At once, utilizing a targeted drug to block a single node, for example, mTOR alone, causes the sarcoma cells to switch on backup growth signals. Blocking both targets works better than a single target. However, cancer might still find a way to survive, so treatment plans need to be smart, favouring dual approaches that tend to produce deeper and more sustained anti-tumor activity [19]. In combination with ifosfamide, Dox, an anthracycline, is a first-line LMS chemotherapy that inhibits topoisomerase II, thereby disrupting DNA repair. Due to the development of resistance, leucopenia, thrombocytopenia, and cardiotoxicity, the use of Dox in the treatment of malignancy is limited. Lately, STS and other solid tumors have been considered as exhibiting PI3K/AKT/mTOR dysregulation. The dysregulation of these pathways not only involves hyperactivation of growth factor signaling but also occurs through activating or loss-of-function mutations. Rising evidence of crosstalk/redundant functions between pathways MAPK and ERK has been linked to the progression of adaptive resistance when targeting PI3K/mTOR pathways. Therefore, it has been recommended that small molecules targeting the pathway, or combining these agents with conventional chemotherapy, may prevent the development of resistance and improve treatment durability. In previous research, scientists checked whether inhibiting/blocking the PI3K/AKT/mTOR pathway could increase the effect of Dox. A study identified a dual inhibitor, BEZ235, that inhibits both PI3K and mTOR in the pathway and yields better outcomes than drugs that block only one part of the pathway. This has a significant effect, as cancer cells can adapt to alternative feedback signals, enabling cell survival. This happens when only mTOR is blocked. In addition, scientists demonstrated in vivo that BEZ235 inhibited LMS growth, and this inhibition was synergistic with Dox, suggesting a potential benefit for LMS patients [19, 20].

MAPK/ERK pathway: The mitogen-activated protein kinase (MAPK) cascade is a critical pathway for dissemination, human sarcoma cell survival, and drug resistance. Important processes such as cell growth, survival, and movement are regulated by the MAPK/ERK signaling cascade, a protein signaling pathway in cells. In the LMS, this pathway is hyperactive, promoting the growth and spread of sarcoma cells to other tissues. Treating LMS by targeting this pathway is being explored because of its role in promoting sarcoma growth and spread [21]. Among the various intracellular signaling pathways, the MAPK pathway plays a crucial role in processes such as cell proliferation, differentiation, apoptosis, angiogenesis, and tumor metastasis. Four main MAPK cascades have been identified in eukaryotic cells: ERK, JNK/stress-activated protein kinase, p38 MAPK, and ERK5. Each of these MAPK signaling pathways consists of at least three levels:

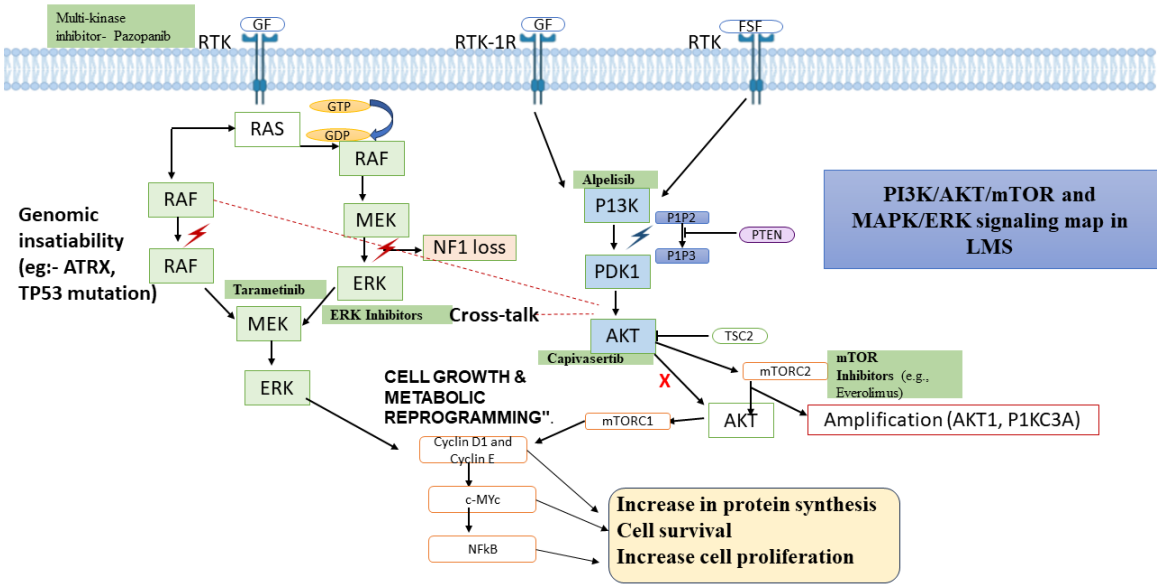

Figure 2. PI3K/AKT/mTOR and MAPK/ERK signaling network in LMS. Schematic illustration of the interconnected PI3K/AKT/mTOR and MAPK/ERK pathways downstream of receptor tyrosine kinases (RTKs) in LMS. Activation of RTKs by growth factors initiates RAS-RAF-MEK-ERK signaling, promoting cell growth and metabolic reprogramming.

MAP3K, MAPKK, and MAPK (**Figure 2**) [22, 23]. Research has shown that the JNK and p38 MAPK pathways are primarily involved in cellular stress responses and apoptosis, whereas the ERK/MAPK pathway, the most extensively studied, is closely associated with cell proliferation and differentiation and plays a pivotal role in the cell signaling network [24].

The MAPK pathway comprises three major kinases: MAPK, MAPK kinase (MAPKK), and MAPK kinase kinase (MAP3K), which activate and phosphorylate downstream proteins. ERK1 and ERK2 are ubiquitous/threonine-type evolutionarily conserved serine/threonine kinases that regulate cell signaling in normal and pathological disease states. ERK expression is critical to development, and hyperactivation of this factor does contribute greatly to the occurrence and acceleration of cancer [25].

Critical evaluation of PI3K/AKT/mTOR pathway and MAPK/ERK pathway interaction: The PI3K/AKT/mTOR pathway and the MAPK/ERK pathway interact reciprocally: a signal from one pathway activates the other, and vice versa. Having recognized the critical roles of the PI3K/AKT/mTOR and MAPK/ERK pathways, it is evident that they act not as individual pathways but as an interlinked network that governs important cellular activities such as growth, survival, and differentiation. Tests conducted in the laboratories have shown that when the only pathway is inhibited, the other pathway shows a natural response. This interaction is relevant in malignancies such as LMS, where deregulation of either pathway can lead to tumor formation. The studies show that DHPI inhibition by a dual inhibitor hinders tumor growth by targeting the most essential survival and proliferation signals used by tumors, thereby inhibiting the PI3K/AKT/mTOR pathway. Indicatively, inhibition of mTORC1 inhibits the negative feedback of the receptor tyrosine kinases and indirectly magnifies the activities of both PI3K and MAPK pathways. In this way, it has been shown that simultaneous inhibition of PI3K/mTOR and MEK exhibits synergistic anticarcinogenic effects. This crosstalk causes therapeutic resistance in sarcomas like LMS. This two-way communication is inclusive, not only defining the pathophysiology of LMS but also providing considerations for further addressing this cancer and enhancing clinical outcomes.

Role of tumor microenvironment

Immune cell infiltration: Two potential biomarkers, tumor-associated macrophages (TAMs) and tumor-infiltrating lymphocytes, may represent promising ceRNA targets in the LMS

immune microenvironment, which is highly infiltrated by these biomarkers. The TME in LMS is important because it determines how the sarcoma proliferates and counteracts the body's normal defense systems. It is composed of various normal cells, such as fibroblasts, blood vessels, and immune cells, which border the sarcoma closely. These cells may, in fact, help the sarcoma by creating a protective neighborhood where sarcoma cells can multiply and spread. On the other hand, treatment is resistant to fibroblasts and other components, reshaping the tissue to support tumor growth in close areas. LMS may be harder to treat due to the complex cellular and multicellular interactions between tumor cells and their microenvironment. It points towards the importance of the treatments that mark not just the tumor but also these supportive structures [26-28].

Tumor-associated macrophages: The LMS microenvironment primarily involves macrophages and T cells. Tumor-linked macrophages generally exist within the range between immunosuppressive M2 macrophages and proinflammatory M1 macrophages. M1 macrophages specifically encourage the Th1 effector response and express high levels of TNF, iNOS, and major histocompatibility complex class II. Most macrophages show the M2 phenotype, as supported by immunostaining showing that most CD14+ cells also express the M2 marker CD163. The presence of M2 macrophages is significant because they are commonly associated with immunosuppressive functions that can support sarcoma progression [29].

Immune evasion mechanisms: In LMS, the response to immunotherapy has been limited to date; numerous possible explanations exist for the underwhelming response. In primary tumors, lower TMB is generally observed in LMS compared with carcinomas [30, 31]. Additionally, as previously discussed, there are mutations and other mechanisms, for example, frequent loss of PTEN protein in LMS, which endorses immune evasion. CD47 is a cell-surface receptor commonly expressed on tumor cells, where it engages with the SIRPα receptor on macrophages and dendritic cells to inhibit their phagocytic activity [32]. CD47 expression has been identified in various cancers, including LMS. Preclinical studies have demonstrated that anti-CD47 antibodies can restore macrophage-mediated detection and engulfment of tumor cells derived from LMS models. In these xenograft experiments, targeting CD47 efficiently inhibited primary tumor development and prevented metastatic disease spread [29].

Macrophages are categorized into two main groups: classically activated macrophages (M1) and alternatively activated macrophages (M2). Each group has distinct roles in immune defense and surveillance, and they can be interconverted based on changes in the internal environment. Macrophages that are recruited to the TME are referred to as TAMs [33]. TAMs are neither M1 nor M2 categories because they represent both M1 and M2 macrophage subpopulations. TAMs have a major implication in control of the TME and immunosuppression, tumor growth, and migration of most tumors, including the sarcomas [34].

Therapeutic implications: Sarcomas represent a heterogeneous family of malignant mesenchymal malignancies (with more than 100 distinct subtypes), including STS and primary bone sarcomas. Sarcomas have complex treatment plans, which depend on the type. The presence of high TAMs can be regarded as a poor prognostic indicator in most types of cancer; hence, they can be targeted for future targeted therapy aimed at sarcoma treatment [35].

Advances in molecular genetic insights

Genomic profiling and mutational landscape

Genomic profiling and the mutational landscape are comprehensive analyses of an organism's genome to reveal genetic changes, including structural variations and chromosomal abnormalities. It can be used to study disease conditions, such as cancer, by plotting the distribution of genome mutations, including extensive chromosomal changes like deletions, duplications, inversions, and translocations. Finally, it discloses the landscape of genetic modifications present in a given sample [36].

Common mutations (e.g., TP53, RB1)

TP53 and RB1 are the most commonly reported gene mutations in LMS. These tumor suppressor genes are usually simultaneously disabled, and this is considered one of the major

genomic characteristics of the disease. Along with LMS, RB1 and TP53 mutations trigger the formation of most other genomically complex sarcomas, every so-so-occasionally coinciding at an already known, increased prevalence relative to translocation-related sarcoma. A comprehensive study revealed that biallelic TP53 and RB1 inactivation in LMS tumors is very high. Patients with Li-Fraumeni syndrome, which is associated with germline defects in TP53 and RB1, have been examined as having an increased risk of developing LMS as a secondary malignancy. It was observed that in the majority of cases, the genes of all of these LMS tumors were eccentric, and these are the very essential initial maturing fundamentals of LMS [37]. In the context of genomic profiling and cancer mutational landscapes, common mutations are typically those in genes such as TP53 (tumor protein 53) and RB1 (retinoblastoma 1). The latter genes are also highly mutated across different cancer types and are important tumor suppressors that regulate the cell cycle. TP53 and RB1 mutations may play an important role in the creation and progression of tumors [38].

Structural variations and chromosomal abnormalities

Numerical and structural chromosome abnormalities, commonly known as chromosomal abnormalities, are one of the typical characteristics of sarcoma. Structural chromosome abnormalities include focal or arm-level gains or losses, whereas chromosome segregation errors can induce numerical chromosome abnormalities during mitosis. New research is underway to uncover the mechanisms by which chromosomal abnormalities can alter cellular fitness, enabling protein and RNA function and tumor formation [39]. Previous cytogenetic studies have demonstrated that LMS is characterized by a wide range of chromosomal changes, with karyotypes that are often complex and lack a single crucial abnormality. Between the chromosomes, structural rearrangements frequently occur at bands 1p36, 1p32, 1p13, 1q32, 7p11.1-p21, 7q32, 10q22, 13q14, and 14p11 on chromosomes 1, 7, 10, 13, and 14, respectively. Also, structural changes involved recurrent losses in chromosomes 4, 9, 14, 15, 16, 18, 21, and 22. It is important to identify these structural and chromosomal abnormalities, as they may predict oncogenes and tumor suppressor genes, such as TP53 and RB1, at these sites. To gain a comprehensive understanding of LMS pathogenesis and identify associated genes, molecular analysis could be helpful [40].

Epigenetic modifications and gene regulation

Epigenetic modifications and gene regulations are well-defined as genetic changes in the gene activity that do not involve changes in the underlying DNA sequence. The oncogenic process is determined by the accumulation of alterations that impact the function and the genome's structure of the cancer. The acquisition of malignant properties is facilitated by epigenetic modifications that modulate gene expression and by genetic mutations that directly disrupt DNA sequencing. The most significant changes involve shifts in DNA methylation and histone modification patterns, which foster subsequent growth and metastasis. In addition to DNA methylation and histone modifications, emerging evidence indicates that non-coding RNAs and chromatin remodeling complexes also regulate gene expression in LMS. Epigenetic dysregulation contributes not only to tumor initiation but also to therapy resistance and disease progression. Importantly, unlike genetic mutations, epigenetic alterations are potentially reversible, making them attractive targets for therapeutic intervention using agents such as DNA methyltransferase inhibitors and histone deacetylase inhibitors [41].

DNA methylation

DNA methylation profiling comprises several functions, including examining the methylation status of CpG sites across the genome and classifying tumors by generating a methylation signature in LMS. The underlying biology of the tumor can be identified, the type of sarcoma can be inferred from these methylation signatures, and these methylation signatures can be leveraged to develop classifiers that help researchers achieve precise diagnosis [42]. Researchers previously introduced a tool based on DNA methylation data and statistical models that process data, such as DNA methylation analysis, using probability for sarcoma classification. For the tumor methylation classes, scientists established a webpage for the

scientific community listing characteristic features of the sarcoma. Employing DNA methylation-based categorization offers valuable aspects. Through this analysis, DNA can be extracted even from tissue embedded in paraffin wax and fixed with formalin. This constitutes a clear advantage over RNA expression profiling, which depends on fresh tumor tissue [43].

Histone modifications

Histone modifications are crucial aspects in chromatin packaging and are responsible for gene regulation during cell fate determination and development. The stability of the genome may be affected by abnormal alterations in histone modifications, which disrupt gene expression patterns and lead to sarcomas and related diseases. The modification is a covalent post-translational modification, such as phosphorylation, glycosylation, acetylation, or methylation of histone tails (H2A, H2B, H3, and H4), catalyzed by proteins. Recently, some of the well-studied histone modifications have been implicated in cancer development, for instance, H3K4me3 and H3K6me3, which are linked with active transcription, and H3K27me3 and H3K36me, which are linked with repressed genes [44]. Modifications to histones, including acetylation and methylation, affect vital cellular processes, including the development and spread of the sarcoma. In LMS, specific histone deacetylases, such as HDAC9, promote sarcoma development by suppressing genes that activate cell death. These modifications present promising targets to aim at converting abnormal changes [45, 46].

Emerging biomarkers and molecular subtypes

Newly identified biological markers, measurable in a patient's body fluids or tissues, enable the classification of diseases into distinct molecular groups based on their genetic and molecular characteristics. This allows for more accurate diagnoses and targeted treatment strategies, especially in cancer, where different subtypes may respond differently to therapies. The discipline is evolving very rapidly with the adoption of genomic sequencing and other molecular profiling methods, leading to the discovery of new biomarkers and more disease subtyping [47]. A biomarker such as circulating tumor DNA (ctDNA) can be a valuable variable when working with patients with LMS. It can be compared with tumor size and disease progression, and used to inform treatment decisions and monitor responses to systemic therapies. Research would help improve patient outcomes by identifying more reliable markers of high-risk diseases like LMS, and established treatments would help forecast treatment success. The identification of ctDNA is an emerging methodology with a promising future, providing oncogenic mutations, tumor burden, clinical outlook, and analysis of therapeutic response. As previously observed, these data suggest that ctDNA, readily detectable with standard sequencing techniques, may serve as a valuable liquid biopsy biomarker for patients with LMS. Higher ctDNA concentrations correlate directly with tumor size and disease progression. Ultimately, it holds promise for guiding treatment decisions, tracking responses to systemic therapy, and differentiating benign and malignant smooth muscle tumors [48]. These molecular insights provide a foundation for translational research approaches aimed at improving disease modeling and therapeutic development.

Translational biology of LMS

Model systems for studying LMS

Patient-derived xenografts (PDX): PDX closely mimic the genetic and phenotypic characteristics of a patient's tumor, enabling personalized treatment evaluation and the study of tumor heterogeneity. However, limitations of PDX models include the potential inability to fully replicate the TME and immune response due to the use of immunocompromised mouse models, as well as the possibility of tumor adaptation during xenografting [49, 50]. One study found that creating a mouse model from patient-derived tumors improved understanding and treatment of a difficult type of STS. It discusses the growth and implications of the PDX model in studying sarcomas. It underscores that PDX models involve transplanting human tumor tissues into immunodeficient mice, allowing tumors to develop in a living organism while maintaining many of their original characteristics. Subcellularly, standardized LMS exhibit a characteristic smooth muscle maturation, with a short to moderate capacity for aggressive neoplasia. Models function as a critical tool for precise investigational studies

and understanding sarcoma pathology, for rare aggressive tumors like LMS [51].

Organoids and 3D culture models: Organoids are three-dimensional systems derived from stem cells that are faithfully reproduced to reflect the key structure and behavior of the parent tissue. Compared with two-dimensional cell cultures, these human organoids will provide a more physiologically relevant experimental model. Organoids can be used to study disease mechanisms and determine drug potency and toxicity because they capture the complexity and heterogeneity of human tissues. When grown from an individual patient's cell, organoids enable the determination of individualized drug efficacy [52]. To summarize the common limitations related to the methodologies usually used to study the pathology of LMS. A study successfully established 3-D models for disease cell lines, using two distinct biocompatible scaffolds. The study used two distinct scaffolding approaches: an agarose gel matrix in standard 6-well plates and an alginate matrix with the Bioprinting INKREDIBL+ system (CELLINK). This advanced bioprinter used a dual-printhead pneumatic base and a UV LED curing system. Both methodologies successfully yielded 3D spheroids for the disease, suitable for morphological analyses. The spheroids successfully multiplied and self-organized into complex structures. Researchers aimed to develop a suitable system that could accurately replicate tissue conditions by accumulating native extracellular matrix (ECM). These models can be useful for improving our understanding of disease mechanisms and the causes of disease, and as a platform for testing novel preventive and therapeutic approaches [53].

Insights from comparative oncology

Comparative oncology also provides insights into the LMS by examining the disease across different species, particularly dogs, and comparing it with human sarcoma. This method enables the researcher to identify commonalities and contrasts in tumor development, progression, and treatment response across different species. Essentially, these smooth muscle tumors naturally occur in both the human and dog species under natural conditions, producing disease pathogenesis comparable to that of human LMS, rather than the traditional laboratory models. Despite morphological differences, human and canine sarcomas exhibit characteristics of aggressiveness, metastasis, and drug resistance. Dogs are therefore outstanding translational models for the study of LMS owing to their similarity with respect to TME and genomic factors [54, 55]. These comparisons could yield new directions for the study and treatment of sarcoma in humans and animals. Some insights relate to the fundamental genetic mechanisms, such as the susceptibility to sarcoma across species, new therapeutic targets, and the use of environmental factors to control and cure cancer, as the animal species showed a wide range of exposure [56, 57].

Therapeutic implications of translational findings

Table 3 represents the integration of molecular carcinogenesis with therapeutic implications in LMS.

Development of targeted therapies

Exclusively for LMS, there are no FDA-approved targeted therapies. The FDA approves the tyrosine kinase inhibitor Pazopanib for treating advanced STS, including LMS, that have progressed after prior chemotherapy; it targets VEGFR, PDGFR, KIT, and FGFR. Aside from Pazopanib, the only other targeted treatments available for LMS are those with tissue-agnostic approval, for example, the checkpoint inhibitors Pembrolizumab and Dostarlimab, especially for tumors characterized by high microsatellite instability or tumor mutational burden. On the other hand, the LMS genome typically exhibits a low tumor mutational burden and few specific, actionable mutations (unlike cancers such as BRAFV600E in melanoma), limiting the clinical benefit of single-agent targeted therapy. As in the development of new targeted therapies, careful consideration must be given to their associated toxicity profiles compared to established medicines, as well as the economic viability of their implementation [29].

Immunotherapy approaches

Immunotherapy approaches for LMS are currently being developed, primarily through che-

Table 2. Genetic and epigenetic alterations in LMS

Alteration type	Gene/region	Functional effect	Impacted pathway	Experimental/clinical relevance
Mutation	TP53, RB1	Loss of function, tumor suppression	Cell cycle, DNA restoration	Prognostic due to aggressive behavior and TP53, RB1 inactivation, poor survival
CNA	RB1 (13q14) PTEN (10q22)	Loss of the cell cycle and tumor suppressor, PI3K/AKT pathway activation	Cell cycle, PI3K/AKT/mTOR	Diagnostic through next-generation sequencing, possible aim through PI3K/AKT/mTOR
Fusion	PGR-NR4A3	Fusion protein	Progesterone receptor gene (Steroid receptor superfamily pathways)	Prognostic implication (limited data), targeted therapies
Methylation	Differentially methylated genes	Hypermethylation on 77	Chromatin remodeling	Potential prognostic indicator
Histone mark	H3, H4 catalyzed by proteins and H3K27me3, H3K36me are linked with repressed genes	Loss of repressive mark, dissimilarity in chromatin	Epigenetic guideline	Therapeutic experimental potential
ncRNA	Limited data	Pro-oncogenic/sarcoma suppressive function	PI3K/MAPK	Potential diagnostic biomarker needs hypothesis

ckpoint inhibition. Even though current Immune Checkpoint Blockade (ICB) trials have shown limited efficacy in LMS, further progress depends on more detailed profiling of the tumor's molecular and immune subtypes, which will determine the evidence base for new immunotherapies. For immunotherapy to advance in the LMS environment, in-depth insight into the TME is pivotal. A major clinical drawback has been the historical methodology of including all LMS cases in ICB trials, which has unintentionally led to the misapprehension that all molecular subtypes of the disease are inherently immune-refractory. Emerging macrophage-targeting drugs currently in Phase I trials are likely to benefit all subtypes of LMS. Yet, an undifferentiated LMS may show a greater response because these tumors exhibit a higher density of infiltrating macrophages [58].

Recent clinical trials have evaluated targeted agents in LMS with varying outcomes. For instance, pazopanib demonstrated improved progression-free survival in advanced STS, including LMS, in the PALETTE trial [59]. Similarly, mTOR inhibitors such as everolimus and dual PI3K/mTOR inhibitors have shown modest activity in early-phase studies [60]. Immunotherapeutic approaches, including checkpoint inhibitors such as pembrolizumab, have demonstrated limited efficacy. Ongoing trials are investigating combination strategies targeting multiple pathways simultaneously to overcome resistance mechanisms [60].

New insights into molecular carcinogenesis

Recent studies discuss molecular heterogeneity and the challenges of transitioning from generalized clinical management to personalized approaches, particularly with respect to genomic profiling and molecular biomarkers. It also highlights the essence of knowing the dynamics of tumor progression to develop treatments that enhance the compatibility for patients [61].

Mechanistic studies elucidating tumor initiation and progression

In a complex manner, mechanistic work on the initiation and progression of LMS tumors examines intricate genetic and epigenetic maladies, yet the complete mechanism remains uncertain. These are tumors that are usually composed of smooth muscle cells or their mesenchymal precursors and are also quite heterogeneous [11]. LMS is a multi-stage process, driven by the accumulation of specific genetic and epigenetic mutations and by complex interactions within the TME (**Table 2**). High frequencies of distant metastasis, the major cause of death among patients with LMS, challenge the management of LMS. To address this, investigators have examined the effects of calcium (Ca^{2+}), one of the accepted regulators of sarcoma signaling, and the role of this receptor in LMS has been poorly defined. New information establishes that the TRPV4 ion channel, a mechanoreceptive calcium channel, is involved in the process. Recent findings explain two

Table 3. Targeted and immune-based therapies in LMS

Target/pathway	Agent class or exemplar drug	Preclinical rationale	Clinical evidence	Biomarker requirement/association
PI3K/AKT/mTOR	Dual PI3K/mTOR inhibitor (BEZ235)	LMS cell lines, growth inhibition and decreased tumor cell survival in vivo [85]	Phase II: progression-free survival in malignant LMS [49]	PTEN protein loss [29]
Cell Cycle (CDK4/6)	CDK4/6 inhibitor example, palbociclib	LMS PDX models	Phase II	RB1loss, TP53 alteration
DNA damage response	PARP inhibitor	LMS cell lines and PDX	Phase II	HR gene alterations [29]
Immune checkpoint inhibitor	PD-1 inhibitors	LMS PDX	Retrospective cohort study	Higher immune infiltration [58]
Tyrosine kinase inhibitor	Multi-Tyrosine kinase inhibitor: Pazopanib	Not available	Phase III [49]	None established
MAPK/ERK	MEK inhibitors	ERK phosphorylate downstream	Limited response	MAPK pathway activation

simultaneous processes: one that moves towards malignant progression and directly stimulates the Ca2+/FAK/PI3K/AKT/GSK3-b signaling pathway, and another that indirectly stimulates it by increasing ECM1 secretion. These findings reveal that the TRPV4/FAK axis is fundamental in the susceptibility and is instrumental in the formation of novel targeted therapy and tools to predict [62].

Crosstalk between genetic alterations and cellular processes

To achieve a full understanding of the aggressive subtype of disease, investigators must not only index individual genomic aberrations but also appropriately use advanced genomic techniques. So, next-generation sequencing is an invaluable tool for discovering the genomic aberration that underlies LMS pathophysiology and which, as a critical factor, helps identify possible outcomes. Next-generation sequencing plays a fundamental role in the study of sarcomas, not only in uncovering genetic alterations that underpin pathophysiology and diagnosis but also in pinpointing therapeutic targets. Deletions in established cancer genes, in addition to those associated with DNA repair mechanisms, are very important in LMS. ctDNA is an optimized NGS-based strategy of vital importance when it is impossible to obtain a tissue biopsy. However, the use of ctDNA detection for LMS poses challenges because typical oncogenic single-nucleotide mutations are insufficient. The study, along with previous studies, has emphasized copy number variations as the dominant biological feature in LMS tumors. Recent discoveries indicate promising approaches based on TP53-related processes, cell cycle regulation, or kinase signaling pathways. Meanwhile, ctDNA has high potential in the context of LMS, particularly at initial diagnosis, risk assessment within prognostic groups, and clinical outcome; however, rigorous prospective trials are necessary to justify its utility [63].

Impact on cell cycle regulation

Deregulation of the cell cycle is a ubiquitous principle of carcinogenesis, and in LMS, the principle identifies a significant target of intervention drugs. The CDK4/6-RB1 pathway is the major regulator of cell division, controlling the major checkpoint, that is, the G1 S transition, and thus proliferation of cells. The existing literature indicates that inhibition of CDK6 (by palbociclib) causes a significant G0/G1-phase block in LMS cells, preventing their progression into S phase. Cell cycle arrest is linked to reduced phosphorylation of the Rb protein, a process necessary to advance through the cell cycle. These results confirm the critical role of CDK4/6 in cell cycle progression in selected LMS cells and underscore the clinical utility of targeting this pathway to arrest sarcoma development [64]. One of the studies also reveals that in LMS, functional deficiency mutations or structural aberrations affecting RB1 bypass these checkpoint processes, leading to uncontrolled cell proliferation, enhancing transmutation. The disruption of RB1-mediated cell cycle control, along with abnormalities in linked signaling pathways, is an established property of LMS tumorigenesis and provides a favorable direction for selective agent development [58].

Interactions with stromal components

TME is characterized by a complex network interconnection of interactions, crosstalk between tumur-associated macrophages (TAMs) and various stromal and immune components, mediated by direct cellular communication and extracellular signals. Evidence suggests that cancer-associated fibroblasts serve as a vital source of chemotactic signals that promote monocyte infiltration and induce macrophage maturation toward an immunosuppressive M2 phenotype. Though tumo-associated macrophages contribute to ECM reorganization, their effect on CAF biology remains comparatively limited, chiefly through induction of the mesenchymal-myoepithelial transition. The prevailing literature has primarily studied the targeting of separate stromal populations in isolation, yet this analytic approach neglects the indispensable role of intercellular communication networks. Future studies must prioritize the characterization of actionable targets within these cellular interactions to refine antitumor outputs [65].

Role of non-coding RNAs in LMS

Non-coding RNAs (ncRNAs) have attracted significant attention due to their unique regulatory roles in biological regulation. NcRNAs are now recognized as vital regulators of numerous developmental processes and disease progression, particularly in human malignancies. Among the various classifications of ncRNAs, specific forms such as microRNAs, long noncoding RNAs, and small nucleolar RNAs mainly influence the complex processes revealed in sarcoma [66]. The perception of ncRNAs has shifted from genomic junk to an essential role in cancer development through regulatory mechanisms of gene expression. NcRNAs may be pro-oncogenic or sarcoma-suppressive. The inherent stability and accuracy of these molecules in the bloodstream support their advancement as viable non-invasive biomarkers and, thus, help fill a special void in tumor genetics and function. Despite current limitations in assay consistency and data analysis, integrating ncRNA biomarkers into clinical practice is essential to enhance the efficacy of precise medication through diagnostic and targeted intervention choices. Future research should aim to prevent current challenges to achieve optimal exploitation of the major intervention targets provided by ncRNAs, particularly with regard to their combined association with personalized cancer recovery outcomes [67]. An integrated view of genomic, epigenetic, and ncRNAs alterations underlying the emergent molecular subtypes of LMS is illustrated in **Figure 3**.

Implications for understanding human disease mechanisms

Lessons from LMS applicable to other malignancies

LMS is a malignant neoplasm arising from smooth muscle and is among the most prevalent types of sarcoma. Although most of the time it involves the retroperitoneum and the uterus, the extremities and trunk are rarely affected. Diagnosis typically requires a core needle biopsy. The choice of treatment plans is based on the tumor's size, grade, and location, the patient's age, and the patient's tolerance to aggressive interventions. The primary treatment method is surgical resection, chemotherapy, and radiation therapy, as side effects are commonly employed [55]. Past research on LMS offers valuable insights into the management of malignancies, which are characterized by genetic complexity, particularly regarding the link between chromosomal instability and the TME. Research on LMS has surpassed the search for single-driver mutations, the regulatory functions of extensive somatic copy-number alterations (SCNAs), and complex karyotypic transformations. The same is currently being applied to other genetically unstable sarcomas to understand how they apply to intervention resistance. Additionally, the analysis of the immune microenvironment of LMS reveals a distribution of immunosuppressive TAMs and not armed T cells. Past experiences align with therapeutic modalities that aim to control myeloid cells to amplify responses in immunosuppressive malignancies that, more often than not, are resistant to checkpoint inhibitors [68]. Finally, the efficacy of conventional chemotherapy in LMS, as a function of the variable, is critical for highlighting the essential importance of using transcriptomic classification to guide targeted therapies. This fact demonstrates that relying specifically on the histological diagnosis is not adequate in predicting the success of the treat-

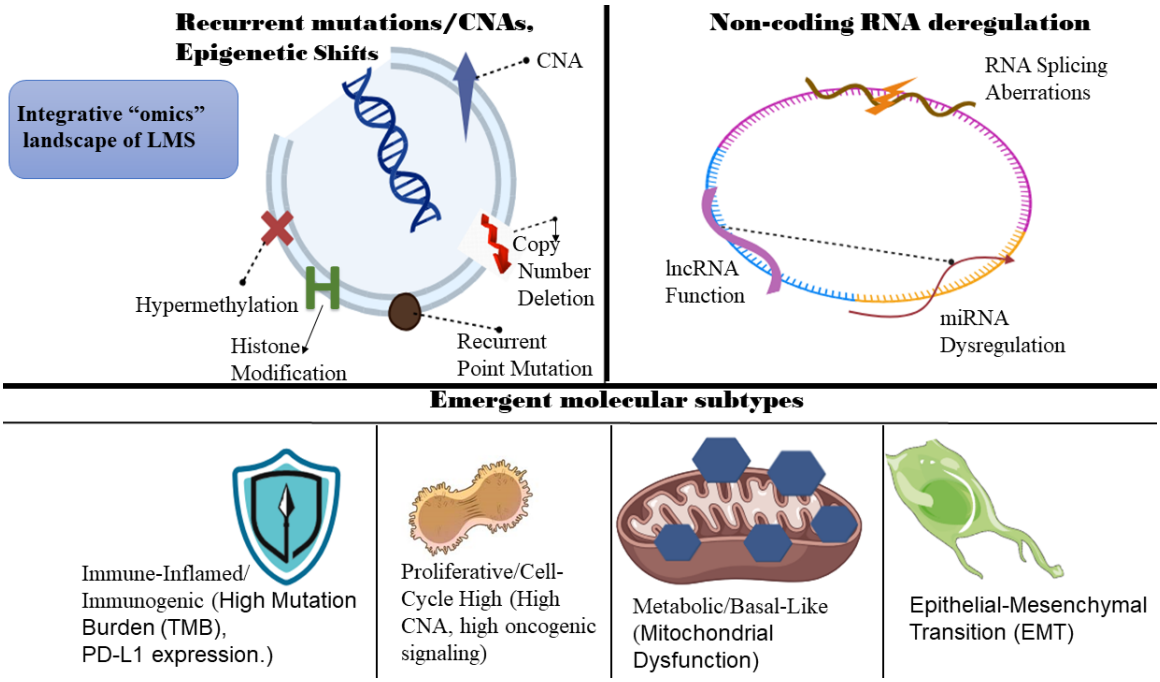

Figure 3. Integrative molecular landscape and emergent subtypes of LMS. Schematic overview illustrating the multi-layered molecular alterations underlying LMS heterogeneity.

ment within the genetically and biologically diverse sarcomas [69].

Insights into fundamental processes in human carcinogenesis

In LMS, loss of both the key tumor suppressors TP53 and RB1 takes center stage as core events leading to genomic instability, which comprises point mutations, small insertions/deletions, copy number variation, and structural disruptions and modifications, triggering karyotypic complexity and adaptive oncogenesis. It is this disruption at these key checkpoints that allows unregulated cell division despite signaling influences, which would otherwise necessitate maturation, usually in specialized phenotypes derived from vascular smooth muscle cells, with smooth muscle structures maintained by serum response factor signaling and cell cycle regulation by RB1. Epigenetic aberrant control is also mediated by extensive DNA demethylation, which catalyzes genomic heterogeneity, selectively activates hypermethylation silencing of critical genes, and enhances hypermethylation by activating expression of growth-inducing factors via hypermethylation catalysts. The interdependent genomic transcriptional and epigenetic alterations confirm the pathogenesis of LMS, highlighting the possible therapeutic outcome, such as pathway inhibitors [70, 71].

Challenges in translating molecular findings to clinical practice

Biological systems are also very complex, and the gap between preclinical models and human patients is a major challenge in translating laboratory findings into patient care. Others are the lack of clinical validation, data integration, unstandardized testing procedures, regulatory challenges, financial issues, constraints in the healthcare systems, and strong research-clinician partnerships. It is these difficulties that make a disparity between the potential improvements in science and their possible clinical use [72].

The main issues are the first biological complexity, because the human body is very complicated, and the introduction of the drug in the context of laboratory experiments may not always accurately reflect the specifics of the disease course in the patient, leading to unexpected results during clinical trials. Also, animal models used in research to study a disease do not necessarily reflect the same out-

comes. In this case, there will be a difference between the outcomes studied in preclinical research and those in the clinic. Moreover, to determine the clinical utility and accuracy of molecular biomarkers, large, well-designed clinical trials are needed to demonstrate their predictive and prognostic value. Data integration is one of the main challenges when integrating different molecular data with clinical information, and it highly demands bioinformatics expertise. The lack of standardized testing procedures across laboratories may also lead to inconsistent outcomes and a complex clinical interpretation of molecular panel findings. Regulatory barriers, such as new molecular tests and therapies, are subject to very rigorous regulatory procedures that can be expensive and time-consuming. The adoption of molecular testing as a clinical tool might be costly, especially given the need for specialized equipment, skills, and data interpretation. Lack of access to sophisticated molecular testing in certain healthcare facilities can act as a barrier to the application of research on patients. The clinicians should be adequately trained to correctly comprehend and interpret molecular information so they can easily incorporate it into their patient management decisions. It should be selected patients by identifying the appropriate population that will be motivated by the intended molecular therapies to maximize treatment effects [73-76].

Future directions

Emerging technologies in molecular and translational research

Single-cell sequencing: The surveillance study identified the vital prognostic systems and the immune profile of LMS by combining single-cell and bulk sequencing measures. As emphasized in the analysis, LMS cells with high stemness exhibit greater invasive potential, a more complex TME, and enhanced cell-cell crosstalk mediated by TGFβ and ECF signaling cascades. Six prognostic genes are identified, yielding a stemness-dependent risk score with significant predictive value for patient outcome. These results reveal new pathways of tumor evolution and immune dysregulation in LMS. Clinical validation, immune-based approaches, and direct inhibitors of such processes should be enforced in future research to address more treatment approaches for patients with LMS [77].

Further opportunities are represented by improving the precision and reliability of the single-cell sequencing technology, the creation of more sensitive and more specific detection methods, minimizing the error rate, and cooperation with artificial intelligence [78].

CRISPR-based functional studies: CRISPR-based systems are a powerful platform for conducting functional studies in molecular and translational research. CRISPR (clustered regularly interspaced short palindromic repeats) is a new editing tool capable of editing DNA in living cells and organisms [62, 63]. A major benefit of CRISPR is that it allows specific targeting of the gene's location in the genome. It is inexpensive, extremely efficient, and user-friendly. Besides, technology can be used to explore noncoding genomic regions. A major drawback of this technology is that it can result in unintended off-target effects. In addition, its ingredients may be potentially carcinogenic. Moreover, its nucleases can trigger an immune response. Besides, the immune improvement through CRISPR would have long-term outcomes on the immune system. These are some of the issues to consider when implementing the approach [62, 63].

Unmet needs and research priorities

Identification of novel therapeutic targets: The novel therapeutic targets are being researched in DNA damage repair, aberrant metabolic processes, PI3K/AKT/mTOR, and UCP2 expression. Also, PARP inhibitors can be considered, given the potential for DNA repair deficiency in certain LMS tumors. The current research aims to identify the best treatment plans that can be specific to the molecular profiles of the tumor [49].

Strategies for early detection and prevention: Nowadays, there is no definite way to prevent LMS. Thus, the primary intervention in early detection is to be aware of possible symptoms and consult a physician if unexplained lumps or growths are present, particularly if they are painful or fast-growing. This involves conducting periodic personal checkups and talking to your health professional about the issues, especially if you have risk factors such as having undergone radiation therapy or a family member with STS [79].

Conclusion

LMS is a rare but aggressive cancer that arises from smooth muscle tissues, commonly affecting the uterus, abdomen, and blood vessels. Its rapid progression and high metastatic potential make early diagnosis and effective treatment critically important. Recent advances in molecular biology and genomics, particularly next-generation sequencing, have significantly improved our understanding of the genetic basis of LMS. Emerging technologies such as CRISPR-Cas9, RNA sequencing, and induced pluripotent stem cells are opening new possibilities for targeted therapies and personalized treatment approaches. Despite these advancements, challenges in early detection and precise treatment remain. Future progress will depend on improving diagnostic sensitivity, overcoming technical limitations, and integrating artificial intelligence to improve disease prediction and management. Overall, continued research and technological innovation hold strong promise for improving the diagnosis, treatment, and clinical outcomes of patients with LMS.

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Disclosure of conflict of interest

None.

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