

Clinical Next-Generation Sequencing in Sarcoma Diagnosis and Classification: A Real-World Cohort Study

Xinyi Zhao

Nanjing Drum Tower Hospital

Jie Liu

Nanjing Drum Tower Hospital

Zhiwen Li

Nanjing Drum Tower Hospital

Ziyu Wang

Nanjing Drum Tower Hospital

Rutian Li

rutianli@njtu.edu.cn

Nanjing Drum Tower Hospital

Research Article

Keywords: Sarcoma, next-generation sequencing, molecular classification, gene fusion, precision oncology

Posted Date: October 1st, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10952508/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Background

Sarcomas are rare and heterogeneous mesenchymal neoplasms in which accurate classification can be challenging because of overlapping histologic and immunophenotypic features. We evaluated the diagnostic contribution of clinical next-generation sequencing (NGS) in a real-world sarcoma cohort and further characterized genomic and fusion findings and their clinical implications.

Methods

We retrospectively reviewed 106 patients with suspected or pathologically confirmed bone and soft tissue tumors who underwent 110 clinical NGS assays. Diagnostic impact was determined by integrated review of histomorphology, immunohistochemistry, molecular findings, and ancillary tests and categorized as reclassification, refinement, molecular confirmation, or no direct diagnostic impact. Differences in altered-gene counts between genomic classes were assessed using Mann–Whitney U tests with Benjamini–Hochberg correction, and outcomes of matched therapy were evaluated.

Results

NGS provided diagnostically relevant information in 61 of 106 patients (57.5%), including reclassification or refinement in 37 (34.9%) and molecular confirmation in 24 (22.6%). Nineteen of the 23 refined tumors had initially been classified as sarcoma, not otherwise specified, or undifferentiated round/small cell sarcoma. Genomically complex or other tumors had more genes affected by short variants ($q = 0.002$) and copy-number alterations ($q < 0.001$) than translocation-associated sarcomas. RNA sequencing detected fusion transcripts in 35 of 53 evaluable patients (66.0%); EWSR1 fusions involved seven partner genes across eight histologic diagnoses. A candidate novel SFXN1::NRG2 fusion in pleomorphic liposarcoma was supported by concordant RNA and genomic breakpoint findings. Treatment-relevant alterations or biomarkers were identified in 35 patients, of whom 11 (31.4%) received matched therapy; among 10 response-evaluable patients, three achieved partial response and six had stable disease.

Conclusions

Clinical NGS was particularly useful for resolving diagnostically ambiguous sarcomas. Partner-level characterization of EWSR1 fusions provided classification information beyond rearrangement status alone, and treatment-relevant molecular findings further linked diagnostic profiling to matched therapy in selected patients.

1. Background

Sarcomas are a diverse group of mesenchymal malignancies arising in bone and soft tissue, collectively representing about 1–2% of all malignancies in adults and 6–15% of those in children under the age of 15

years [1]. Although they are uncommon, sarcomas constitute more than 100 distinct histologic subtypes [2]. Accurate classification can be challenging because different sarcoma entities may show overlapping histologic and immunophenotypic features despite substantial differences in clinical behavior, prognosis, and treatment [2]. Surgery remains the mainstay of treatment for localized disease, whereas systemic treatment options for recurrent, unresectable, or metastatic disease remain limited and are highly histology dependent [3].

Advances in molecular pathology have reshaped sarcoma classification. The fifth edition of the World Health Organization (WHO) Classification of Tumours, Soft Tissue and Bone Tumours, incorporates recurrent gene fusions and other characteristic genetic alterations into the definitions or diagnostic criteria of multiple entities [2]. DNA- and RNA-based next-generation sequencing (NGS) can collectively detect short variants, copy-number alterations, and gene fusions, thereby providing information relevant to diagnostic classification and treatment selection [4, 5]. The National Comprehensive Cancer Network (NCCN) Soft Tissue Sarcoma Guidelines indicate that NGS may be considered in selected clinical circumstances, including potential eligibility for clinical trials, disease refractory to or progressing on standard therapies, and histologies in which testing is likely to yield clinically actionable findings [6].

The Cancer Genome Atlas (TCGA) provided a landmark multiplatform analysis of 206 adult soft tissue sarcomas representing six major histologic types, establishing a reference framework for the genomic architecture of common adult sarcomas [7]. Subsequent clinical sequencing studies extended genomic profiling to a broader range of histologic subtypes and routine clinical settings [5, 8–10]. In this study, we assessed the diagnostic impact of clinical NGS in a histologically diverse, single-center Chinese sarcoma cohort and further characterized genomic and fusion patterns and the real-world uptake and outcomes of matched therapy.

2. Methods

2.1 Study Cohort

Patients with suspected or pathologically confirmed bone and soft tissue tumors who underwent clinical NGS at or through Nanjing Drum Tower Hospital between April 2020 and March 2026 were retrospectively reviewed. Patients with sequencing quality-control failure or a benign final diagnosis were excluded.

2.2 Clinical Sequencing

Clinical NGS was performed as part of routine care using multiple commercially available sequencing assays from several clinical laboratories, including targeted DNA panels, combined DNA/RNA assays, and whole-exome sequencing. Assay design, gene content, and analytical pipelines varied among laboratories and over the study period. Genomic alterations and sequencing-derived biomarkers were obtained from the final clinical NGS reports without centralized reanalysis of raw sequencing data.

2.3 Diagnostic Assessment

Initial and final diagnoses were compared after integrated review of histomorphology, immunohistochemistry, molecular findings, and ancillary tests. Diagnostic impact was classified as reclassification, refinement,

molecular confirmation, or no direct diagnostic impact. Reclassification denoted assignment to a different tumor entity. Refinement denoted assignment of a broad, descriptive, not otherwise specified (NOS), or differential diagnosis to a more specific related entity. Molecular confirmation denoted detection of a characteristic molecular alteration without a change in diagnosis.

2.4 Genomic Analysis

Genomic alterations and reported tumor mutational burden (TMB) values were extracted from the clinical NGS reports. Descriptive genomic analyses included variants of uncertain significance. Individual genomic alterations listed in the clinical reports were counted as separate events, whereas each altered gene was counted once per assay when calculating gene frequencies. For cross-platform comparisons, analyses were restricted to a 79-gene DNA set common to the Geneseeq, OrigiMed, and Simcere Diagnostics panels. Seventy-three patients had panel-based sequencing eligible for this harmonized analysis; when multiple eligible assays were available for a patient, the assay from the primary tumor was selected (Supplementary Table S2 in Additional file 1).

RNA-based fusion analysis was performed when RNA sequencing was available. Fusion transcripts detected by RNA sequencing other than established sarcoma-associated fusions were further reviewed using PubMed, the Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer [11], the Catalogue of Somatic Mutations in Cancer (COSMIC) [12], and FusionGDB [13]. Fusion pairs that had been described in the literature or curated databases were grouped into previously reported uncommon fusions, whereas those without prior reports were grouped into candidate novel fusions.

2.5 Treatment Relevance

Treatment relevance was assigned when a genomic alteration or sequencing-derived biomarker supported biomarker-directed systemic therapy based on regulatory approval, clinical guidelines, published clinical evidence, or OncoKB [6, 14]. Best overall response was assessed according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 [15]. Progression-free survival (PFS) was measured from initiation of NGS-matched therapy to disease progression or death, with patients without an event censored at last follow-up.

2.6 Statistical Analysis

Differences in altered-gene counts between genomic classes were assessed using two-sided Mann–Whitney U tests, with Benjamini–Hochberg correction for three comparisons. P or q values < 0.05 were considered statistically significant. Analyses were performed using R version 4.6.0 and Python version 3.12.13. During manuscript preparation, ChatGPT (OpenAI) was used to assist with language editing and translation. All outputs were critically reviewed and verified by the authors, who take full responsibility for the final content.

3. Results

3.1 Cohort and sequencing characteristics

A total of 110 patients with bone and soft tissue tumors underwent clinical NGS during the study period, of whom 106 were included in the study cohort (Fig. 1). Both primary and metastatic tumors were sequenced in

four patients, yielding 110 evaluable sequencing assays. At the patient level, the median age at diagnosis was 49 years (range, 10–76 years), and 56 patients (52.8%) were male. The most common primary sites were the extremities (n = 40, 37.7%) and the abdominopelvic, retroperitoneal, or visceral sites (n = 37, 34.9%) (Table 1).

There were 39 different diagnoses in the cohort. Undifferentiated pleomorphic sarcoma (UPS) was the most common diagnosis (n = 18, 17.0%), followed by dedifferentiated liposarcoma (DDLPS; n = 10, 9.4%), synovial sarcoma (SS) and leiomyosarcoma (LMS) (n = 7 each, 6.6%) (Supplementary Figure S1).

At the assay level, NGS was used across the disease course, with 57 of 110 assays (51.8%) performed during the initial diagnostic work-up and 53 (48.2%) performed later in the disease course, including at recurrence or progression before systemic therapy and after systemic therapy. The median interval from initial diagnosis to sequencing was 1.8 months (range, 0–111.3 months). Primary tumor tissue was used in 75 assays (68.2%), and 79 assays (71.8%) were performed on surgical specimens. The median turnaround time from sample receipt to report issuance was 10 days (range, 4–28 days) (Table 1).

Table 1
Clinical and sequencing characteristics of the study cohort.

	n, %	Median, range
PATIENT-LEVEL CHARACTERISTICS (n = 106)		
Age at diagnosis, years		49 (10–76)
Sex		
Male	56 (52.8%)	
Female	50 (47.2%)	
Tumor site		
Head and neck	11 (10.4%)	
Trunk (chest wall, abdominal wall, flank, paraspinal, buttock)	12 (11.3%)	
Intrathoracic/mediastinal	6 (5.7%)	
Extremity	40 (37.7%)	
Intra-abdominal, pelvis, retroperitoneum, visceral	37 (34.9%)	
ASSAY-LEVEL SEQUENCING CHARACTERISTICS (n = 110)		
Time to tumor sequencing from initial diagnosis		1.8 months (0–111.3)
Time from sample receipt to NGS report		10 days (4–28)
Timing of NGS testing during the disease course		
At initial diagnosis*	57 (51.8%)	
At recurrence or progression before systemic therapy	15 (13.6%)	
After systemic therapy	38 (34.5%)	
Sequencing sample source		
Primary tumor	75 (68.2%)	
Recurrent tumor	12 (10.9%)	
Metastatic lesion	23 (20.9%)	
Specimen type		
Surgical specimen	79 (71.8%)	
Biopsy specimen	30 (27.3%)	
Other	1 (0.9%)	

NGS, next-generation sequencing. *Among the 57 assays performed at initial diagnosis, 16 (28.1%) were from patients with metastatic disease at initial diagnosis.

3.2 Diagnostic impact of NGS

NGS provided diagnostically relevant information in 61 of 106 patients (57.5%), including diagnostic reclassification in 14 patients (13.2%), diagnostic refinement in 23 (21.7%), and molecular confirmation without a change in diagnosis in 24 (22.6%) (Fig. 2A; Supplementary Table S1). Representative reclassifications included Ewing sarcoma to alveolar rhabdomyosarcoma following detection of a PAX3::FOXO1 fusion. Conversely, the absence of the expected EWSR1::ATF1/EWSR1::CREM fusions in a presumed clear cell sarcoma prompted pathologic reassessment, and the tumor was subsequently reclassified as undifferentiated pleomorphic sarcoma. Diagnostic refinement predominantly involved tumors with broad or nonspecific initial diagnoses. Nineteen of the 23 refined cases (82.6%) had initially been classified as sarcoma, NOS (n = 14), or undifferentiated round/small cell sarcoma (n = 5). Among the 14 sarcoma, NOS cases, four were further classified as dedifferentiated liposarcoma, three as CIC-rearranged sarcoma, and two as NTRK-rearranged spindle cell neoplasm. All five tumors initially classified as undifferentiated round/small cell sarcoma were assigned to specific diagnoses (Fig. 2B).

Some of the diagnostic changes also had implications for choice of subsequent treatments. A tumor was initially diagnosed as fibrosarcoma but was subsequently reclassified as a KIT p.V559G-mutant gastrointestinal stromal tumor (GIST), enabling treatment with imatinib [16]. Also, two tumors with a primary diagnosis of sarcoma, NOS were further classified as NTRK-rearranged spindle cell tumors, for which tropomyosin receptor kinase (TRK) inhibitor therapy may be considered [6, 14].

3.3 Genomic alteration landscape

Sequencing of 110 valid assays identified 2,020 genomic alteration events involving 1,323 genes, with a median of five events per assay. Short variants accounted for 40.9% of all events, followed by copy-number alterations (34.0%), structural variants (18.0%), splicing alterations (6.8%), and other alterations (0.3%). For assay-level genomic analyses, tumors were grouped into translocation-associated sarcomas and a combined group of genomically complex sarcomas and other tumors with recurrent molecular alterations (Supplementary Figure S1) [8]. Assays from genomically complex or other tumors (n = 68) showed recurrent alterations involving TP53 (26/68, 38.2%), CDKN2A (13/68, 19.1%), and ATRX, CDKN2B, and NF1 (9/68 each, 13.2%). Beyond recurrent tumor-type-specific rearrangements, translocation-associated sarcomas (n = 42 assays) harbored additional non-rearrangement alterations, most frequently involving TERT (7/42, 16.7%); MYCN amplification was observed in two of three alveolar rhabdomyosarcomas, and concurrent CDKN2A/CDKN2B loss was observed in both NTRK-rearranged spindle cell neoplasms (Supplementary Figure S2).

Representative histologic subtypes shared with the TCGA sarcoma cohort were examined (Fig. 3A) [7]. DDLPS showed the characteristic 12q13–15 amplification pattern, with MDM2 gain in all 11 assays and CDK4, HMGA2, and TSPAN31 gain in nine assays each (81.8%). LMS and the combined UPS and myxofibrosarcoma (MFS) group showed copy-number losses involving TP53, RB1, and CDKN2A/B. SS showed few additional alterations beyond SS18::SSX, with only PTPN11 and TERT recurring, each in two assays. When the analysis was extended to the full genomic classes at the patient level, genomically complex or other tumors showed higher numbers of altered genes than translocation-associated sarcomas for short variants (median, 1 vs 0; q

= 0.002), copy-number alterations (median, 1 vs 0; $q < 0.001$), and all non-rearrangement alterations (median, 2 vs 0; $q < 0.001$; Fig. 3B). TMB was generally low in the 71 evaluable assays, with a median of 1.50 mut/Mb (interquartile range, 0.97–3.14), and only three assays (4.2%) had values of ≥ 10 mut/Mb.

3.4 Fusions and rearrangements

RNA-based fusion analysis identified fusion transcripts in 35 of 53 patients (66.0%). In addition to established sarcoma-associated fusions, four previously reported uncommon and 12 candidate novel gene-gene fusion transcripts were detected (Fig. 4A). Among the candidate novel events, SFXN1::NRG2 was supported by concordant RNA- and DNA-level findings in a pleomorphic liposarcoma. RNA sequencing identified fusion of SFXN1 exons 1–2 to NRG2 exons 2–10, with corresponding genomic breakpoints in SFXN1 intron 2 and NRG2 intron 1. The predicted transcript retained the NRG2 epidermal growth factor (EGF)-like domain, which mediates binding to ERBB3 and ERBB4 [17], and showed the same exon 2–based 3' configuration as several previously reported in-frame NRG2 α fusions [18, 19]. These structural features support SFXN1::NRG2 as a candidate fusion warranting further investigation.

Diagnostically relevant EWSR1 fusions were identified in 17 patients, involving seven partner genes across eight histologic diagnoses (Fig. 4B). EWSR1::FLI1 and EWSR1::FEV occurred exclusively in Ewing sarcoma in this cohort, whereas the cAMP response element-binding protein (CREB)-family fusions EWSR1::ATF1 and EWSR1::CREM were observed across multiple histologic entities. These findings highlight the additional entity-specific molecular information provided by fusion-partner identification compared with EWSR1 rearrangement status alone [20].

Figure 4. **Fusion profiles and genomic alterations in selected sarcoma subtypes.** (A) Previously reported uncommon and candidate novel gene–gene fusion transcripts identified by RNA-based sequencing, grouped by histologic diagnosis. (B) Distribution of EWSR1 fusion partners across histologic diagnoses. (C) Genomic alterations in undifferentiated small round cell sarcomas of bone and soft tissue, including Ewing sarcoma, CIC-rearranged sarcoma, round cell sarcoma with EWSR1–non-ETS fusion, and sarcoma with BCOR genetic alterations. Each column represents one patient. Colors indicate alteration types.

3.5 Therapeutic outcomes of matched therapy

Treatment-relevant alterations were identified in 35 patients, of whom 11 (31.4%) received matched therapy (Fig. 1; Table 2). Four patients received approved or guideline- recommended regimens, whereas seven received off-label treatments. Most patients who did not receive matched therapy continued standard management or had no current indication for matched treatment (Fig. 1). Among the 11 treated patients, 10 were evaluable for response; three achieved partial responses, six had stable disease, and one had progressive disease, yielding an objective response rate of 30.0% and a disease control rate of 90.0%. Observed PFS durations ranged from 1.1 to 19.8 months, with five patients remaining progression-free at the data cutoff. The patient with the reclassified KIT p.V559G-mutant GIST achieved a partial response to imatinib, with a PFS of 19.8 months. The other two partial responses occurred in a patient with undifferentiated pleomorphic sarcoma harboring MET amplification and rearrangement who received crizotinib and a patient with ERBB2-amplified malignant peripheral nerve sheath tumor who received trastuzumab deruxtecan (Table 2).

Table 2
Therapeutic outcomes of NGS-matched treatment.

Patient ID	Diagnosis	Treatment-relevant finding	Drug	Best RECIST response	PFS, months	Category
P36	Dedifferentiated liposarcoma	TMB-high	Ivonescimab	SD	8.0+	Off-label drug
P43	Gastrointestinal stromal tumor	KIT p.A502_Y503dup	Imatinib	SD	2.4+	Approved drug
P48	Gastrointestinal stromal tumor	KIT p.V559D	Imatinib	NE	4.6+	Approved drug
P50	Dedifferentiated liposarcoma	CDK4 amplification	Palbociclib	SD	5.3+	Off-label drug
P53	Gastrointestinal stromal tumor	KIT p.V559G	Imatinib	PR	19.8	Approved drug
P65	Dermatofibrosarcoma protuberans	COL1A1::PDGFB fusion	Imatinib	SD	11.9	Approved drug
P72	Leiomyosarcoma	MET amplification/ rearrangement	Crizotinib	SD	2.3	Off-label drug
P73	Dedifferentiated liposarcoma	CDK4 amplification	Palbociclib	SD	4.9	Off-label drug
P74	Dedifferentiated liposarcoma	CDK4 amplification	Palbociclib	PD	1.1	Off-label drug
P88	Undifferentiated pleomorphic sarcoma	MET amplification/ rearrangement	Crizotinib	PR	6.0	Off-label drug
P101	Malignant peripheral nerve sheath tumor	ERBB2 amplification	Trastuzumab deruxtecan	PR	7.5+	Off-label drug

NGS, next-generation sequencing; RECIST, Response Evaluation Criteria in Solid Tumors; TMB, tumor mutational burden; NE, not evaluable; PR, partial response; PD, progressive disease; SD, stable disease; PFS, progression-free survival. + indicates that the patient remained progression-free at the data cutoff (May 31, 2026).

4. Discussion

This study provides a real-world assessment of clinical NGS in a histologically diverse sarcoma cohort. Among 106 patients representing 39 histologic diagnoses, NGS provided diagnostically relevant information in 57.5% and identified treatment-relevant alterations in 35 patients, 11 of whom received matched therapy. Genomic analysis also showed distinct alteration patterns in translocation-associated sarcomas and genomically complex or other tumors. Together, these findings highlight the contribution of clinical NGS to

integrated sarcoma diagnosis and classification, while also demonstrating its value for molecular characterization and treatment selection.

Diagnostic impact comprised reclassification, refinement, and molecular confirmation. Reclassification or refinement occurred in 37 of 106 patients (34.9%), and 19 of the 23 refined tumors had initially been classified as sarcoma, NOS or undifferentiated round/small cell sarcoma, suggesting that the diagnostic contribution of NGS was particularly evident when morphology and immunophenotyping did not allow a specific diagnosis, as also reported in previous molecular diagnostic studies of sarcoma [9, 21–24]. NGS was also frequently used early in the diagnostic work-up: 51.8% of assays were performed during the initial diagnostic work-up, with a median interval from diagnosis to NGS of 1.8 months and a median report turnaround time of 10 days. In addition, the identification of seven EWSR1 partners across eight diagnoses illustrated that EWSR1 rearrangement is not specific to a single entity; identification of the fusion partner can narrow the differential diagnosis and aid more specific classification when considered in light of histopathologic and immunophenotypic findings [20].

The TCGA sarcoma study showed that adult soft-tissue sarcomas generally have low mutational burdens, whereas complex-karyotype tumors are characterized by extensive copy-number alterations and recurrent mutations in a limited set of genes, including TP53, ATRX, and RB1 [7]. Subsequent sequencing studies likewise found greater copy-number alterations and more frequent p53/ retinoblastoma (RB) pathway alterations in genomically complex than in translocation-associated sarcomas [4, 8]. These features were also evident in our harmonized 79-gene analysis, with TMB remaining low overall. Our series additionally included 39 diagnoses, among them several uncommon molecularly defined entities, providing real-world molecular data for sarcoma types that remain underrepresented in large genomic studies.

Notably, both EWSR1::NFATC2-positive round-cell sarcomas in our cohort showed copy-number gains involving 20q and 22q. Previous studies have identified recurrent gains involving the NFATC2 locus at 20q13 and the EWSR1 locus at 22q12, together with a distinct DNA methylation profile, in EWSR1::NFATC2-positive sarcomas [25, 26]. These features further distinguish this molecular subtype from conventional Ewing sarcoma and illustrate that, in selected fusion-defined sarcomas, genomic features accompanying the rearrangement can provide classification information beyond identification of the fusion alone.

RNA sequencing also identified uncommon and candidate novel fusion transcripts, among which SFXN1::NRG2 in a pleomorphic liposarcoma was supported by concordant RNA- and DNA-level breakpoints and predicted retention of the NRG2 EGF-like domain. NRG2 encodes a neuregulin ligand whose EGF-like domain mediates signaling through ERBB receptors [17]. Previously reported NRG2α fusions are rare and have been described predominantly in epithelial malignancies; reported fusion structures retain the 3' NRG2 EGF-like domain [18, 19]. In a CD74::NRG2α-positive lung adenocarcinoma, selective ERBB4 phosphorylation provided evidence that the retained domain can engage ERBB signaling [18]. The concordant breakpoints and preserved receptor-binding domain make SFXN1::NRG2 a structurally plausible candidate fusion and support further investigation of NRG2-associated signaling in mesenchymal tumors.

Beyond its diagnostic contribution, NGS findings also guided treatment options. Nearly one third of patients with treatment-relevant alterations received matched therapy in routine practice, with objective responses observed in three of the ten evaluable patients and disease control in nine. In one patient, reclassification of a

KIT-mutant tumor as GIST directly enabled treatment with imatinib, illustrating a direct clinical consequence of diagnostic reclassification. The presence of a treatment-relevant alteration did not necessarily translate into an immediate indication for matched therapy, a gap also reported in previous sarcoma profiling studies [27–30]. The clinical utility of genomic findings therefore depends not only on molecular actionability but also on disease status, available standard therapy, and treatment timing.

Several limitations should be considered. This was a retrospective, single-center study in which NGS was performed when clinically indicated rather than systematically in an unselected sarcoma population; the observed diagnostic impact may therefore not be generalizable to all patients with sarcoma. The clinical panels differed in gene content and analytical approaches. A common 79-gene set was therefore used for cross-platform genomic analyses, while RNA sequencing and TMB were not available for all assays. Lastly, due to the small numbers of each subtype and the lack of a comparator group, treatment outcomes are descriptive. These findings need to be confirmed with multicenter prospective studies using standardized DNA and RNA sequencing and consistent collection of diagnostic and clinical outcomes.

5. Conclusions

In this heterogeneous real-world cohort, clinical NGS played a significant role in integrated sarcoma diagnosis and classification, particularly in tumors with broad or nonspecific initial diagnoses. Genomic and fusion profiling also clarified molecularly defined entities and offered opportunities for treatment, further encouraging the use of NGS in the evaluation of sarcomas.

Abbreviations

CNA

copy-number alteration

COSMIC

Catalogue of Somatic Mutations in Cancer

CREB

cAMP response element-binding protein

DDLPS

dedifferentiated liposarcoma

DNA

deoxyribonucleic acid

EGF

epidermal growth factor

FISH

fluorescence in situ hybridization

GIST

gastrointestinal stromal tumor

LMS

leiomyosarcoma

MFS

myxofibrosarcoma

NCCN

National Comprehensive Cancer Network

NE

not evaluable

NGS

next-generation sequencing

NOS

not otherwise specified

PD

progressive disease

PFS

progression-free survival

PR

partial response

RB

retinoblastoma

RECIST

Response Evaluation Criteria in Solid Tumors

RNA

ribonucleic acid

SD

stable disease

SS

synovial sarcoma

SV

structural variant

TCGA

The Cancer Genome Atlas

TMB

tumor mutational burden

TRK

tropomyosin receptor kinase

UPS

undifferentiated pleomorphic sarcoma

WHO

World Health Organization.

Declarations

Ethics approval and consent to participate

This study was approved by the Medical Ethics Committee of Nanjing Drum Tower Hospital (No. 2026 – 0470; approval date, May 13, 2026) and was conducted in accordance with the Declaration of Helsinki. Informed consent to participate was obtained by telephone, and the requirement for a written signature on the informed consent form was waived by the ethics committee.

Consent for publication

The requirement for consent for publication was waived by the Medical Ethics Committee of Nanjing Drum Tower Hospital.

Competing interests

The authors declare that they have no competing interests.

Funding

No funding was received for this study.

Author Contribution

XZ collected the cases, analyzed the data, and drafted the manuscript. JL contributed to data analysis. ZL reviewed the pathological diagnoses. ZW curated the sequencing data. SW critically revised the manuscript. RL conceived and designed the study, supervised the work, and critically revised the manuscript. All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

Data Availability

The de-identified processed genomic dataset generated and/or analysed during the current study is available in the ScienceDB repository, <https://www.scidb.cn/anonymous/QXJRRjdm>. The sequencing results analysed in this study were derived from final clinical NGS reports, and raw sequencing data were not centrally available to the investigators.

References

1. van der Graaf WTA, Orbach D, Judson IR, Ferrari A. Soft tissue sarcomas in adolescents and young adults: a comparison with their paediatric and adult counterparts. *Lancet Oncol*. 2017;18:e166–75. 10.1016/S1470-2045(17)30099-2.

2. WHO Classification of Tumours Editorial Board. Soft Tissue and Bone Tumours. Volume 3, 5th ed. Lyon: International Agency for Research on Cancer; 2020.
3. Gronchi A, Miah AB, Dei Tos AP, Abecassis N, Bajpai J, Bauer S, et al. Soft tissue and visceral sarcomas: ESMO-EURACAN-GENTURIS Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol.* 2021;32:1348–65. 10.1016/j.annonc.2021.07.006.
4. Scheipl S, Brcic I, Moser T, Fischerauer S, Riedl J, Bergovec M, et al. Molecular profiling of soft-tissue sarcomas with FoundationOne® Heme identifies potential targets for sarcoma therapy: a single-centre experience. *Ther Adv Med Oncol.* 2021;13:17588359211029125. 10.1177/17588359211029125.
5. Cote GM, He J, Choy E. Next-generation sequencing for patients with sarcoma: a single center experience. *Oncologist.* 2018;23:234–42. 10.1634/theoncologist.2017-0290.
6. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®): Soft Tissue Sarcoma. Version 5.2026.
https://www.nccn.org/professionals/physician_gls/pdf/sarcoma.pdf. Accessed 30 Jul 2026.
7. Cancer Genome Atlas Research Network. Comprehensive and integrated genomic characterization of adult soft tissue sarcomas. *Cell.* 2017;171:950–e96528. 10.1016/j.cell.2017.10.014.
8. Gounder MM, Agaram NP, Trabucco SE, Robinson V, Ferraro RA, Millis SZ, et al. Clinical genomic profiling in the management of patients with soft tissue and bone sarcoma. *Nat Commun.* 2022;13:3406. 10.1038/s41467-022-30496-0.
9. Watkins JA, Trotman J, Tadross JA, Harrington J, Hatcher H, Horan G, et al. Introduction and impact of routine whole genome sequencing in the diagnosis and management of sarcoma. *Br J Cancer.* 2024;131:860–9. 10.1038/s41416-024-02721-8.
10. 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. 10.1038/s41467-022-30453-x.
11. Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer. (2026). Mitelman F, Johansson B, Mertens F and Paulsson K, editors. <https://mitelmandatabase.isb-cgc.org>. Accessed 30 Jul 2026.
12. Sondka Z, Bindal Dhir N, Carvalho-Silva D, Jupe S, Madhumita, McLaren K, et al. COSMIC: a curated database of somatic variants and clinical data for cancer. *Nucleic Acids Res.* 2024;52:D1210–7. 10.1093/nar/gkad986.
13. Kim P, Tan H, Liu J, Lee H, Jung H, Kumar H, et al. FusionGDB 2.0: fusion gene annotation updates aided by deep learning. *Nucleic Acids Res.* 2022;50:D1221–30. 10.1093/nar/gkab1056.
14. Chakravarty D, Gao J, Phillips SM, Kundra R, Zhang H, Wang J, et al. OncoKB: a precision oncology knowledge base. *JCO Precis Oncol.* 2017;1:1–16. 10.1200/PO.17.00011.
15. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer.* 2009;45:228–47. 10.1016/j.ejca.2008.10.026.
16. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®): Gastrointestinal Stromal Tumors. Version 1.2026.
https://www.nccn.org/professionals/physician_gls/pdf/gist.pdf. Accessed 30 Jul 2026.

17. Carraway KL 3rd, Weber JL, Unger MJ, Ledesma J, Yu N, Gassmann M, et al. Neuregulin-2, a new ligand of ErbB3/ErbB4-receptor tyrosine kinases. *Nature*. 1997;387:512–6. 10.1038/387512a0.
18. Kohsaka S, Hayashi T, Nagano M, Ueno T, Kojima S, Kawazu M, et al. Identification of novel CD74-*NRG2α* fusion from comprehensive profiling of lung adenocarcinoma in Japanese never or light smokers. *J Thorac Oncol*. 2020;15:948–61. 10.1016/j.jtho.2020.01.021.
19. Ou SI, Xiu J, Nagasaka M, Xia B, Zhang SS, Zhang Q, et al. Identification of novel CDH1-*NRG2α* and F11R-*NRG2α* fusions in NSCLC plus additional novel *NRG2α* fusions in other solid tumors by whole transcriptome sequencing. *JTO Clin Res Rep*. 2021;2:100132. 10.1016/j.jtocrr.2020.100132.
20. Krystel-Whittemore M, Taylor MS, Rivera M, Lennerz JK, Le LP, Dias-Santagata D, et al. Novel and established *EWSR1* gene fusions and associations identified by next-generation sequencing and fluorescence in-situ hybridization. *Hum Pathol*. 2019;93:65–73. 10.1016/j.humpath.2019.08.006.
21. Schipper LJ, Monkhorst K, Samsom KG, Bosch LJW, Snaebjornsson P, van Boven H, et al. Clinical impact of prospective whole genome sequencing in sarcoma patients. *Cancers (Basel)*. 2022;14:436. 10.3390/cancers14020436.
22. Italiano A, Di Mauro I, Rapp J, Pierron G, Auger N, Alberti L, et al. Clinical effect of molecular methods in sarcoma diagnosis (GENSARC): a prospective, multicentre, observational study. *Lancet Oncol*. 2016;17:532–8. 10.1016/S1470-2045(15)00583-5.
23. Öfverholm I, Wallander K, Haglund C, Chellappa V, Wejde J, Gellerbring A, et al. Comprehensive genomic profiling alters clinical diagnoses in a significant fraction of tumors suspicious of sarcoma. *Clin Cancer Res*. 2024;30:2647–58. 10.1158/1078-0432.CCR-24-0384.
24. Sreenath ND, Singh K, Rastogi S, Biswal N, Singh A, Barwad A, et al. Clinical impact of integrating RNA-based next-generation sequencing into the diagnostic evaluation of soft tissue sarcomas: insights from a single-center multidisciplinary workflow. *JCO Glob Oncol*. 2026;12:e2500440. 10.1200/GO-25-00440.
25. Szuhai K, Ijszenga M, de Jong D, Karseladze A, Tanke HJ, Hogendoorn PCW. The *NFATc2* gene is involved in a novel cloned translocation in a Ewing sarcoma variant that couples its function in immunology to oncology. *Clin Cancer Res*. 2009;15:2259–68. 10.1158/1078-0432.CCR-08-2184.
26. Koelsche C, Kriegsmann M, Kommoss FKF, Stichel D, Kriegsmann K, Vokuhl C, et al. DNA methylation profiling distinguishes Ewing-like sarcoma with *EWSR1-NFATc2* fusion from Ewing sarcoma. *J Cancer Res Clin Oncol*. 2019;145:1273–81. 10.1007/s00432-019-02895-2.
27. Nakata E, Ennishi D, Osone T, Ninomiya K, Tomida S, Itano T, et al. Real-world data of comprehensive cancer genomic profiling tests performed in the routine clinical setting in sarcoma. *Cancer Med*. 2025;14:e71098. 10.1002/cam4.71098.
28. Groisberg R, Hong DS, Holla V, Janku F, Piha-Paul SA, Ravi V, et al. Clinical genomic profiling to identify actionable alterations for investigational therapies in patients with diverse sarcomas. *Oncotarget*. 2017;8:39254–67. 10.18632/oncotarget.16845.
29. Boddu S, Walko CM, Bienasz S, Bui MM, Henderson-Jackson E, Naghavi AO, et al. Clinical utility of genomic profiling in the treatment of advanced sarcomas: a single-center experience. *JCO Precis Oncol*. 2018;2:1–8. 10.1200/PO.18.00096.
30. Gushe CA, Weiss MC, Lee L, Gitelis S, Blank AT, Wang D, et al. The clinical utility of next-generation sequencing for bone and soft tissue sarcoma. *Acta Oncol*. 2022;61:38–44.

Figures

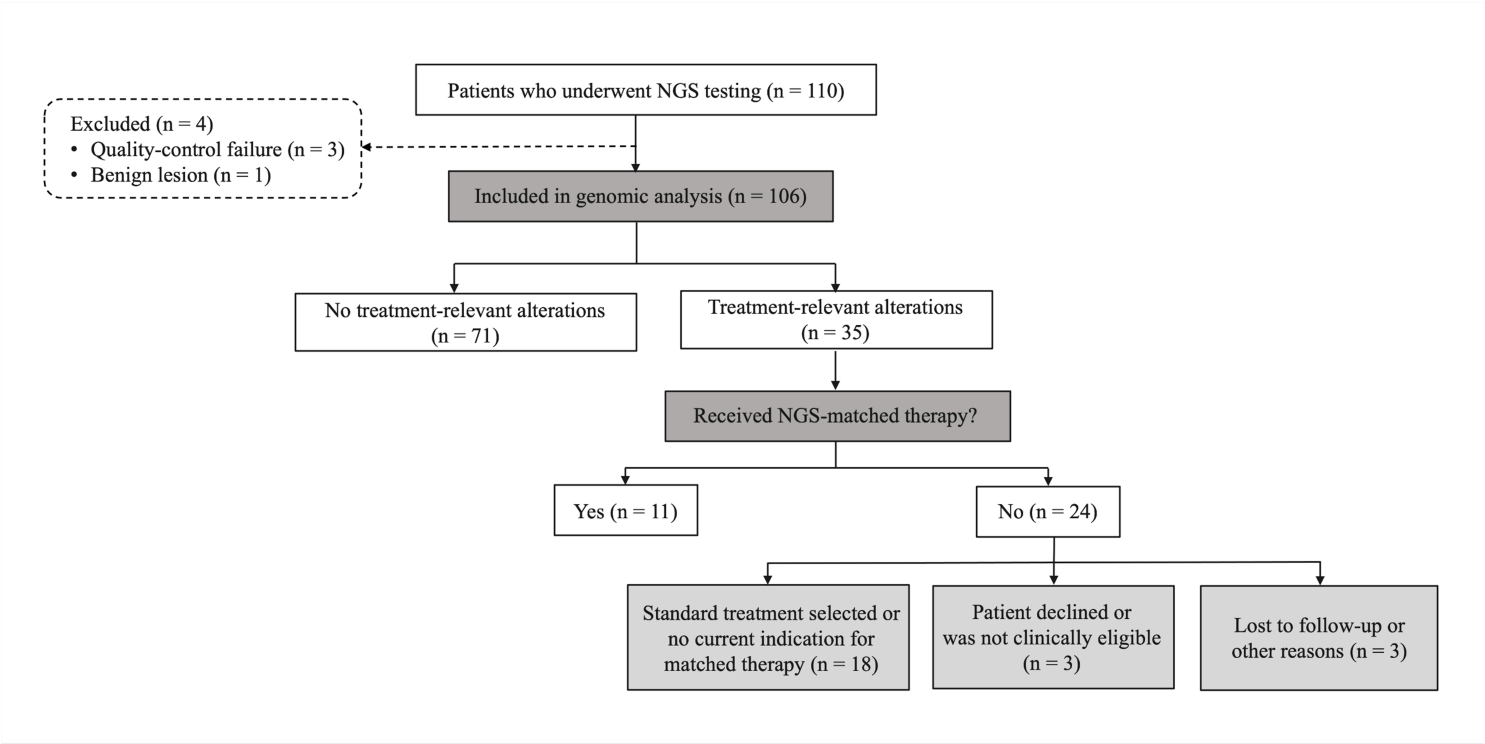


Figure 1

Patient selection and NGS-matched therapy workflow. NGS, next-generation sequencing.

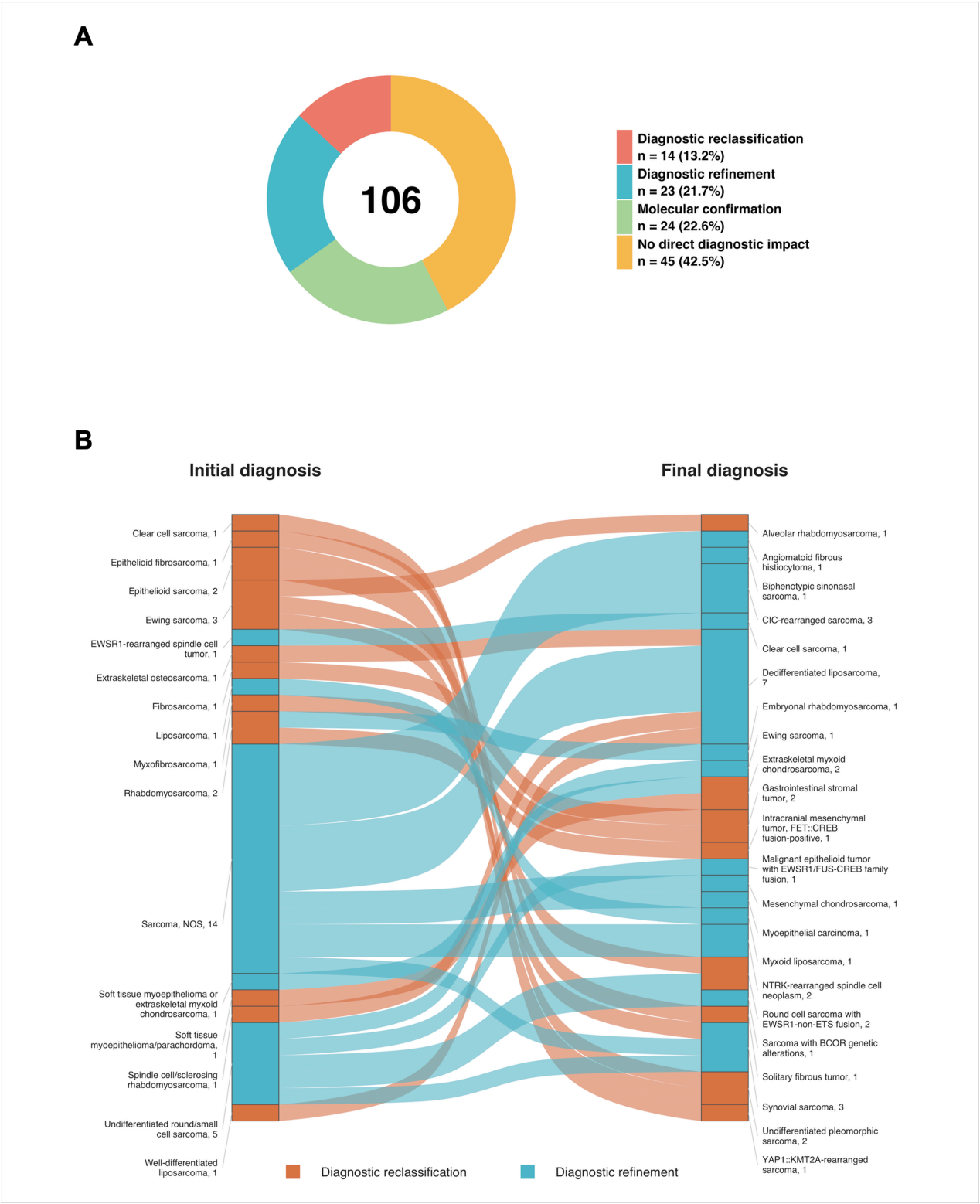
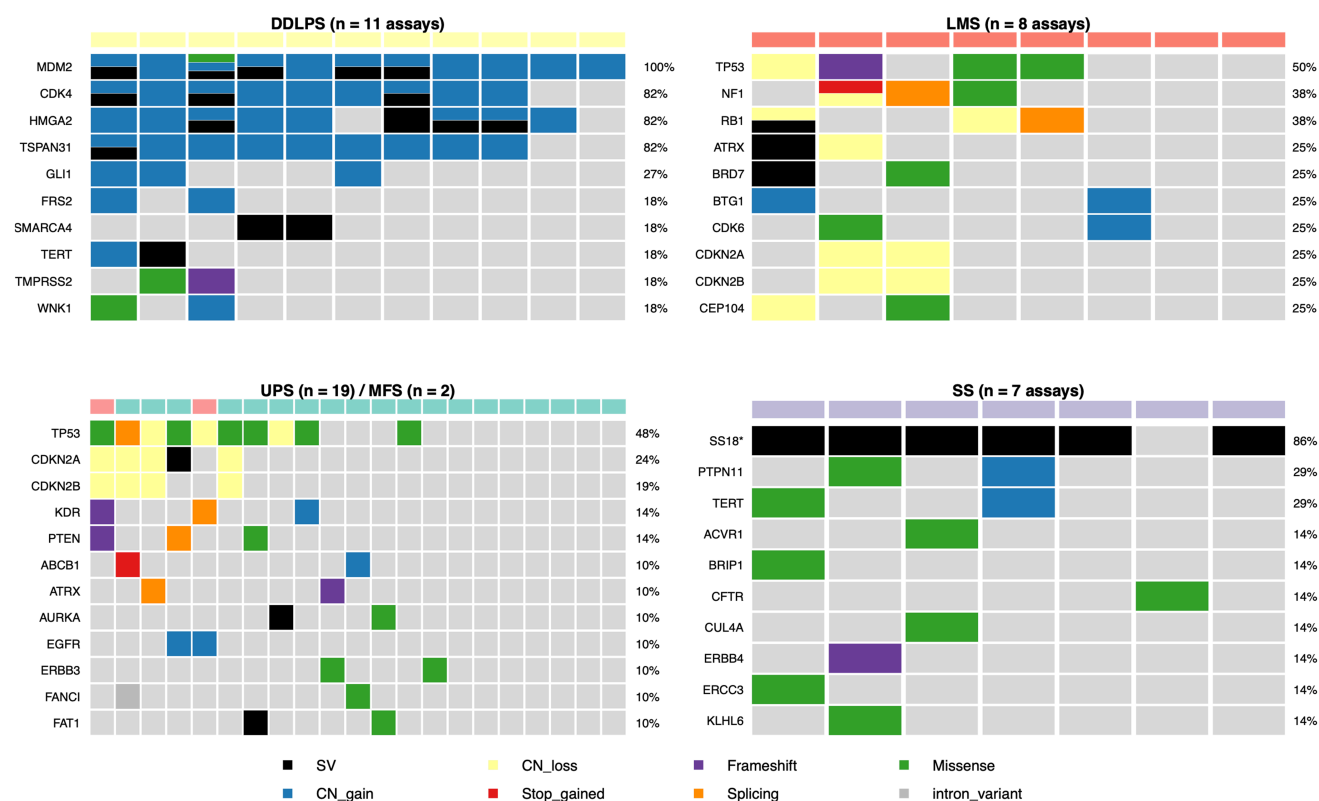


Figure 2

Diagnostic impact of next-generation sequencing. (A) Distribution of diagnostic impact categories among 106 patients. (B) Sankey diagram illustrating diagnostic reclassification or refinement in 37 of 106 patients (34.9%). Left, the number of patients assigned to each initial diagnosis; right, the final diagnoses after integrated pathologic and genomic review. NOS, not otherwise specified.

A



B

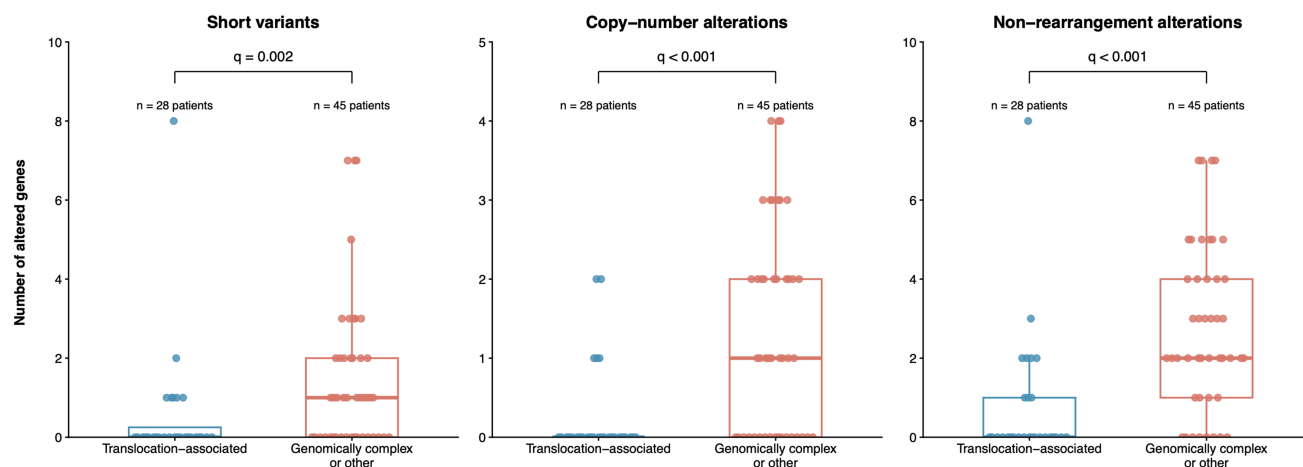


Figure 3

Genomic alteration patterns in representative sarcoma subtypes and across genomic classes. (A) Oncoplots of DDLPS, LMS, UPS/MFS, and SS. Columns represent sequencing assays, rows represent genes, and colors indicate alteration types. Percentages indicate the proportion of altered assays within each subtype. (B) Short variant, copy-number alteration, and non-rearrangement alteration burdens within a 79-gene common DNA set in translocation-associated versus genomically complex or other tumors. Non-rearrangement alterations

comprised short variants and CNAs. Points represent individual patients, and boxes indicate medians and interquartile ranges. Two-sided Mann–Whitney U tests were used, with Benjamini–Hochberg correction for three comparisons; adjusted values are shown as q values. *In one of the seven SS cases, an SS18 rearrangement was confirmed by break-apart FISH but was not detected by NGS. CNA, copy-number alteration; DDLPS, dedifferentiated liposarcoma; FISH, fluorescence in situ hybridization; LMS, leiomyosarcoma; MFS, myxofibrosarcoma; NGS, next-generation sequencing; SS, synovial sarcoma; SV, structural variant; UPS, undifferentiated pleomorphic sarcoma.

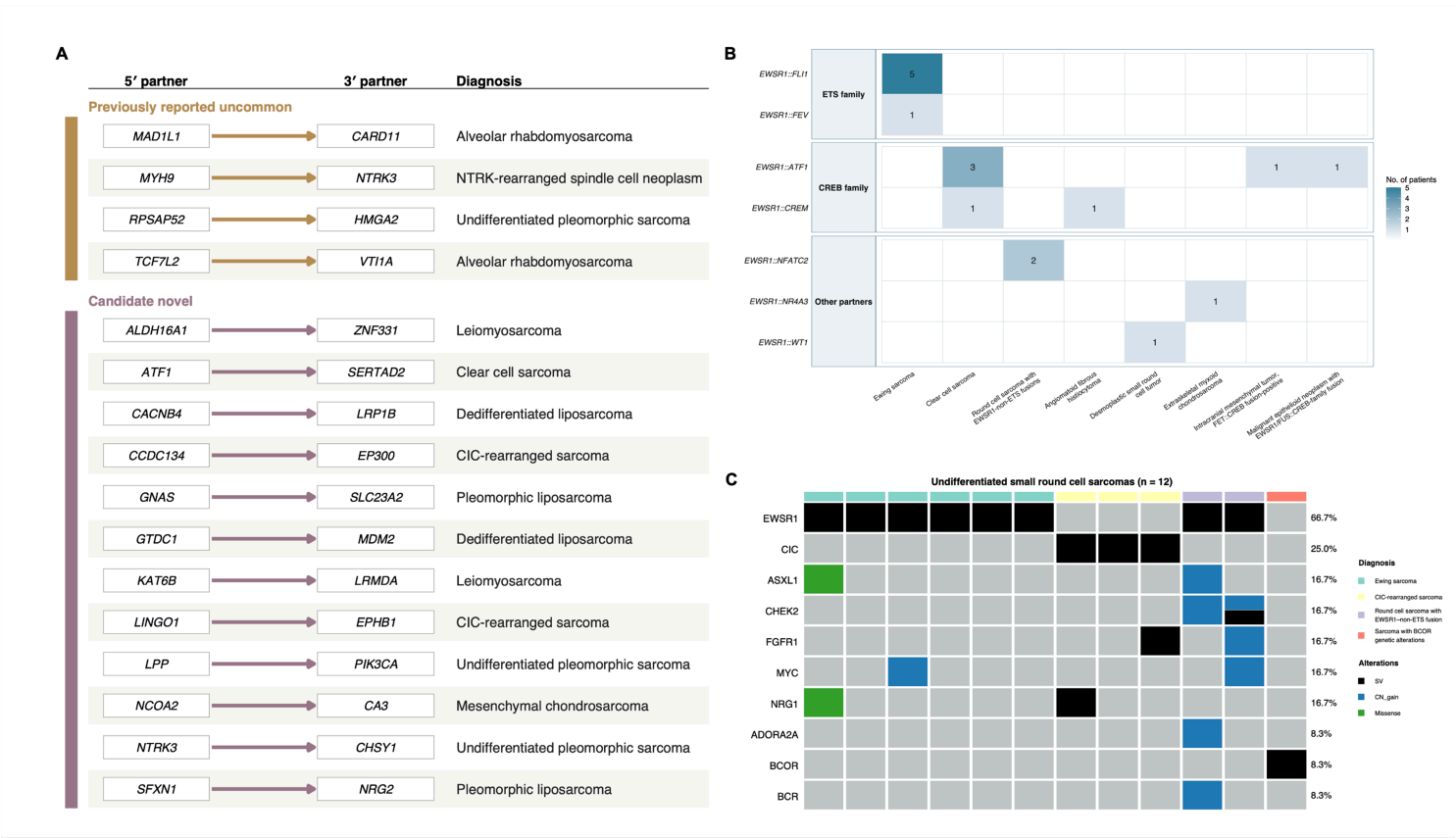


Figure 4

Fusion profiles and genomic alterations in selected sarcoma subtypes. (A) Previously reported uncommon and candidate novel gene–gene fusion transcripts identified by RNA-based sequencing, grouped by histologic diagnosis. (B) Distribution of EWSR1 fusion partners across histologic diagnoses. (C) Genomic alterations in undifferentiated small round cell sarcomas of bone and soft tissue, including Ewing sarcoma, CIC-rearranged sarcoma, round cell sarcoma with EWSR1–non-ETS fusion, and sarcoma with BCOR genetic alterations. Each column represents one patient. Colors indicate alteration types.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Additionalfile1.pdf](#)