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Molecular biomarkers impact survival after neoadjuvant chemoradiation in locally advanced soft tissue sarcoma patients

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Research Article

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Molecular biomarkers impact survival after neoadjuvant chemoradiation in locally advanced soft tissue sarcoma patients

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Abstract

Background: Locally advanced, marginally resectable soft tissue sarcomas (STS) are frequently treated with multimodal neoadjuvant strategies to enable limb-sparing, R0 resection. However, pathological response (PR) to neoadjuvant chemoradiation is heterogeneous, and robust molecular predictors of response are lacking. We performed comprehensive DNA/RNA profiling to identify genomic correlates of PR and clinical outcomes in a real-world cohort treated with a uniform neoadjuvant chemoradiation regimen.

Methods: We retrospectively analyzed pre-treatment biopsies from 27 patients with high-grade, marginally resectable STS treated at a single sarcoma center (median age 55 years;

range 28–74; 62.96% male). Histological subtypes included undifferentiated pleomorphic sarcoma (44.44%), myxofibrosarcoma (29.63%), malignant peripheral nerve sheath tumor (14.82%), myxoid liposarcoma (7.41%), and pleomorphic liposarcoma (3.70%). Targeted DNA/RNA sequencing was performed using the TruSight Oncology 500 assay to identify single-nucleotide variants (SNV), copy number variation (CNV), and gene fusions. Pathological response was assessed in resection specimens based on the proportion of viable tumor. Overall survival (OS), local recurrence-free survival (LRFS), and distant metastasis-free survival (DMFS) were estimated using the Kaplan–Meier method.

Results: The cohort displayed a complex genomic landscape with recurrent SNV/CNV events and frequent fusion calls across STS subtypes. Presence of gene fusions was associated with improved OS ($p = 0.043$), whereas PR itself was not significantly associated with survival ($p = 0.427$). Exploratory analyses identified molecular features related to PR: *ROS1*-associated gene fusions were observed exclusively in tumors with favorable PR, while *MDM2* gene amplification and *PTEN* deletion were linked to poor PR. Additionally, likely pathogenic variants of *TP53, PLCG2, PIK3C2B, GABRA6,* and *KMT2C* genes were clustered in poor-response cases. Molecular profiling also flagged potential diagnostic reclassification in selected tumors via highly specific fusion events, underscoring its value beyond prognostication.

Conclusions: Comprehensive DNA/RNA profiling in locally advanced STS treated with neoadjuvant chemoradiation suggests that fusion status and specific actionable alterations may stratify response biology and survival. These hypothesis-generating biomarkers, particularly *ROS1*-associated gene fusions, *MDM2* gene amplification, and *PTEN* loss — require validation in larger, histology-controlled cohorts and may inform future molecularly guided neoadjuvant strategies.

Trial registration: NCT03651375.

Keywords: neoadjuvant treatment, soft tissue sarcoma, treatment response, genetic profile, gene fusion, CNV, SNV, diagnostic biomarker, predictive biomarker

Background

Soft tissue sarcomas (STS) are a rare but highly aggressive group of malignant neoplasms accounting for less than 1% of all solid malignancies in adults. Due to rarity and

heterogeneity, the diagnosis of STS is challenging. More than 100 subtypes of STS have been described (1, 2). However, soft tissue malignancies are divided into about 70 subtypes, each distinguished by morphology that often leads to different clinical characteristics and requires specific therapeutic approaches (2). Gene fusions are common in STS and occur in one-third of STS cases. Gene fusion is an important engine mutation of STS and has thus emerged as a potential treatment target (3). Even with widespread immunohistochemical markers, genetic analysis of certain fusions is essential, especially in rare cases or in difficult differential diagnosis (3). Some types of gene fusion are subtype-specific. The *SS18::SSX* family is the type of gene fusion characteristic of synovial sarcomas (3-5), *FUS::DDIT3* is specific for myxoid liposarcomas (3, 4, 6-8), *COL1A1::PDGFB* is dermatofibrosarcomas protuberans-specific (3, 8, 9). The *PAX3::FOXO1* and *PAX7::FOXO1* gene fusions specifically occur in alveolar rhabdomyosarcomas (3, 4, 8, 10), *NAB2::STAT6* is specific for solitary fibrous tumors (3, 11, 12), and *EWSR1::FLI1* is the type of Ewing sarcoma-specific (3, 13, 14).

Locally advanced marginally resectable primary extremity high-risk STS require intensive preoperative treatment before possible limb-sparing surgery (15). Therefore, unresectableand marginally resectable sarcoma remains a treatment challenge for doctors due to the absence of a standard, highly effective targeted treatment. At present, most patients are treated with anthracycline-based neoadjuvant chemotherapy and/or radiation therapy (15, 16). Although chemotherapy radiotherapy is highly toxic, there are no biomarkers that predict the reaction of neoadjuvant therapy. The identification of molecular biomarkers of pathological response (PR) can help to select personalized treatments (17-20). Standard therapies are palliative in the metastatic disease, where the median overall survival (OS) is 12–24 months (21-24). Few molecular-guided therapeutics have proven efficacy for sarcomas, such as imatinib for gastrointestinal stromal tumors (GIST) with *KIT* or *PDGFRA* variants and tyrosine kinase inhibitors for *ALK* and *NTRK*-associated fusion-driven inflammatory myofibroblastic tumors (IMT) and sarcoma not otherwise specified (NOS) (24). However, molecular biology and STS genetic characteristics are still unknown.

Currently, the most frequently altered genes in STS are known to be *TP53*, *CDKN2A*, *RB1*, *NF1*, *ATRX*, *BRCA2*, *ATM*, *ATR*, and *ERCC2* genes (25). Based on a study by Nacev et al., a comparative genetic analysis of 2,138 sarcomas across 45 subtypes showed that the most common aberrations were in the cell cycle control genes, *TP53*, receptor tyrosine kinases, genes related to PI3K/Akt and MAPK pathways, and the epigenetic regulators (26). In addition,Zhang and colleagues found that the most common changes in STS were found in the

TP53, CDKN2A, CDKN2B, MAP3K1, LRP1B, MDM2, RB1, PTEN, MYC, and *CDK4* genes (27). At the same time, no biomarkers could predict the potential effect of chemotherapy and radiotherapy (20, 28).

In this work, we illustrate the genomic alterations in STS, revealing the potential clinical utility of targeted next-generation sequencing (NGS) for the diagnosis, prognosis, and treatment of connective tissue malignancies.

Methods

Patient cohort and inclusion criteria

Our translational study enrolled 27 patients (Table 1) diagnosed with marginally resectable STS. Patients started multidisciplinary treatment between 1/1/2017 and 31/12/2023 with the UNRESARC regimen, as previously described (29, 30). Of all patients enrolled in the translational study, 16 patients participated in the phase II clinical trial (NCT03651375) conducted in our department (31), while the next 11 were treated in the expansion cohort under the regular National Health Insurance Reimbursement system (32). Date of last follow-up was 26/01/2026.

The inclusion criteria for the study were: age >18 years, STS diagnosis confirmed by a sarcoma pathologist, availability of pre-treatment primary tumor tissue, and completion of UNRESARC treatment as per protocol. All patients had at least one year of follow-up. Exclusion criteria included prior perioperative therapy with chemotherapy and/or radiotherapy or recurrent disease after primary tumor resection. The project received approval from the local Bioethics Committee (No. 8/2017/1/2018 and 52/2024).

Table 1. *Baseline patients characteristics*

Characteristics	**Value, n (%)**
Age at diagnosis	
Median	55
Range	28-74
Gender	**n (%)**
Female	10 (37.04)
Male	17 (62.96)
Sarcoma subtype	**n (%)**

Undifferentiated pleomorphic sarcoma (UPS)	12 (44.44)
Myxofibrosarcoma (MFS)	8 (29.63)
Malignant peripheral nerve sheath tumor (MPNST)	4 (14.82)
Myxoid liposarcoma (MLPS)	2 (7.41)
Pleomorphic liposarcoma (PLPS)	1 (3.7)
Tumor localization	**n (%)**
Trunk wall	3 (11.11)
Chest	1 (3.7)
Arm/shoulder	1 (3.7)
Forearm	1 (3.7)
Thigh	19 (70.37)
Lower leg	2 (7.41)
Largest tumor dimension before treatment	**n (%)**
5-10 cm	7 (25.93)
>10-15 cm	9 (33.33)
>15-20 cm	6 (22.22)
>20-25 cm	1 (3.7)
>25-30 cm	2 (7.41)
>30 cm	2 (7.41)

Sample collection and preparation

The study material consisted of tissues fixed in buffered 10% formalin (fixation time no longer than 48 hours) and embedded in formalin-fixed paraffin-embedded (FFPE) blocks. To validate the molecular analysis, material from available healthy tissue from FFPE blocks (including lymph node, skin, or lung) was used. The evaluation of PR was conducted as we described before in our protein biomarker study (33).

Nucleic acid extraction

DNA and RNA were isolated from tissue sections (generally 6 × 10 μm) using the Agencourt FormaPure Kit (Beckman Coulter). DNA concentrations were measured using the Qubit High Sensitivity kit (Thermo Fisher Scientific, Waltham, MA). For library preparation, 40 ng DNA /RNA was used as input.

Next-generation sequencing

DNA and RNA library preparation was performed using the TruSight™ 500 hybrid capture–based library preparation kit, following the manufacturer's protocol (34). Sequencing was performed on an Illumina NextSeq 500 and an Illumina NovaSeq 6000 system.

Previously extracted RNA was used to prepare a cDNA library with FusionPlex® Sarcoma v2 (Archer™, CA, USA) (35). The final targeted amplicons were sequenced on an Illumina NextSeq 500 system.

Bioinformatic analysis

Gene fusion analysis was performed using the RNA sequencing files and the same bioinformatics workflow for both TruSight™ 500- and FusionPlex® Sarcoma v2-sequenced samples and controls (36). However, for the second FusionPlex® Sarcoma v2 panel, analysis began with FASTQ files, which were further converted according to the analysis workflow using the Arriba workflow (Supplementary Figure SF1) (37).

Control samples' data was used to filter somatic mutations and sequencing artifacts. DNA sequencing files were used for copy number variation (CNV) and single-nucleotide variant (SNV) analysis. For CNV analysis, mapped samples were filtered to exclude results detected in control samples. After analysis in PureCN, an additional filtration step was performed (Supplementary Figure SF2).

For SNV analysis, variant calling was performed with FreeBayes and Mutect2. The results of both analyses were intersected, excluding results present in only one of the two analyses and keeping flags from Mutetc2 variant calling. Funcotator, which, like Mutect2, is part of the Genome Analysis Toolkit (GATK) package, was used for annotation (38). The .vcf files were then analyzed using OpenCRAVAT (Supplementary Figure SF3). To exclude potentially germline variants, we filtered out results from the dbSNP Common database and the gnomAD Gene Global database with a frequency greater than 1%. To exclude potentially germline variants, we filtered out results from the dbSNP Common database and the gnomAD Gene Global database with a frequency greater than 1%.

Statistical analysis

Data are described using descriptive statistics for continuous variables, while categorical variables were reported as frequencies and percentages. The death date was confirmed in the Polish National Healthcare Registry using the personal identification number

(26/01/2026). The patients alive or without disease progression at the date of data retrieval were censored. OS was calculated from the start of treatment to the date of death or last follow-up (censored). Local recurrence-free survival (LRFS) was calculated from the date of the radical resection of the primary tumor up to the date of radiologically confirmed local recurrence/date of death, or last follow-up (censored). Distant metastasis-free survival (DMFS) was calculated from the start of treatment to the date of radiologically confirmed distant metastases/date of death, or to the last follow-up (censored). Relapse-free survival (RFS) was calculated from the start of treatment to the date of radiologically confirmed local recurrence or distant metastases/date of death, or to the last follow-up (censored). The Kaplan–Meier method with the log-rank test and Gehan–Breslow–Wilcoxon test were used to plot and assess differences in survival curves. With all point estimates, 95% confidence intervals (CI) were reported. No adjustment for multiple testing was performed. Differences were considered statistically significant when $p < 0.05$.

Fisher's exact test was used to compare univariate proportions and the non-parametric Mann–Whitney U to compare continuous distributions. Statistical tests and computations were performed using GraphPad Prism 9.4.0.

Results

General molecular characterization of patients

In the study group, we reported an average of almost 9 aberrations per patient. The most common aberration was CNV, accounting for 53% of all alterations detected (Supplementary Figure SF4). Among CNVs, deletions were more frequent. The most frequently altered genes were *PLCG2* and *HMGA2* (Supplementary Figure SF5). However, all seven *HMGA2* gene translocations were detected in a single patient.

Fusion detection

In total, we detected 82 gene fusions in the study group. In more than 20% of the patients, no gene fusion was detected (n = 6). After excluding low-coverage positions, we identified 53 gene fusions. On average, there were two gene fusions per patient. Of these, more than 30% involved intergenic regions (n = 17) (Supplementary Figure SF6). Of all detected gene fusions, 17 gene fusions were detected in the study group, which had one gene partner with a coverage of less than 10x (Supplementary Table 1). These gene fusions were not excluded due to high coverage of the second gene partner (coverage above 300x, often over 1000x). Interestingly, however, in the case of the *FUS::DDIT3* gene fusion, two patients were diagnosed with myxoid liposarcoma (MLPS), and one patient with this fusion was

diagnosed with myxofibrosarcoma (MFS). In the case of the *NAB2::STAT6* gene fusion - it was detected in an MFS case. Over 90% of gene fusions were detected in sarcomas not typically considered as fusion-related sarcomas (Supplementary Figure SF9-12). A large fragment DNA deletion and 2 duplications were detected, resulting in the formation of fusion proteins as estimated by further analysis. The remaining fusion proteins were produced by translation, including about 30% inversions (n = 16) (Supplementary Figure SF7).

The most common gene fusion was *FUS::DDIT3*. Most gene fusions included genes related to cell signalling – MAPK or PI3K-AKT-mTOR pathways (e.g., *PIK3CA, ROS1, JAK2, AKT3, FGFR1*) and genes that encode transcriptional factors such as *FOXO1, TP53,* and *HGMA2* (Figure 1).

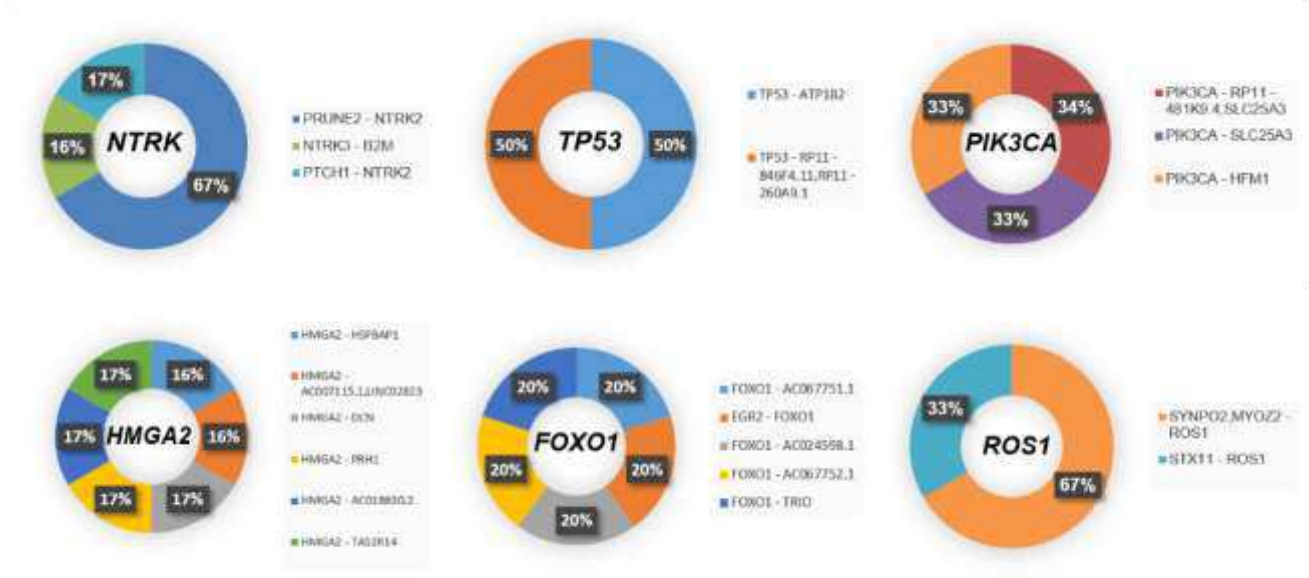
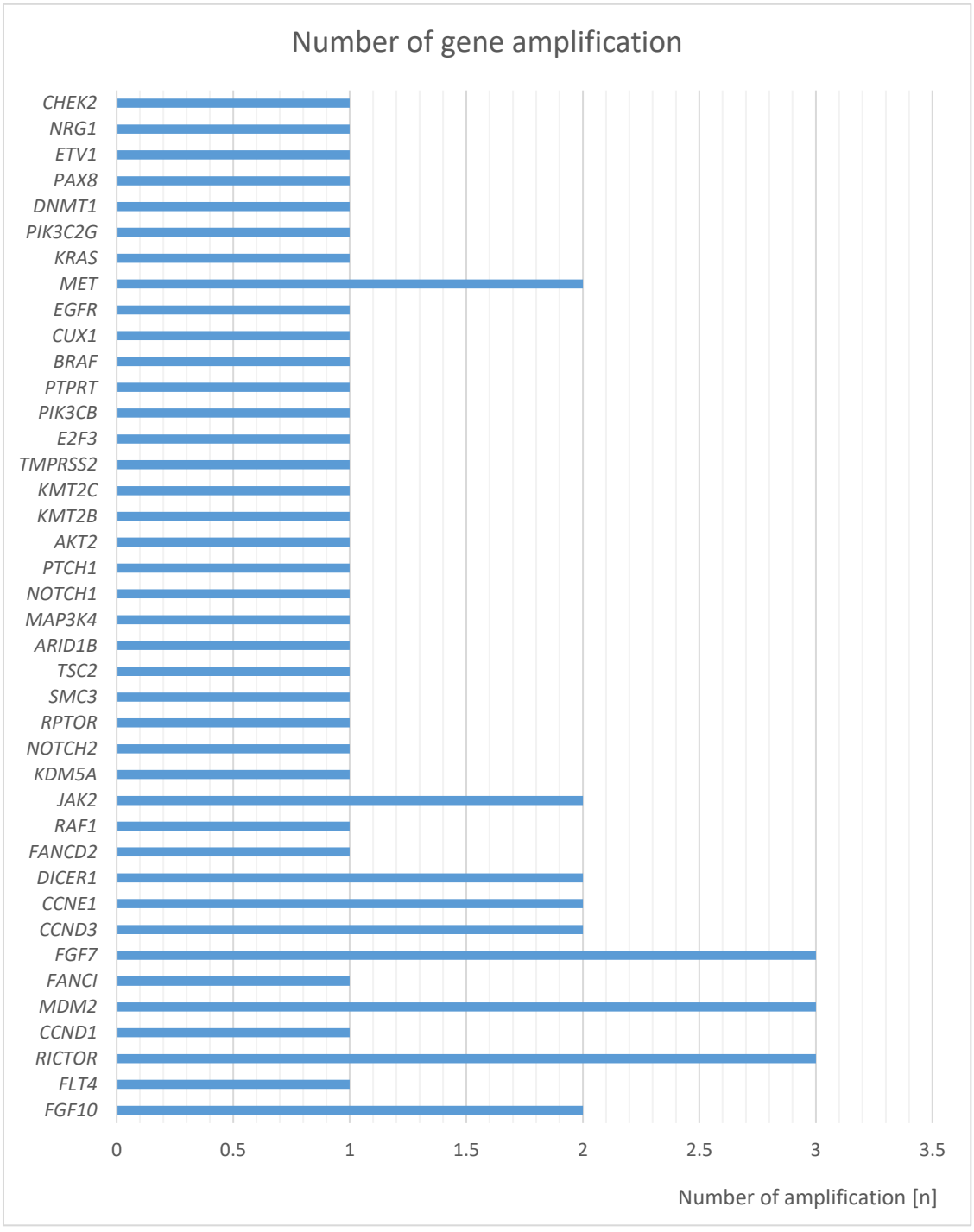


Figure 1. The most common gene fusion detected in the study cohort

Copy number variation analysis

We detected 124 gene copy number alterations in the study cohort. Over 30% of patients had no change in gene copy number (n = 9). We detected 52 high-level gene amplifications of more than 5 copies. More than 40% of detected amplifications exceeded 10 gene copies. In less than 45% of patients, no gene amplification was detected (n = 12). The most frequently amplified genes were *MDM2, FGF7*, and *RICTOR* (5,8%, respectively) (Figure 2A). More than 50% of the amplifications involved genes related to signaling pathways. *RICTOR* and *FGF7* gene amplifications were detected exclusively in patients with UPS, and these were often among the most common amplifications. Interestingly, in two

223 cases, *RICTOR* and *FGF10* amplification occurred in the same patient, suggesting a co-
224 occurrence.



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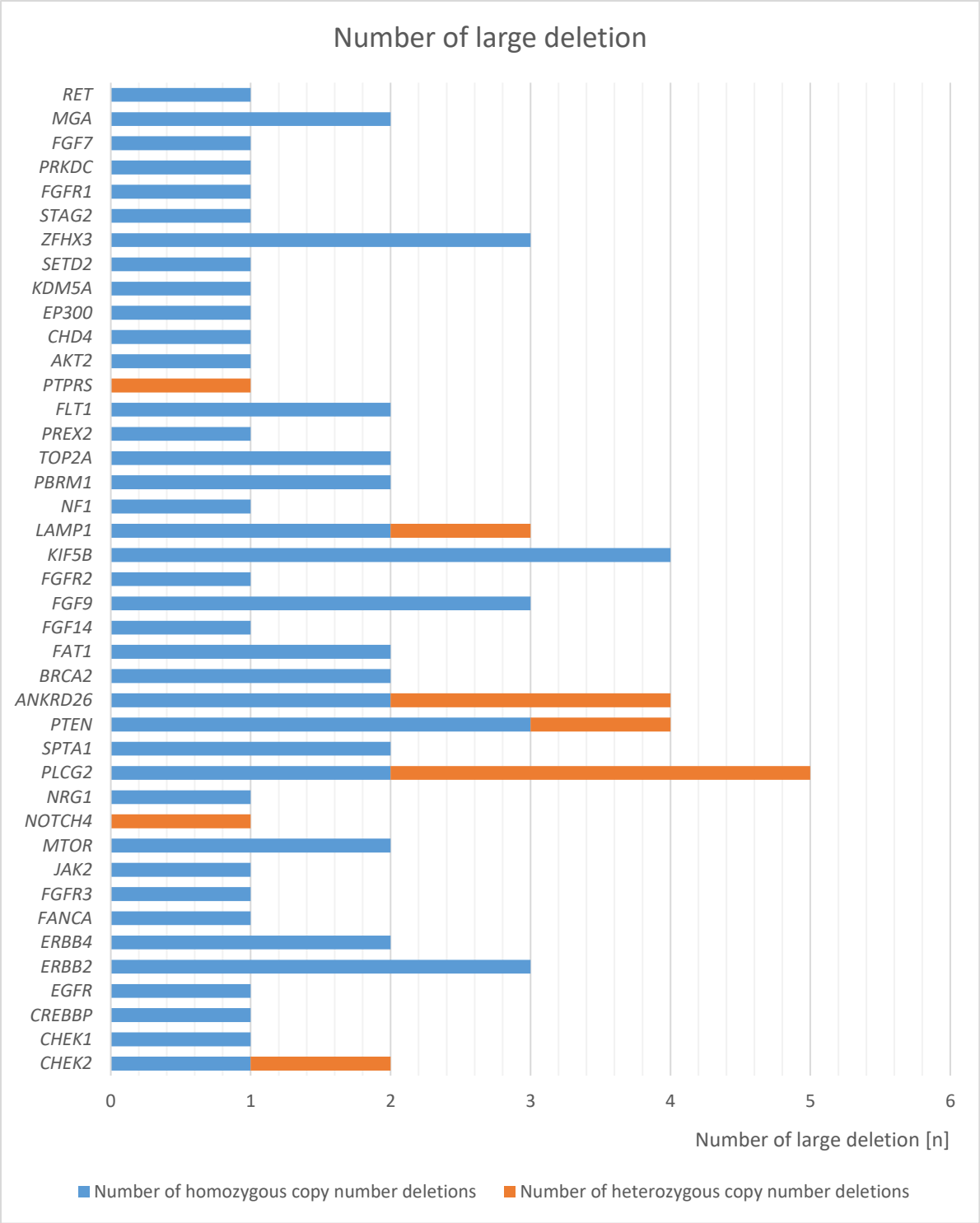


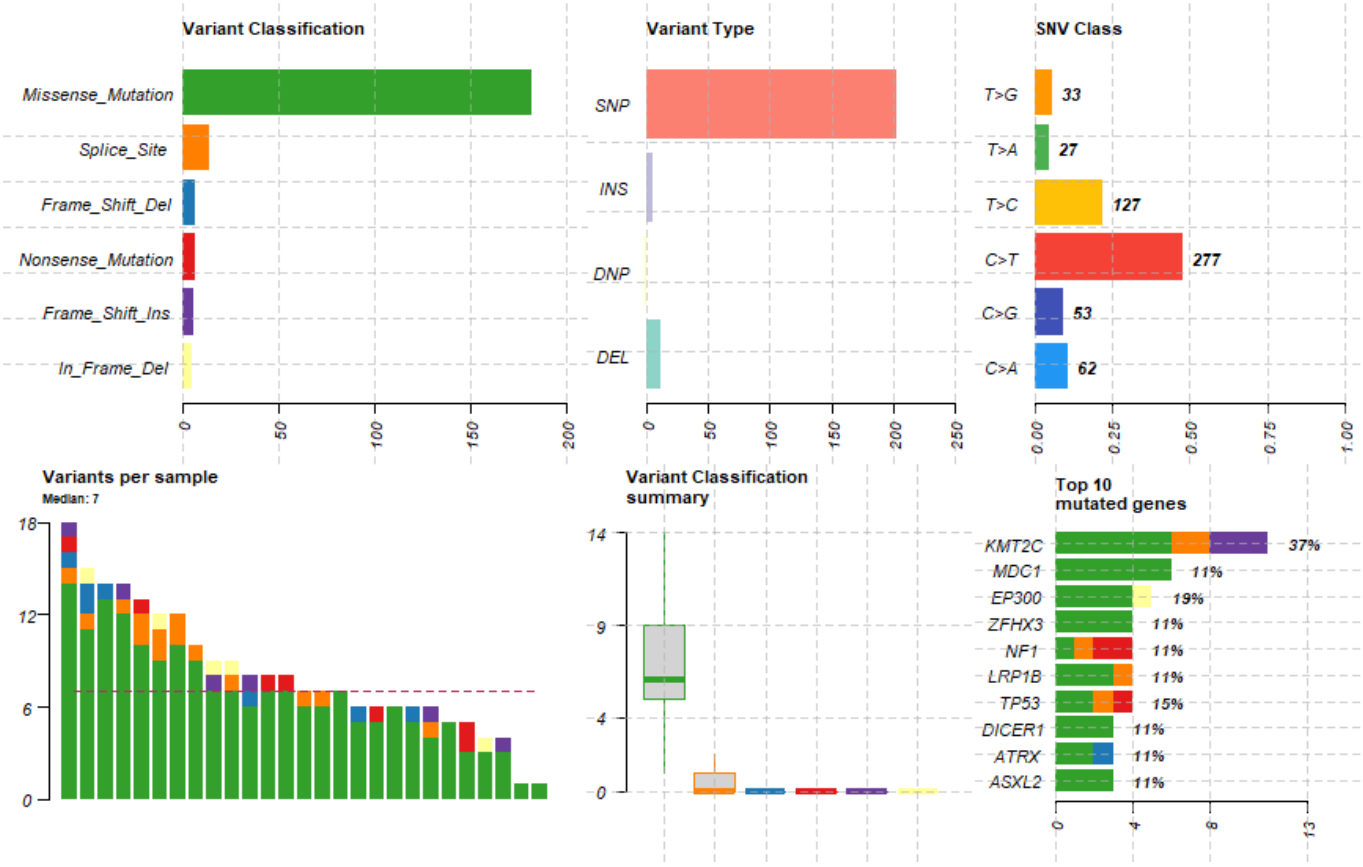
Figure 2. Gene amplifications (A) and large deletions (B) detected in the study cohort

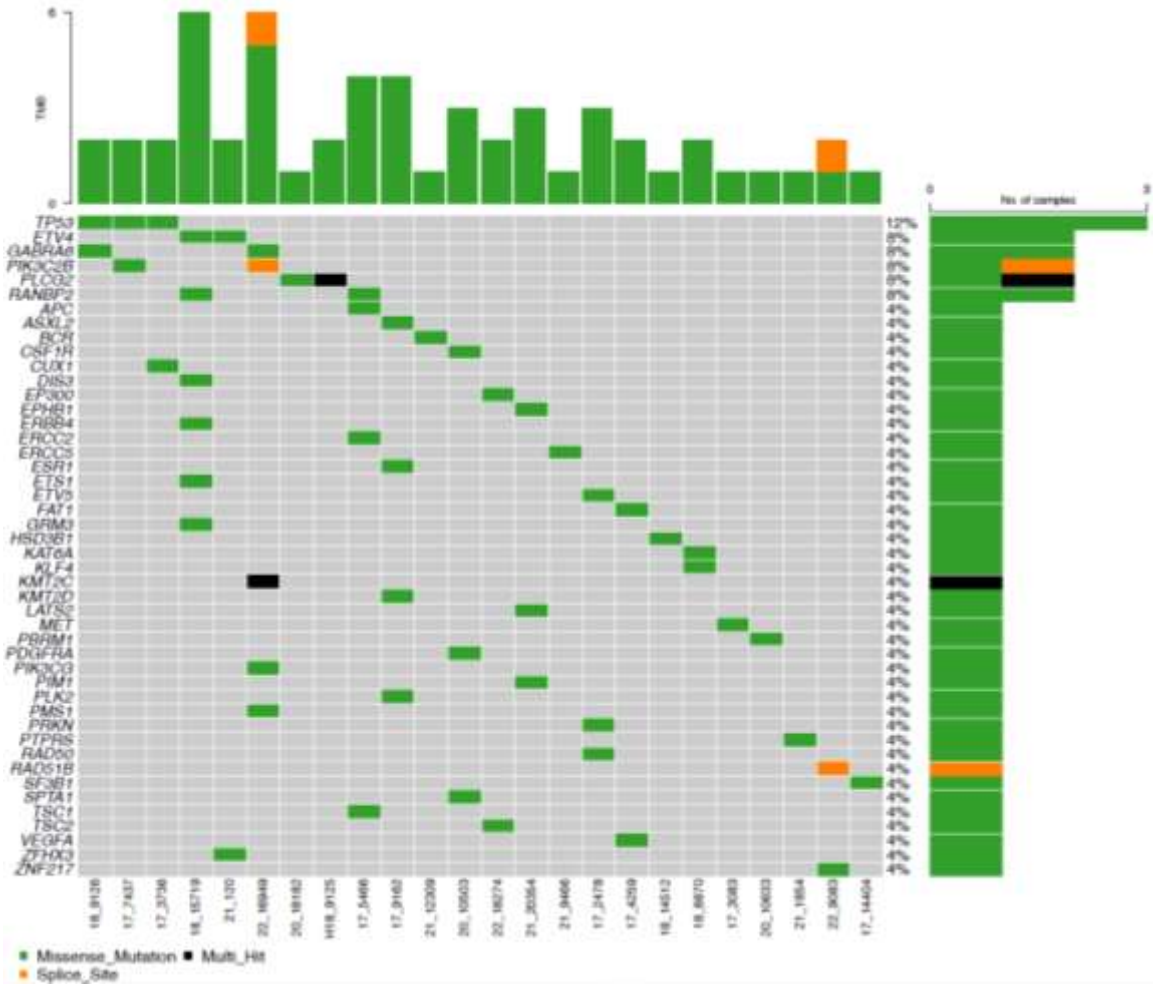
We detected 72 gene deletions (Figure 2B). Of these, fewer than 14% were homozygous copy-number deletions. In over 40% of patients, no gene copy deletion was detected (n = 11). The most frequently deleted genes were *PLCG2* (6.9%) and *ANKRD26, KIF5B*, and *PTEN* (5.5% each). Interestingly, in each case, the *ANKRD26* gene deletion co-

occurred with the *KIF5B* gene deletion. Less than 50% of the deletions involved genes involved in signaling pathways.

Single-nucleotide variant analysis

We detected 680 variants. Most were non-coding variants, including almost 29% intronic variants of all detected variants. Additionally, over 31% were synonymous (silent) variants. Only about one-third of the variants (32.36%) were missense variants (Supplementary Figure SF8). One frameshift deletion and one in-frame deletion were also detected. Of the remaining 343 variants, we identified 58 likely pathogenic and 58 variants of uncertain significance (VUS), resulting in an average of 2 point mutations per patient.





242

243 **Figure 3**. (A) - Single-nucleotide variants classification of all detected variants; (B) - Oncoplot of likely
244 pathogenic and ambiguous variants detected in the analyzed cohort

245 The most common was a missense variant, and the most common substitution was the
246 change of the nucleotide C>T (Figure 3A). The recurring variants were the *TP53, PLCG2,*
247 *RANBP2, PIK3C2B* and *GABRA6* genes (Figure 3B). The most common mutations involved
248 genes associated with signaling pathways, followed by genes encode transcription factors and
249 proteins involved in chromatin remodeling.

250 Clinical outcomes

251 The median OS since the start of treatment was 7.3 years, while the median DMFS
252 and RFS was 2.8 years. The median LRFS since the date of radical resection was 7.1 years.
253 The rate of 3-year and 5-year OS after the initiation of UNRESARC treatment was 70.4%
254 (95% CI: 49.4% - 83.9%) and 65.3% (95% CI: 43.6% - 80.4%), respectively (Figure 4A). The
255 rate of 3-year LRFS after radical resection was 70.4% (95% CI: 49.4% - 83.9%) and and 5-
256 year was 58.5% (95% CI: 35.2% - 75.9%) (Figure 4B). The rate of 3-year and 5-year DMFS
257 after the initiation of UNRESARC treatment was 48.2% (95% CI: 28.7% - 65.2%) (Figure

4C). The rate of 3-year RFS after the initiation of UNRESARC treatment was 48.2% (95% CI: 28.7% - 65.2%) and 5-year was 42.1% (95% CI: 22.5% - 60.6%) (Figure 4D).

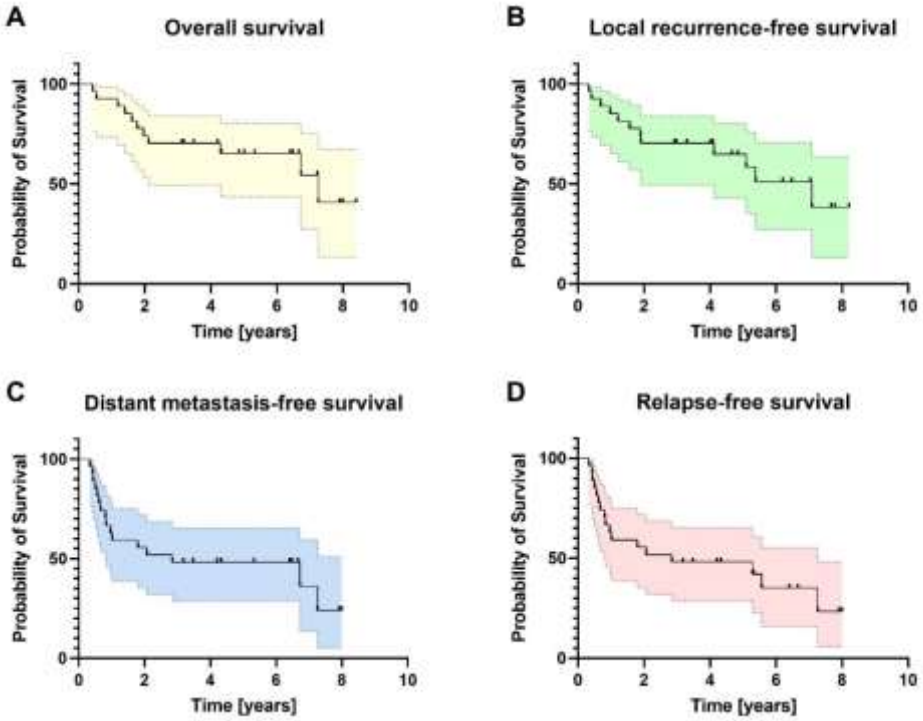


Figure 4. Survival analysis: (A) Overall survival, (B) Local recurrence-free survival, (C) Distant metastasis-free survival, (D) Relapse-free survival

Sarcoma subtype did not affect the OS, LRFS, and DMFS (Supplementary Figure SF13). The survival and local recurrence rate did not differ by PR (Figure 5A and 5B). However, there were significant differences in DMFS (Figure 5C, p = 0.002), and RFS (Figure 5D; p = 0.004) by PR.

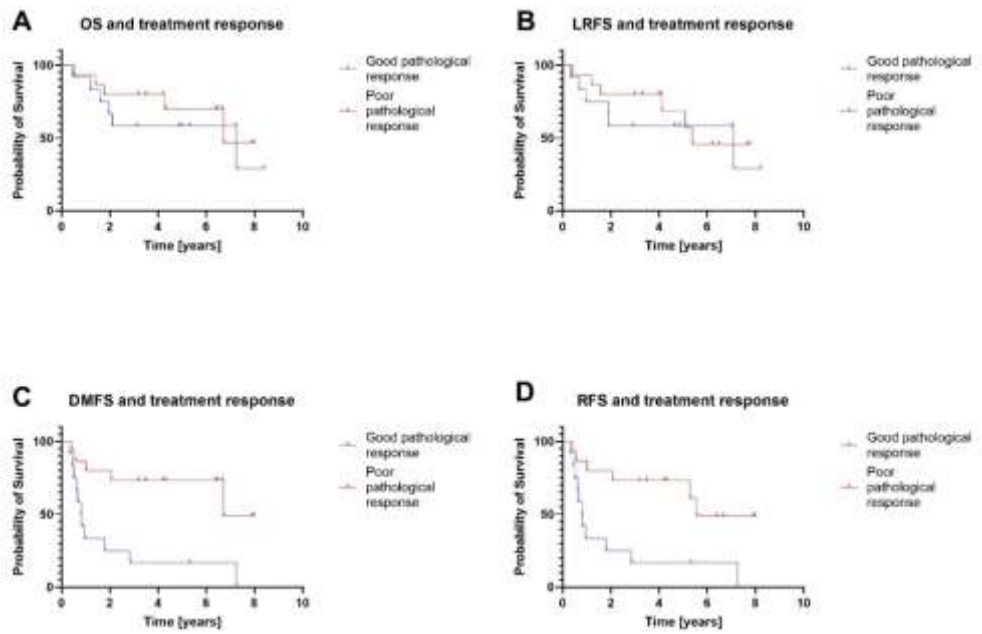


Figure 5. Survival analysis and treatment response: (A) Overall survival, (B) Local recurrence-free survival, (C) Distant metastasis-free survival, (D) Relapse-free survival

Correlation between molecular features and clinical outcomes

Fusion–related sarcoma patients showed a significant difference in survival rate (Figure 6A; $p = 0.043$). The median OS for patients with fusion-related sarcoma was 1.9 years, whereas for patients with other sarcomas it was 7.26 years. The presence of fusions also had an impact on LRFS (Figure 6B; $p = 0.030$) or DMFS (Figure 6C; $p = 0.001$). No single aberration showed a significant effect on long-term OS, LRFS, or DMFS ((Supplementary Figure SF14).

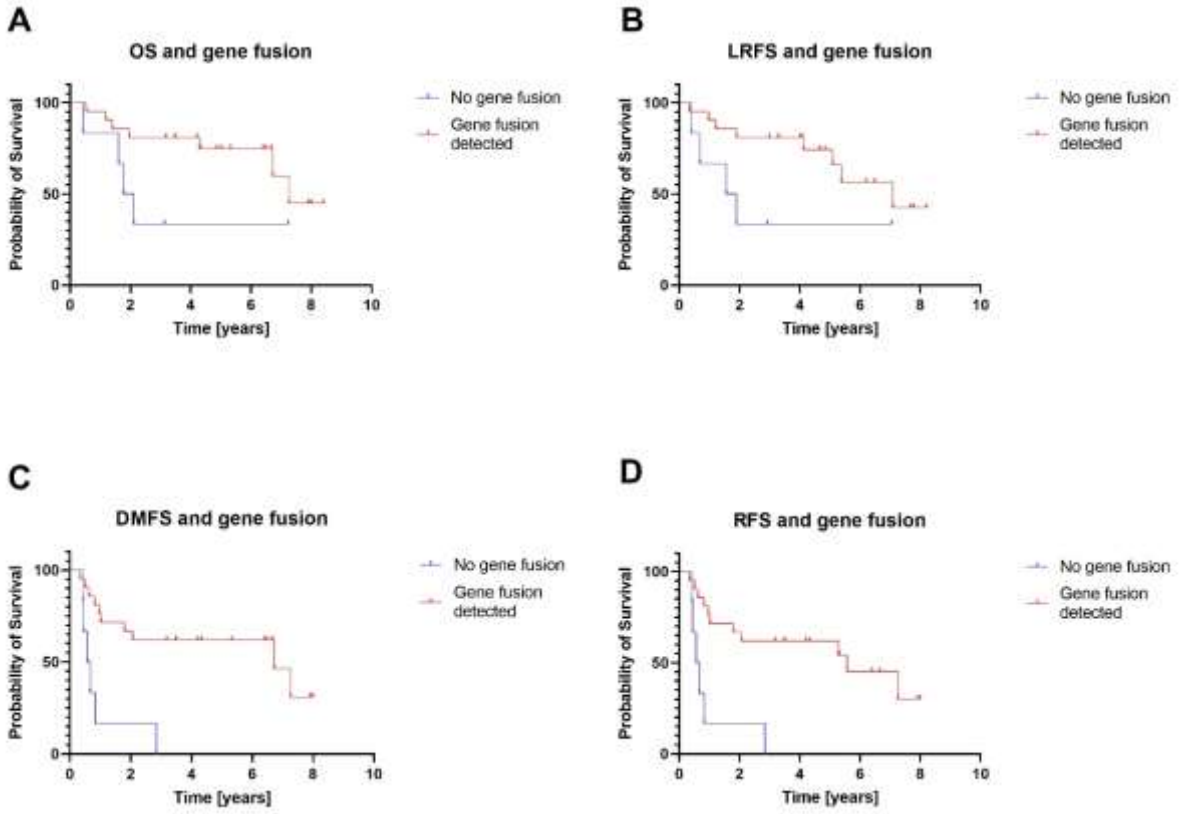
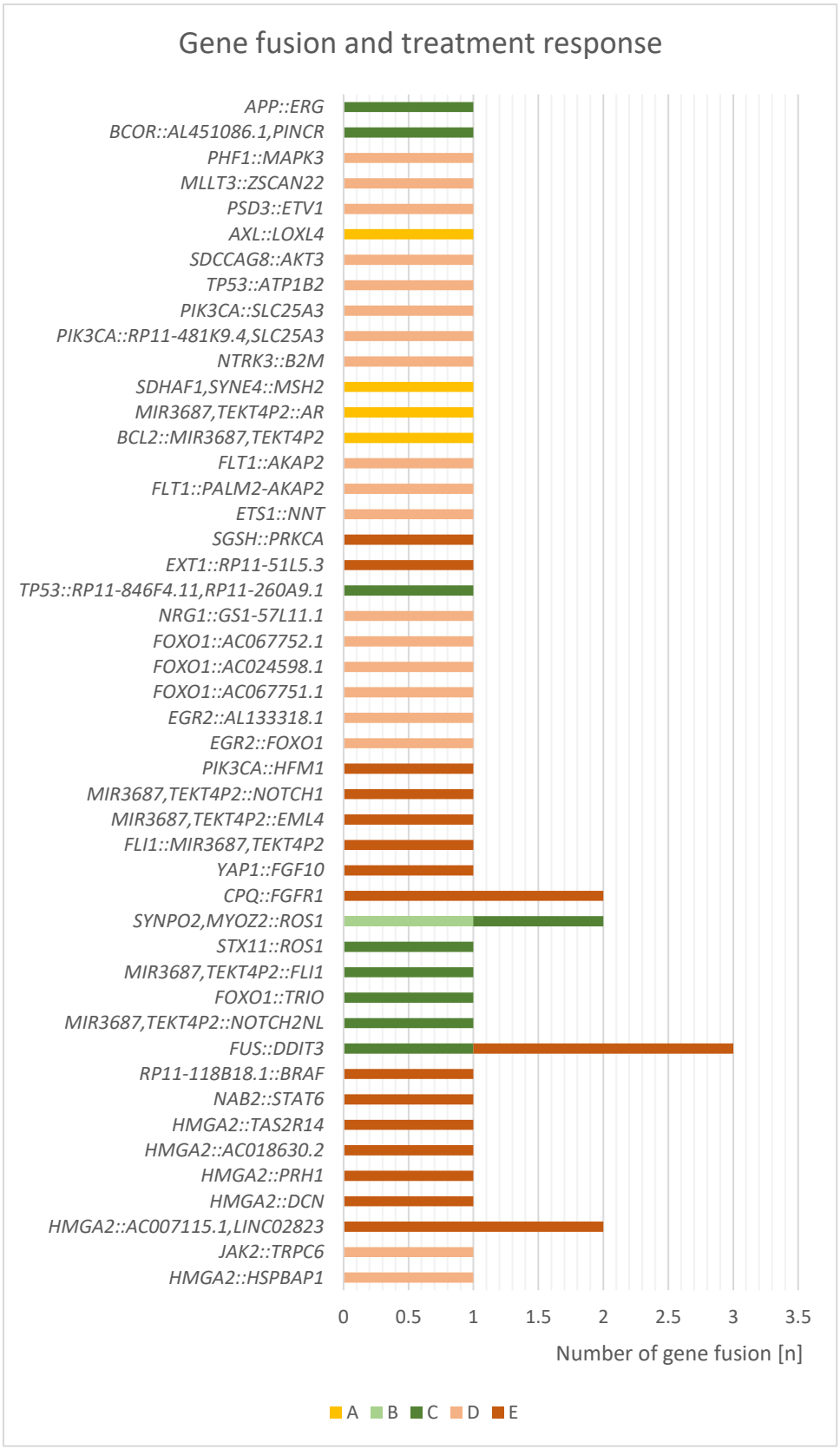


Figure 6. Survival analysis and gene fusion: (A) Overall survival, (B) Local recurrence-free survival, (C) Distant metastasis-free survival, (D) Relapse-free survival

Genetic alteration and treatment response

Sequencing results, compared with neoadjuvant response and PR, showed that all patients with *ROS1*-associated gene fusion demonstrated good PR (in more than 10% of cases) (Figure 7A). In contrast, all patients with *MDM2* gene amplification (>10%) or *PTEN* gene deletion demonstrated poor PR (<15%) (Figure 7B and 7C). Additionally, patients with likely pathogenic variants in the *TP53, PLCG2, PIK3C2B, GABRA6*, and *KMT2C* genes demonstrated poor PR (>7%) (Figure 7D). Of all detected genetic aberrations, alteration of 7 genes were potentially drugable (Table 2).


Gene fusion and treatment response
APP::ERG
BCOR::AL451086.1,PINCR
PHF1::MAPK3
MLLT3::ZSCAN22
PSD3::ETV1
AXL::LOXL4
SDCCAG8::AKT3
TP53::ATP1B2
PIK3CA::SLC25A3
PIK3CA::RP11-481K9.4,SLC25A3
NTRK3::B2M
SDHAF1,SYNE4::MSH2
MIR3687,TEKT4P2::AR
BCL2::MIR3687,TEKT4P2
FLT1::AKAP2
FLT1::PALM2-AKAP2
ETS1::NNT
SGSH::PRKCA
EXT1::RP11-51L5.3
TP53::RP11-846F4.11,RP11-260A9.1
NRG1::GS1-57L11.1
FOXO1::AC067752.1
FOXO1::AC024598.1
FOXO1::AC067751.1
EGR2::AL133318.1
EGR2::FOXO1
PIK3CA::HFM1
MIR3687,TEKT4P2::NOTCH1
MIR3687,TEKT4P2::EML4
FLI1::MIR3687,TEKT4P2
YAP1::FGF10
CPQ::FGFR1
SYNPO2,MYOZ2::ROS1
STX11::ROS1
MIR3687,TEKT4P2::FLI1
FOXO1::TRIO
MIR3687,TEKT4P2::NOTCH2NL
FUS::DDIT3
RP11-118B18.1::BRAF
NAB2::STAT6
HMGA2::TAS2R14
HMGA2::AC018630.2
HMGA2::PRH1
HMGA2::DCN
HMGA2::AC007115.1,LINC02823
JAK2::TRPC6
HMGA2::HSPBAP1
0
0.5
1
1.5
2
2.5
3
3.5
Number of gene fusion [n]
A
B
C
D
E

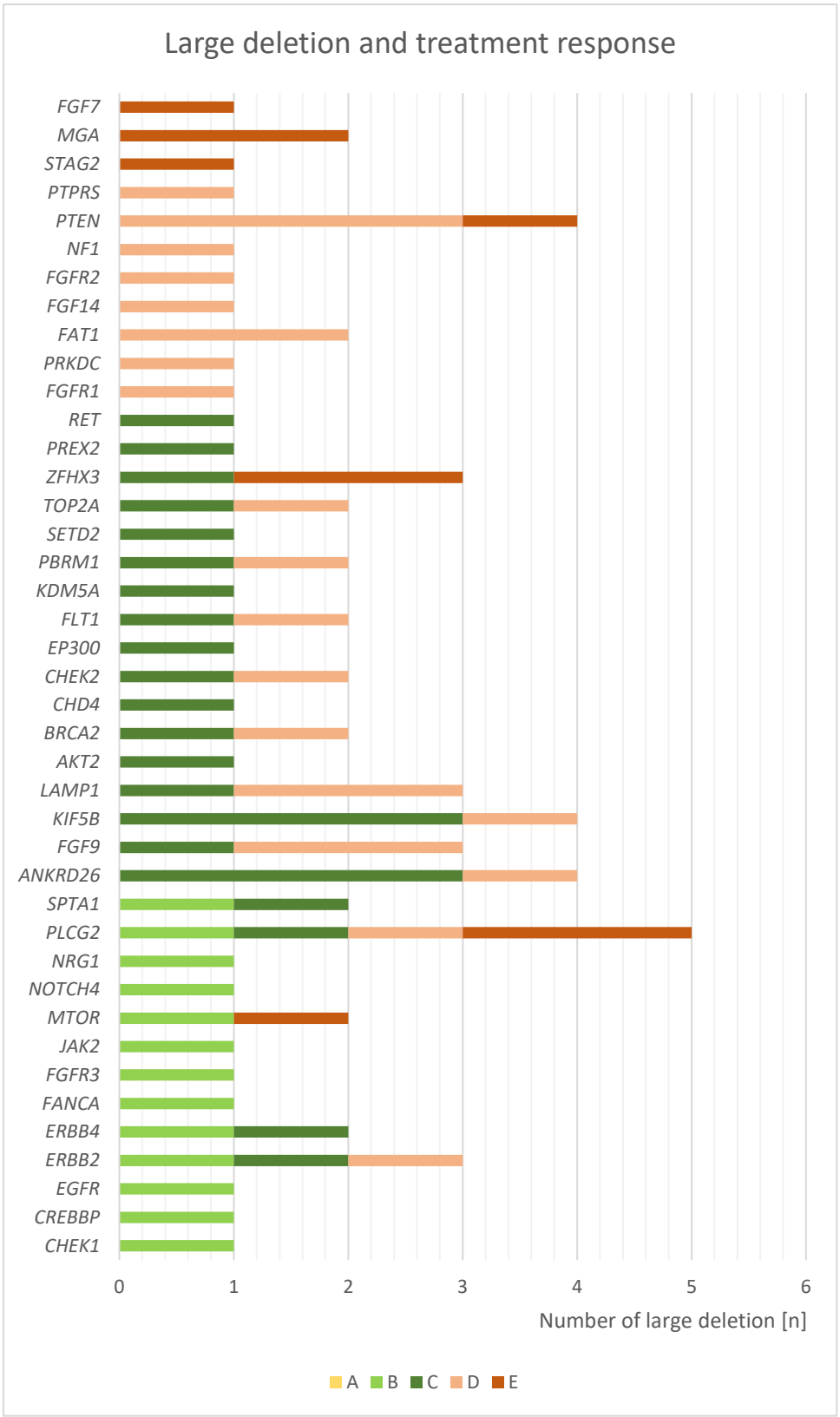
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Gene amplification and treatment response
RAF1
FANCD2
PAX8
DNMT1
PTPRT
PIK3CB
E2F3
TMPRSS2
KMT2C
KMT2B
AKT2
TSC2
SMC3
RPTOR
NOTCH2
KDM5A
JAK2
FANCI
FLT4
NRG1
ETV1
MDM2
CCND1
RICTOR
PTCH1
NOTCH1
MAP3K4
FGF10
ARID1B
PIK3C2G
KRAS
MET
EGFR
CUX1
BRAF
CHEK2
FGF7
DICER1
CCNE1
CCND3
0
0.5
1
1.5
2
2.5
3
3.5
Number of gene amplification [n]
A
B
C
D
E

291


Large deletion and treatment response
FGF7
MGA
STAG2
PTPRS
PTEN
NF1
FGFR2
FGF14
FAT1
PRKDC
FGFR1
RET
PREX2
ZFHX3
TOP2A
SETD2
PBRM1
KDM5A
FLT1
EP300
CHEK2
CHD4
BRCA2
AKT2
LAMP1
KIF5B
FGF9
ANKRD26
SPTA1
PLCG2
NRG1
NOTCH4
MTOR
JAK2
FGFR3
FANCA
ERBB4
ERBB2
EGFR
CREBBP
CHEK1
0
1
2
3
4
5
6
Number of large deletion [n]
A
B
C
D
E

292

293


SNV and treatment response
KEL
PIM1
EPHB1
LATS2
ZNF217
RAD51B
ERCC5
ETV4
ETS1
DIS3
PREX2
GRM3
ERBB4
HSD3B1
ATM
TP53
PLCG2
ESR1
KMT2D
ASXL2
SH2B3
PLK2
FAT1
VEGFA
ETV5
PRKN
RAD50
PMS1
KMT2C
PIK3CG
PIK3C2B
GABRA6
PTPRS
ZFHX3
KLF4
KAT6A
CREBBP
BLM
ROS1
SPTA1
PDGFRA
CSF1R
EP300
TSC2
RANBP2
ERCC2
TSC1
APC
SF3B1
PALB2
PBRM1
0
0.5
1
1.5
2
2.5
Number of SNV [n]
A
B
C
D
E

294

Figure 7. (A) Gene fusion, (B) Gene amplification, (C) Large deletion, (D) Single-nucleotide variant and pathological response to the neoadjuvant treatment.

A, B, C – good pathological response, D, E –poor pathological response

Table 2. Potentially druggable mutations identified in the study.

Alteration detected in the UNRESARC study	Alteration type	Why potentially druggable (example agent class)	ClinicalTrials.gov (NCT)	EU Clinical Trials Register (EudraCT)
ROS1 gene fusion/rearrangement (e.g., *STX11::ROS1; SYNPO2/MYOZ2::ROS1*)	Fusion	ROS1 kinase targetable with ROS1 TKIs (e.g., crizotinib, entrectinib, repotrectinib).	NCT03093116 (repotrectinib; TRIDENT-1) NCT04094610 (repotrectinib; pediatrics/YA)	2016-003616-13 (repotrectinib; ALK/ROS1/NTRK rearrangements)
NTRK2 or *NTRK3* gene fusion (e.g., *PTCH1::NTRK2; B2M::NTRK3*)	Fusion	TRK fusions are targetable with TRK inhibitors (larotrectinib, entrectinib; next-gen repotrectinib).	NCT02576431 (larotrectinib; NAVIGATE) NCT02568267 (entrectinib; basket) NCT03093116 (repotrectinib; TRIDENT-1)	2015-003582-28 (larotrectinib; NTRK fusion-positive tumors) 2016-003616-13 (repotrectinib; includes NTRK rearrangements)
BRAF fusion / activating RAF alteration (e.g., intergenic::BRAF fusion detected)	Fusion	Selected RAF/BRAF fusions/activating RAF alterations may be targetable with RAF/MAPK pathway inhibitors (e.g., tovorafenib).	NCT04985604 (tovorafenib; RAF-altered tumors) NCT03429803 (tovorafenib/DAY101; gliomas & other tumors)	2020-003657-30 (tovorafenib; RAF fusion/alteration cohorts)
FGFR1–3 gene fusion / rearrangement (reported among signaling-pathway fusions)	Fusion	FGFR fusions are targetable with FGFR inhibitors (e.g., Debio 1347; other FGFR TKIs depending on context).	NCT03834220 (Debio 1347; FUZE; FGFR1–3 fusions)	2018-003584-53 (Debio 1347; FGFR1–3 fusion/rearrangement)
MDM2 amplification	CNV (amplification)	MDM2-amplified sarcomas are targetable with MDM2 inhibitors (e.g., brigimadlin/BI 907828;	NCT03449381 (brigimadlin/BI 907828; MDM2-amplified sarcoma cohort) NCT05218499 (brigimadlin; Brightline-1;	2021-002392-20 (brigimadlin; Brightline-1 in EU)

		milademetan; other MDM2 inhibitors).	DDLPS) NCT05012397 (milademetan; basket, incl. MDM2-amplified tumors) NCT05180695 (HDM201 + pazopanib; STS with MDM2 amplification cohort) NCT06414434 (BTX-A51; liposarcoma with MDM2 amplification)	
PTEN deletion / PTEN loss	CNV (deletion)	PTEN loss may confer sensitivity to PI3K/AKT/mTOR-pathway inhibition; registry-identified alteration-directed subprotocols exist.	NCT02465060 (NCI-MATCH; subprotocols for PTEN loss)	No registry matches identified.
RICTOR amplification	CNV (amplification)	Potentially actionable via dual mTORC1/2 kinase inhibitors; however, no RICTOR-amplification–selected trials were identified in the registries during this search.	No alteration-selected trials identified in this search.	No registry matches identified.

Discussion

The goal of personalized medicine is to implement biomarker testing and molecularly targeted treatment into patient care. NGS should be considered an integral part of histological diagnosis for malignancies lacking standardized therapeutic options. In the single-arm MOSCATO trial, the use of multigene sequencing and comparative genomic hybridisation (CGH) arrays was associated with an improved progression-free survival (PFS) in 30% of patients and an objective response rate (ORR) of 11% (39). However, diagnostic errors in

sarcoma remain exceedingly common, with rates up to 20-30% even among expert sarcoma pathologists (2, 24). Our study identified two cases diagnosed with MFS in which we detected gene fusions characteristic of other sarcoma subtypes. In the study group, three cases of the *FUS::DDIT3* fusion were reported, with only two patients diagnosed with MLPS. This fusion occurs in over 90% of MLPS cases and is characteristic of this sarcoma subtype (40), suggesting the possibility of a change in diagnosis. In both cases diagnosed with MLPS, the FISH assay was previously ordered, which confirmed the presence of a *DDIT3* translocation. In the case of a patient diagnosed with MFS, genetic testing was not ordered, confirming the need for routine genetic testing. Moreover, in the case diagnosed as MFS, we detected the *NAB2::STAT6* gene fusion, which is specific for the solitary fibrous tumors (SFT) subtype (41).

Furthermore, the use of genomic profiling in clinical practice has steadily increased as the number of biomarkers of therapeutic relevance has grown. Several cancer types were evaluated based on finding specific changes such as *NTRK*-related gene fusions, leading to the development of FDA-approved targeted treatments, such as larotrectinib and entrectinib, which showed effectiveness by providing personalized treatment options (42-44). In our study group, we detected a patient with an *NTRK3*-associated gene fusion, which could have an impact on therapeutic decisions. Patients must meet certain criteria to receive targeted treatment, including all available treatment regimens in accordance with the recommendations of the National Comprehensive Cancer Network (NCCN). However, in case of a lack of further standard therapeutic methods, the patient may benefit from targeted therapy, which is an additional treatment option. In our molecularly profiled cohort, we identified a spectrum of alterations with plausible therapeutic tractability, dominated by oncogenic kinase rearrangements and copy number–driven vulnerabilities. Recurrent tyrosine kinase fusions included *ROS1*-associated and *NTRK2/3*-associated rearrangements, constituting the most directly druggable lesions given the established clinical activity of matched kinase inhibitors (ROS1-directed and TRK inhibitors, respectively) and the availability of genotype-selected basket programs for rare fusion-positive solid tumors. In addition, MAPK-pathway activation through *RAF* alterations was represented by *BRAF/RAF* fusion events, supporting consideration of pathway-directed strategies (e.g., RAF/MAPK-targeted approaches in appropriately selected fusion-positive contexts). A further actionable kinase category comprised *FGFR1–3* fusion signals, providing a mechanistic rationale for FGFR inhibitor–based treatment in eligible patients. Among copy-number events, *MDM2* amplification

emerged as a clinically relevant target class in sarcoma biology, aligning with the therapeutic concept of pharmacologic p53 reactivation via MDM2 inhibition and with multiple ongoing MDM2 inhibitor development programs. Additional pathway alterations with context-dependent actionability included *PTEN* loss, consistent with potential susceptibility to PI3K–AKT–mTOR axis modulation in molecularly assigned trials, and *RICTOR* amplification, which suggests mTORC2 pathway engagement but currently requires cautious interpretation as a selection biomarker due to less consistent alteration-driven clinical validation. Collectively, these findings delineate a subset of patients for whom biomarker-guided therapy—preferentially within clinical trials—may be rational, particularly for kinase fusion–positive and *MDM2*-amplified disease. In particular, approximately 15% of our cases harbored alterations in the *ROS1* gene - gene fusions and pathogenic SNV. In each case, patients demonstrated good PR, suggesting that the biomarker is a potential predictive biomarker for neoadjuvant therapy. However, studies in larger cohorts are necessary. Currently approved ROS1 tyrosine kinase inhibitors (TKIs) are crizotinib and entrectinib (45).

In our study, we observed a statistically significant difference in survival rate between fusion-related sarcomas and other sarcomas (p = 0.043), but not by histopathologic sarcoma subtype. Patients with gene fusions showed a higher survival rate (mOS = 7.26 years), but also a lower rate of local recurrence or distant metastasis. Moreover, in some retrospective analyses, Ewing sarcoma patients with *EWSR1::FLI1* showed better prognosis than those with other translocations [41, 42], but in prospective trials comparing survival in Ewing sarcoma patients, a significantly better survival in patients with *EWSR1::FLI1* was not confirmed (46, 47). On the other hand, *FOXO1*-associated gene fusions could be a poor-prognosis predictive factor in lymph node-positive alveolar rhabdomyosarcoma (46, 48). Moreover, alveolar rhabdomyosarcoma-specific translocations, *PAX3::FOXO1* and *PAX7::FOXO1,* are suggested to be associated with shorter survival (46, 49). However, given the small sample size and exploratory character of the analysis, this finding should be interpreted with caution. Validation in a larger cohort is needed to confirm these results.

The most frequently altered gene in our cohort was *PLCG2*. Zhengtian et al. found that PLCG2 expression was positively correlated with survival in STS patients and suggested it as a prognostic factor. They showed that genes in the highly expressed *PLCG2* group were enriched in immune-related activities, and patients had a better survival rate. In the low-expression PLCG2 group, genes were enriched in the E2F, G2M, and MYC pathways. Difference and correlation analyses showed that CD8+ T cells, gamma delta T cells,

monocytes, and M1 macrophages were positively correlated with PLCG2 expression, suggesting that PLCG2 may reflect the immune status of the tumor microenvironment (TME) (50). On the other hand, in our study, two out of six patients with an altered *PLCG2* gene died.

This study has potential limitations. Firstly, its retrospective nature and small experimental group make the study exploitative. The statistical finding should be interpreted with caution, because of the smaller sample sizes within some experimental groups. Validation in a larger cohort is needed to confirm. Secondly, the NGS testing was performed exclusively on patients' tumor tissue, which made precise filtering of germline variants difficult. No paired healthy tissue was available, but five other healthy tissue samples were used as controls.

Conclusions

In summary, this study provides a detailed description of the genomic landscape of high-risk STS. We present evidence from specific cases in which genetic testing influences diagnosis and may provide an additional treatment option, thereby contributing to personalized therapy for patients. In addition to chemotherapy, other targeted treatment or clinical trial enrollment may be considered, depending on the patient's genetic testing results.

List of abbreviations

CGH - comparative genomic hybridisation

CI - confidence intervals

CNV – copy number variation

DMFS – distant metastasis-free survival

FFPE - formalin-fixed paraffin-embedded

GATK - Genome Analysis Toolkit

GIST - gastrointestinal stromal tumors

IMT - inflammatory myofibroblastic tumors

LRFS – local recurrence-free survival

MFS - myxofibrosarcoma

MLPS – myxoid liposarcoma

NCCN - National Comprehensive Cancer Network

NGS – next-generation sequencing

NOS - not otherwise specified

ORR - objective response rate

OS – overall survival

PFS – progression-free survival

PR- pathological response

RFS – relapse-free survival

SFT - solitary fibrous tumor

SNV - single-nucleotide variant

STS – soft tissue sarcoma

TKIs - tyrosine kinase inhibitors

TME - tumor microenvironment

UPS – undifferentiated pleomorphic sarcoma

Declarations

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of the Maria Sklodowska-Curie National Research Institute of Oncology (approval number: 8/2017/1/2018 and 52/2024). Written informed consent was obtained from all participants in a phase 2 clinical trial UNRESARC (NCT03651375).

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analysed during the current study are available in the European Nucleotide Archive (ENA) repository, [https://www.ebi.ac.uk/ena/browser/view/PRJEB107237].

Competing interests

The authors declare no conflict of interest.

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Authors' contributions

Conceptualization: MJS, AMC, PR; Data curation: KB, JP, PB, AT, MJS; Formal analysis: KB, JP, PG, PB; Funding acquisition: MJS, AMC; Investigation: KB, KAS, AT, JP, PG, ASC, MW, AB, HKP; Methodology: AMC, JP, KB, AT; Project administration: MJS, AMC; Resources: MJS, KB, AT, KAS, JP, PG, ASC, MW; Software: KB, JP, AT; Supervision: AMC, PR, PG; Validation: KB, JP, AT, KAS; Visualization: KB, JP; Writing – original draft: KB; Writing – review and editing: all authors

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