# Genetic profiling of osteosarcoma in an adolescent using a next-generation sequencing panel and Sanger sequencing: A case report and review of the literature

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**Abstract.** Osteosarcoma (OS) is the most common malignant bone tumor affecting adolescents and young adults and it usually occurs in the long bones of the extremities. The detection of cancer-related genetic alterations has a growing effect in guiding diagnosis, prognosis and targeted therapies. However, little is known about the molecular aspects involved in the etiology and progression of OS, which limits options for targeted therapies. The present study described a case of an adolescent patient (16-years-old) who was diagnosed with conventional central OS in the right distal femur without the evidence of pulmonary metastases; the patient was treated with surgery and adjuvant chemotherapy. Genetic alterations in resected tumor tissue were investigated via next-generation sequencing (NGS) technology using a targeted NGS panel. Sanger sequencing was also performed to investigate somatic and germline *TP53* mutations (exons 4-8). NGS analysis revealed an intratumor heterogeneity signature in OS tumor, including several single nucleotide variants identified in genes encoding tyrosine kinase proteins. No PCR products for *TP53* exon 5 were detected in the tumor sample by PCR analysis prior to Sanger sequencing, suggesting a significant deletion in this exon. Sanger sequencing analysis revealed the missense variant *TP53* c.712T>A (p.Cys238Ser) in tumor tissue sample, thus reinforcing the role of *TP53* somatic mutations in OS development. Additionally, the *TP53* c.215C>G (p.Pro72Arg) germline missense variant was identified in the peripheral blood sample. In conclusion, the findings provided new information on genetic aspects that may contribute to OS development, especially in pediatric patients.

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## Introduction

Osteosarcoma (OS) is the most common primary malignant bone tumor affecting adolescents and young adults and it predominantly occurs in the long bones of the extremities, notably in the femur (1,2). The lungs are the most common metastatic site for OS and indicate a poor prognosis. OS incidence is rare, accounting for <1% of all human cancers and ~2% of childhood and adolescent cancers (3). OS clinical evaluation includes medical history and physical examination followed by radiologic imaging and tissue biopsy for histological diagnosis (4). The treatment modality generally includes surgery and combination of chemotherapy (5). However, some patients do not respond effectively to therapy; therefore, new treatment modalities are needed. Several other oncological treatments showing promising results in a number of cancers are being investigated for sarcomas, including the use of poly(ADP-ribose) polymerase (PARP) inhibitors (PARPi), new adjuvant therapies, immunotherapy with immune checkpoint inhibitors (blockage of the programmed cell death protein 1/programmed death-ligand 1 axis) and epigenetic therapies (6-14). Despite these efforts, the efficacy or clinical benefits of these treatment strategies for sarcomas remain controversial.

Advances in understanding cancer-related genetic alterations have had substantial effects on precision oncology (15,16). Next-generation sequencing (NGS) is a high-throughput sequencing offering robust genomic data for tumor genotyping that is able to drive diagnosis and treatment decisions (16,17). Despite this, little is known about the molecular aspects involved in OS etiology and progression; thus, genomic testing and targeted therapy to improve treatment are still rare scenarios for patients with OS. Mutations in the *TP53* and *RB1* tumor suppressor genes are commonly described for OS (18,19). However, the clinical complexity of OS suggests additional genetic drivers of this neoplasm.

The present study described the clinical course and the genomic profiling of an adolescent patient who was diagnosed with OS and treated with a standard protocol. It was approved by the Ethics and Research Committee of the National Institute of Traumatology and Orthopedics (approval no. CAAE: 60632822.4.0000.5273).

**Case presentation**

A 16-year-old female was referred to the Specialized Care Center for Orthopedic Oncology (National Institute of Traumatology and Orthopedics, Rio de Janeiro, Brazil) in November 2022 due to a mass, swelling and increasing pain in the right knee which began two months earlier. Patient had no known family history of cancer. The physical exam revealed a palpable mass in the right knee and restricted range of motion (10-90˚) associated with pain, as major findings. Radiography revealed a blastic lesion affecting the metadiaphyseal region of the right distal femur with a sunburst periosteal reaction (Fig. 1A). Magnetic resonance imaging revealed infiltration of the lesion through the distal third of the femur and mild involvement of soft tissues, resulting in a lesion that appeared hypointense on T1 and hyperintense on T2 (Fig. 1B). Computed tomography of the chest did not reveal evidence of distant metastases (Fig. 1C). A needle biopsy was performed and the diagnosis of conventional central OS, grade III, with a chondroblastic area was confirmed by histological examination (Fig. 2A and B). Tissue preparation for histological examination was performed by hematoxylin and eosin (H&E) staining. Briefly, the deparaffinization procedure of the tissue section was performed with xylene followed by alcohol rinses and then by rinsing in tap water to hydrate the section. Next, the Harris hematoxylin solution was applied for 2 min to stain the nuclear elements, followed by rinsing in tap water. After, a treatment with 5% acid alcohol was carried out followed by rinsing in tap water and subsequent addition of a 70% alcohol solution for 1 min. Finally, the eosin solution was applied for 15 sec to stain nonnuclear elements, followed by 100% ethanol rinses for dehydration and xylene treatment. For microscopy images, the stained tissues were cut into 7-$\mu$m slices using a microtome.

In January 2023, the patient started treatment with a neoadjuvant chemotherapy regimen of six cycles of cisplatin 60 mg/m$^2$, doxorubicin 75 mg/m$^2$, cardioxane 375 mg/m$^2$ and high doses of methotrexate (12 g/m$^2$). In May 2023, the patient underwent a wide resection and endoprosthetic reconstruction (Fig. 1D). Histopathological analysis of the surgical specimen revealed tumor necrosis of 60% (Huvos grade II), tumor-free resection margin and absence of angiolymphatic or perineural invasion (Fig. 2C and D). In June 2023, the patient started adjuvant chemotherapy with twelve cycles of cisplatin 60 mg/m$^2$, doxorubicin 75 mg/m$^2$, cardioxane 375 mg/m$^2$ and high doses of methotrexate (12 g/m$^2$). Until the last follow-up in April 2024, the patient still had good clinical signs, with no evidence of recurrence or pulmonary metastases.

The tumor tissue sample for DNA sequencing analysis was obtained from the surgical resection, 4 months after neoadjuvant chemotherapy. A peripheral blood sample was obtained at the same time. NGS analysis of tumor tissue was performed using the AmpliSeq for Illumina Focus Panel (Illumina, Inc.). Briefly, tumor tissue was fragmented using the L-Beader 24 tissue disruptor (Loccus do Brasil Ltda). Then, genomic DNA was extracted using Quick-DNA Miniprep Kit (Zymo Research Corp.). For NGS, the library preparation process was performed with 10 ng of DNA input, in which DNA targets were amplified by PCR using the AmpliSeq Focus DNA Panel and the AmpliSeq for Illumina Library PLUS (cat. no. 20019101; Illumina, Inc.). The AmpliSeq Focus DNA Panel contains 29 kb and the DNA amplicon size contains an average size of 107 bp in length. Afterwards, the amplicons were partially digested and subsequently ligated with index combination for dual-index sequencing using the AmpliSeq for Illumina CD Indexes Set A (cat. no. 20019105; Illumina, Inc.). All DNA library preparation assay was performed according to the manufacturer's protocol, available on the Illumina website (https://support.illumina.com/downloads/ampliseq-for-illumina-focus-panel-reference-guide-1000000039456.html). The quality of DNA library was visualized on 2% agarose gel electrophoresis using a DNA ladder 100 bp (Ludwig Biotecnologia ltda) and stained with ethidium bromide, revealing a size distribution corresponding to ~300 bp. The quantification of DNA library was assessed by Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, Inc.). Finally, after denaturing and diluting steps, the library was loaded onto the reagent cartridge and transferred automatically to a flow cell (NextSeq 500/550 Mid-Output v2.5 Kit; cat. no. 20024905; Illumina, Inc.) for paired-end sequencing reads based on sequencing by synthesis (SBS) technology on the NextSeq 550 Sequencing System. For bioinformatics analysis, genomics data were analyzed using the DNA Amplicon App (Version 2.1.1) on BaseSpace Sequence Hub (Illumina, Inc.) (https://www.illumina.com/products/by-type/informatics-products/basespace-sequence-hub/apps/dna-amplicon.html), resulting in genomic variants identified in the following databases: Single Nucleotide Polymorphism Database (dbSNP) (https://www.ncbi.nlm.nih.gov/snp/?cmd=search), Catalogue of Somatic Mutations in Cancer (COSMIC) (https://cancer.sanger.ac.uk/cosmic) and ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/). In the present study, the single nucleotide variants (SNVs) in tumor tissue were identified in coding and intronic regions described in the following genes: *JAK1*, *ALK*, *FGFR3*, *PDGFRA*, *FGFR4*, *EGFR*, *RET* and *KRAS*.

The AmpliSeq for Illumina Focus Panel (Illumina, Inc.) includes genes with known relevance to solid tumors. However, the *TP53* gene is not part of this panel. Since *TP53* mutations are commonly described for OS, mutational hotspot exons of *TP53* (exons 4-8) were investigated in the present study by Sanger sequencing. To investigate somatic and germline *TP53* mutations, DNA samples were obtained from tumor and peripheral blood. Briefly, genomic DNA was extracted from blood using QIAamp Blood Mini Kit (cat. no. 51104; Qiagen GmbH). The PCR products of *TP53* (exons 4-8) were purified by using the PureLink Quick Gel Extraction and PCR Purification Combo Kit (cat. no. K220001; Invitrogen; Thermo Fisher Scientific, Inc.), followed by Sanger sequencing reaction using the BigDye Terminator v3.1 Cycle Sequencing kit on an ABI 3730XL DNA Analyzer (Applied Biosystems; Thermo Fisher Scientific, Inc.), as previously described (20). DNA sequencing results were analyzed with ChromasPro software (Technelysium Pty Ltd), version 2.1.10.1, using the reference sequence from NCBI (NM_000546.6).

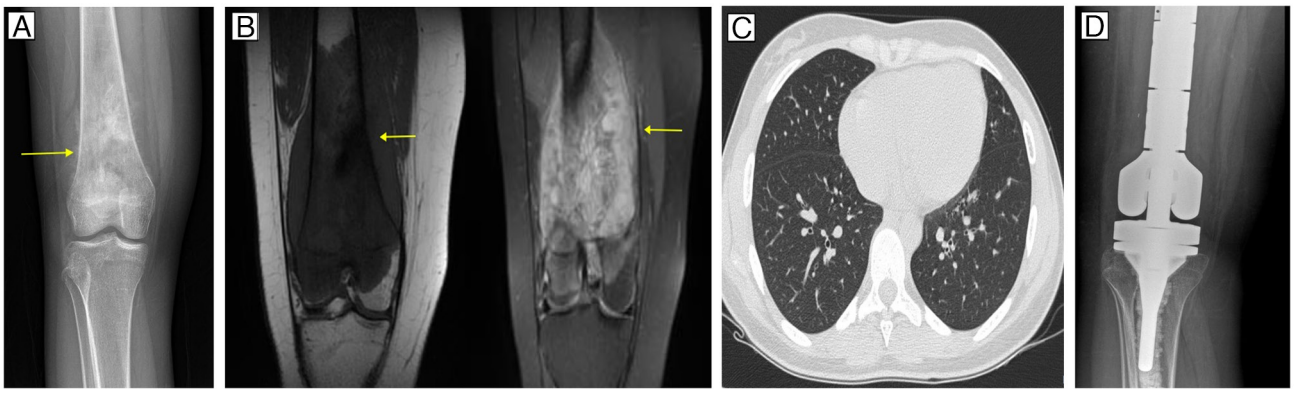


Figure 1. Imaging findings of the patient at diagnosis and after tumor resection. (A) Radiography revealed a blastic lesion located in the metadiaphyseal region of the right distal femur with a sunburst periosteal reaction (arrow). (B) Magnetic resonance imaging of coronal section showing a solid mass lesion accompanied by mild involvement of soft tissues (arrows): T1-weighted image (left); fat-suppressed T2-weighted image (right). (C) Axial computed tomography of the chest revealed no pulmonary metastasis. (D) Endoprosthetic reconstruction after tumor resection.

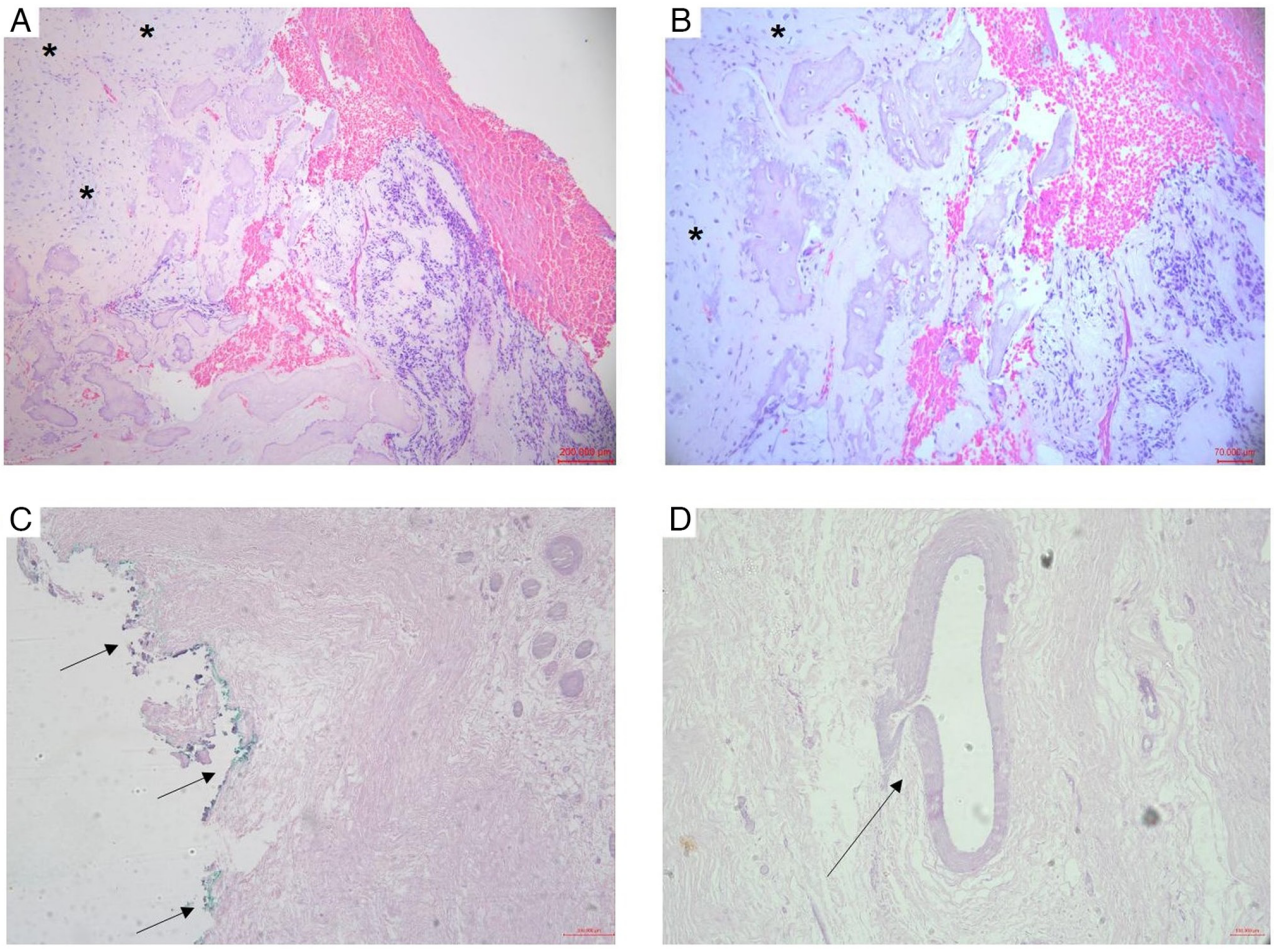


Figure 2. Morphological features of conventional central OS. (A and B) H&E stained sections revealed chondroblastic and osteoblastic patterns from the patient at diagnosis. Chondroblastic area (black asterisks) characterized by malignant cells showing nuclear pleomorphism in chondroid matrix (hyaline cartilage). The osteoblastic area is showing a sclerosing osteoid matrix with hyperchromatic, irregular and pleomorphic neoplastic cells (A; magnification, x100; B; magnification, x200). (C) Morphological features of OS tissue after chemotherapy, showing tumor-free resection margin (arrows) (H&E; magnification, x100). (D) Morphological features of OS tissue following chemotherapy, showing absence of angiolymphatic or perineural invasion (arrow) (H&E; magnification, x200). OS, osteosarcoma; H&E, hematoxylin and eosin.

As shown in Fig. 3, the PCR products of *TP53* were initially visualized on polyacrylamide gels. Only *TP53* exon 5 was not amplified from the tumor tissue sample, which suggested a significant somatic exonic deletion of *TP53* in this OS tumor. Sanger sequencing analysis revealed the missense variant *TP53* c.712T>A (p.Cys238Ser) in the tumor sample (Fig. 4) and the missense variant *TP53* c.215C>G (p.Pro72Arg) in the peripheral blood sample (Fig. 5).

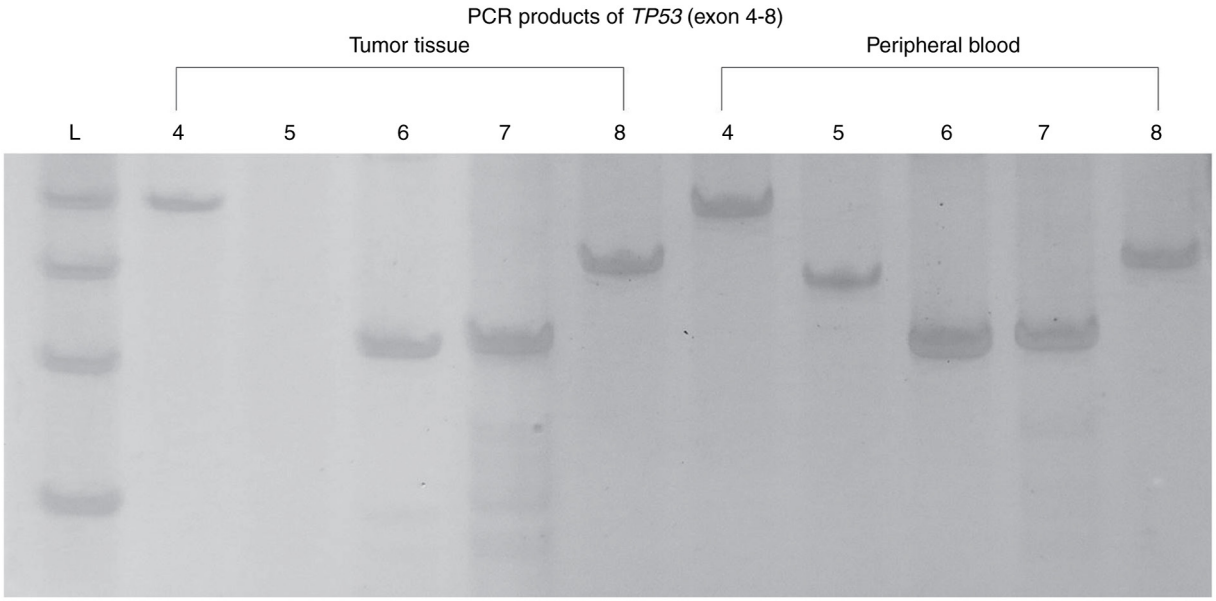


Figure 3. Representative nondenaturing 10% polyacrylamide gel of PCR products for *TP53* (exons 4-8) from tumor and peripheral blood samples. Lanes: L, DNA ladder (100 bp); 4, exon 4 (413 bp); 5, exon 5 (310 bp); 6, exon 6 (224 bp); 7, exon 7 (237 bp); 8, exon 8 (337 bp).

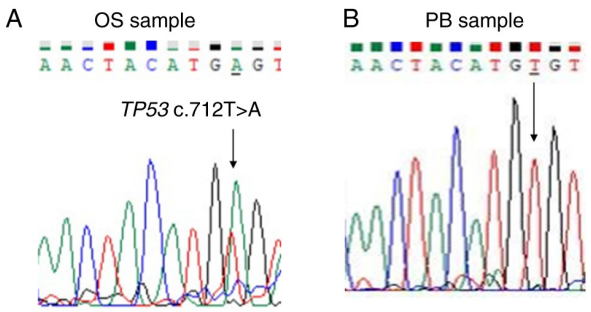


Figure 4. Sanger sequencing chromatograms using ChromasPro software. (A) The missense variant *TP53* c.712T>A (p.Cys238Ser) identified in OS tumor tissue sample is indicated by arrow. (B) The same *TP53* region investigated in the PB sample showing a normal sequence (absence of base change), as indicated by arrow. Reference sequence of *TP53* from NCBI: NM_000546.6. OS, osteosarcoma; PB, peripheral blood.

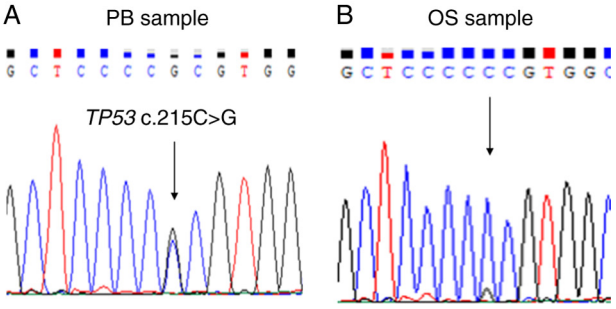


Figure 5. Sanger sequencing chromatograms using ChromasPro software. (A) The germline missense variant *TP53* c.215C>G (p.Pro72Arg) identified in PB sample is indicated by arrow. (B) The same *TP53* region investigated in the OS tumor tissue sample showing a normal sequence (absence of base change), as indicated by arrow. Reference sequence of *TP53* from NCBI: NM_000546.6. PB, peripheral blood; OS, osteosarcoma.

All genetic variants identified in the present study are described in Table I.

## Discussion

OS is the most common primary malignant bone tumor and it is characterized by its rare incidence and occurrence in the long bones of the extremities, notably in the femur. Adolescents and young adults are usually affected by the disease and improved 5-year overall survival rates are observed for younger patients (1). Molecular tests based on potential cancer-related genomic drivers could improve precision oncology for OS (21). However, knowledge about the genomic hallmarks related to OS etiology and progression is insufficient; thus, treatment options are still limited. In the present study, NGS analysis and Sanger sequencing were applied to investigate the genomic landscape of an adolescent patient diagnosed with OS and treated with a standard protocol. NGS analysis revealed that OS tumor sample harbored an intratumor heterogeneity signature and identified somatic variants in the following genes: *JAK1*, *ALK*, *FGFR3*, *PDGFRA*, *FGFR4*, *EGFR*, *RET* and *KRAS*.

Janus kinase (JAK) is a family of non-receptor tyrosine kinase proteins involved in the signal transduction of multiple cellular events, such as proliferation and differentiation (22,23). Some *JAK* mutations have been identified in different types of cancer. It has been reported that tumors with *JAK1* mutations may exhibit high mutation burden and microsatellite instability, which may result in immune response alterations and contribute to tumor immune evasion (24). In the present study, a coding synonymous variant in the *JAK1* gene (rs2230588; COSM3751351) was identified. Carvalho *et al* (25) reported the same *JAK1* genetic variant in 25% (5/20) of tumor samples from patients diagnosed with head and neck squamous cell carcinoma.

Anaplastic lymphoma kinase (ALK) gene encodes a receptor tyrosine kinase and *ALK* mutations, rearrangements/fusions, or amplifications have been identified in several

Table I. Summary of the genetic variants identified in the present study by NGS and Sanger sequencing analyses.

A, NGS analysis-OS tumor tissue

| Gene ID | Chr | Pos | Ref | Alt | Type | Context | Consequence | dbSNP | COSMIC | ClinVar | Qual |
|---|---|---|---|---|---|---|---|---|---|---|---|
| JAK1 | chr1 | 65310489 | T | C | SNV | Coding | synonymous_variant | rs2230588 | COSM3751351 | | 100 |
| ALK | chr2 | 29416572 | T | C | SNV | Coding | missense_variant | rs1670283 | | benign | 100 |
| ALK | chr2 | 29445458 | G | T | SNV | Coding | synonymous_variant | rs3795850 | COSM5351767 | benign | 100 |
| FGFR3 | chr4 | 1805799 | T | C | SNV | Intron | intron_variant | rs3135888 | | | 100 |
| FGFR3 | chr4 | 1807894 | G | A | SNV | Coding | synonymous_variant | rs7688609 | | | 100 |
| PDGFRA | chr4 | 55097835 | G | C | SNV | Intron | intron_variant | rs4864504 | | | 100 |
| FGFR4 | chr5 | 176517326 | T | C | SNV | Intron | intron_variant | rs422421 | | | 100 |
| FGFR4 | chr5 | 176517797 | C | T | SNV | Coding | missense_variant | rs376618 | | | 100 |
| FGFR4 | chr5 | 176519516 | A | G | SNV | Intron | splice_region_variant, intron_variant | rs3135925 | | | 100 |
| FGFR4 | chr5 | 176523562 | C | A | SNV | Intron | intron_variant | rs31777 | | | 100 |
| FGFR4 | chr5 | 176523597 | A | G | SNV | Intron | splice_region_variant, intron_variant | rs31776 | | | 100 |
| EGFR | chr7 | 55219909 | G | T | SNV | Intron | intron_variant | rs41364648 | | | 100 |
| EGFR | chr7 | 55228053 | A | T | SNV | Intron | intron_variant | rs1558544 | | | 100 |
| RET | chr10 | 43613843 | G | T | SNV | Coding | synonymous_variant | rs1800861 | COSM4418405, COSM4418406 | benign | 100 |
| KRAS | chr12 | 25400206 | G | T | SNV | Intron | intron_variant | rs10842518 | | | 100 |

B, Sanger sequencing (TP53 gene)-tumor tissue and peripheral blood

| *TP53* region | Sample | Type | Consequence |
|---|---|---|---|
| *TP53* exon 5 deletion | OS tumor | Deletion | deletion |
| *TP53* c.712T>A (p.Cys238Ser) | OS tumor | SNV | missense variant |
| *TP53* c.215C>G (p.Pro72Arg) | peripheral blood | SNV | missense variant |

NGS, next-generation sequencing; OS, osteosarcoma; SNV, single nucleotide variant; dbSNP, Single Nucleotide Polymorphism Database.

human cancers (26). ALK inhibitors represent an effective treatment strategy for patients with malignancies exhibiting *ALK* rearrangement and their effectiveness in patients with OS should be further explored. Ordulu *et al* (27) described a 73-year-old male with high-grade OS and lung metastases who showed sensitivity to ALK-targeted therapy harboring the *EML4::ALK* fusion and the $ALK^{L1196M}$ mutation. By contrast, Takeyasu *et al* (28) identified the *ITSN2::ALK* fusion in a 17-year-old male patient with OS showing poor sensitivity to alectinib therapy and progressive disease. In the present study, only DNA targets were investigated; therefore, gene fusions in RNA samples were not assessed. NGS analysis revealed a coding missense variant (rs1670283) and a synonymous variant (rs3795850; COSM5351767) in the *ALK* gene and both variants were classified as benign in the ClinVar database: https://www.ncbi.nlm.nih.gov/clinvar/variation/VCV000133472.35 and https://www.ncbi.nlm.nih.gov/clinvar/variation/VCV000335694.20, respectively. The *ALK* variant rs1670283 has also been associated with hereditary cancer-predisposing syndrome, breast cancer (29), anaplastic large cell lymphoma (30), gastric cancer (31) and benign tumor of the central nervous system (32). The *ALK* variant rs3795850 (COSM5351767) identified in the present study has also been associated with neuroblastoma susceptibility and detected in other cancer studies, including breast cancer (29), lung cancer (33,34) and Wilms tumor (35).

The fibroblast growth factor receptors (FGFRs) are a family of receptor tyrosine kinases involved in signaling pathways of different biological processes, in which the FGF/FGFR pathway plays a role in bone development and homeostasis (36,37). *FGFR* genomic alterations, including gene amplifications and gene mutations, have been described in OS (38,39). Additionally, clinical trials and case studies have evaluated the efficacy of FGFR inhibitors as a treatment option for patients with OS and reported improvements in progression-free survival (40,41). *FGFR3* gene is a FGFR family member and *FGFR3* mutations have been reported in several skeletal dysplasias (42-44). In the present study, the *FGFR3* variants rs3135888 (intron variant) and rs7688609 (synonymous variant) were identified. By using NGS technology, Mansour *et al* also identified the *FGFR3* rs7688609 variant in a patient with lung carcinoma (45). Kassem *et al* identified the *FGFR3* rs7688609 variant in seven patients with brain tumors through liquid biopsy using NGS (46). The *FGFR3* rs7688609 variant was also identified by panel-based NGS in a case of glioblastoma (47). FGFR4 also belongs to the FGFR family and the present study identified five *FGFR4* variants: rs422421 (intron variant), rs376618 (missense variant), rs3135925 (splice region variant, intron variant), rs31777 (intron variant) and rs31776 (splice region variant, intron variant). In a meta-analysis study conducted by Moazeni-Roodi *et al*, the *FGFR4* rs376618 variant was described in three studies and no association with overall cancer risk was found (48).

The platelet-derived growth factor receptor-alpha (*PDGFRA*) gene encodes a receptor tyrosine kinase involved in multiple cellular events. *PDGFRA*-mutated tumors can be found in several cancers and targeted inhibitors have been described as a treatment option to improve overall clinical outcomes (49-51). *PDGFRA* mutations commonly occur in the exons 12/14/18, notably in the exon 18 (D842V). Armstrong *et al* described a refractory metastatic OS case (7-year-old) with partial response to sorafenib (52). NGS technology revealed a *PDGFRA* D846V mutation in the initial tumor sample but not in the relapse sample, suggesting that this specific *PDGFRA* mutation is a sorafenib target. Using a custom NGS panel, a Brazilian study also identified molecular heterogeneity in OS tumor tissues, in which copy number variations (CNVs) were identified in the *PDGFRA* gene (53). In the present study, the intronic variant rs4864504 in the *PDGFRA* gene was identified.

The epidermal growth factor receptor (EGFR) is a receptor tyrosine kinase involved in downstream signaling cascades resulting in cellular growth and proliferation. *EGFR* deregulation has been identified in a number of cancers, especially lung cancer and frequently includes activating mutations in functional domains and amplifications (54,55). Treatment with EGFR tyrosine kinase inhibitors (TKIs) has emerged as an important strategy in cancer therapy to benefit patients harboring *EGFR* mutations (56). Two *EGFR* intronic variants (rs41364648 and rs1558544) were identified in the present study. Geißler *et al* (57) reported the *EGFR* rs1558544 variant in 7 of 25 patients with colorectal cancer. This *EGFR* rs1558544 variant was also found in a patient with cutaneous squamous cell carcinoma resistant to conventional treatments but successfully treated with anti-EGFR targeted therapy (58).

Rearranged during transfection (*RET*) is a proto-oncogene that encodes a transmembrane receptor tyrosine kinase involved in several cellular signaling pathways. *RET* gene mutations are commonly found in medullary thyroid carcinoma but can also be found in other cancers (59). Germline activating mutations and somatic amplifications in the *RET* gene have been described in patients with OS (60). The *RET* rs1800861 (COSM4418405) synonymous variant was identified in the present study. This *RET* variant was also found by NGS analysis in a patient with chronic myeloid leukemia (61).

Kirsten rat sarcoma viral oncogene homologue (*KRAS*) is a well-known proto-oncogene playing a central role as a signal transducer. *KRAS* mutations are commonly found in numerous human cancers and targeted therapies with KRAS inhibitors have emerged as promising treatment strategies (62). The uncommon *KRAS* intronic variant rs10842518 (g.25400206 G>T) was identified in the present study.

Most of the somatic SNVs described in the present study by NGS analysis were notably identified in genes encoding tyrosine kinase proteins, revealing an important intratumor heterogeneity signature that may contribute as an additional event for OS development. The application of TKIs in patients with OS has been evaluated as a strategy to achieve improved therapeutic efficacy, suggesting the simultaneous inhibition of several relevant receptor tyrosine kinases in OS (63,64).

*TP53* is a tumor suppressor gene that encodes the p53 protein, which plays a key role in cell cycle control and genome integrity; thus, it is referred to as the guardian of the genome (65). Therefore, genetic alterations in the *TP53* gene leading to its malfunction are hallmarks of several human cancers. Regarding OS, mutations in *TP53* are commonly described (18,19,66). Additionally, OS is commonly diagnosed in Li-Fraumeni syndrome (LFS), a cancer predisposition syndrome characterized by inherited pathogenic germline

variants in the *TP53* gene (66,67). Mutational hotspots in the *TP53* gene notably occur by nucleotide substitution in the coding sequence within exons 4-8 (68). Wunder *et al* (69) investigated *TP53* mutations (exons 4-10) in 196 OS tumors by single-strand conformation polymorphism and sequencing, identifying 38 mutations (19.4%) described as 23 missense mutations, 11 nonsense mutations, three splice site changes and one in-frame insertion. Chen *et al* (18) investigated somatic mutations in pediatric osteosarcoma samples by whole-genome sequencing and identified multiple somatic chromosomal alterations (notably structural variations) and SNVs. High rates of *TP53* mutations, including *TP53* rearrangements (50%; 16/32), missense mutations (22%; 7/32), nonsense mutations (16%; 5/32) and *TP53* deletions (6%; 2/32), have been reported. By whole-genome sequencing, Ribi *et al* (70) identified patients with OS with a deletion in intron 1 of *TP53*, including exon 1, and a patient harboring a deletion, including the entire *TP53* gene. By whole-exome sequencing, Bousquet *et al* (71) identified *TP53* mutations in OS samples from three young patients, being described as STOP gained mutation, nonsynonymous mutation, deletions and splice site mutation. In the present study, no PCR products for *TP53* exon 5 were detected in the tumor tissue sample, suggesting that a deletion event in exon 5 can lead to somatic *TP53* inactivation and contribute to OS development. Sanger sequencing analysis revealed that the patient harbored the missense variant *TP53* c.712T>A (p.Cys238Ser) in tumor tissue sample and the *TP53* c.215C>G (p.Pro72Arg; rs1042522) germline missense variant in the peripheral blood sample. The variant *TP53* c.712T>A (p.Cys238Ser) is reported to be pathogenic in the ClinVar database and was also identified in a family with LFS (72). The *TP53* c.215C>G (p.Pro72Arg) is a common single nucleotide polymorphism and has been reported to be benign for LFS in the ClinVar database. Taken together, these findings reinforce the idea that *TP53* mutations represent key oncogenic drivers in patients with OS.

The present study reported a case of an adolescent patient with OS harboring an intratumor heterogeneity signature. OS is a rare and challenging malignant tumor. In Brazil, patients diagnosed with OS are still treated with a standard protocol, reinforcing the need for genetic testing to guide diagnosis and treatment. The present study has several limitations. First, although the National Institute of Traumatology and Orthopedics is a reference center for musculoskeletal sarcomas care, DNA sequencing was not performed in all patients, which could improve the investigation of the association between the genetic findings and the development of pediatric OS or even variations in therapy response. Second, the present study faced the lack of diagnostic molecular genetic tests to help select patients at highest risk of disease progression for this NGS-based study. It was hypothesized that patients with OS at higher risk could harbor genetic alterations that contribute to both disease development and progression. Third, only DNA targets were investigated in the present study; therefore, the detection of clinically actionable gene fusions that have been described in OS was not assessed. Despite this, the findings of the present study had the potential to help to unravel part of the genetic landscape that constitutes the biologic heterogeneity of OS, providing extensive genomic information through a refined and advanced NGS-based technology. Additionally, all genetic variants identified in the present study had not previously been described in OS cases and might have contributed to disease development.

Notably, the number of genome-targeted therapies and the percentage of patients with cancer responding to these therapies have increased over the years (73). By contrast, patients with OS continue to be ineligible for targeted therapy due to lack of genetic testing in clinical routine, being a consequence of little investment and visibility, which notably represents a knowledge gap. As researchers, it was hypothesized that the present study facilitates understanding on the need for OS genetic investigation. In the next 5 years, it is hoped that more efforts with global collaboration initiatives in the scientific and medical communities will include genetic testing in clinical routine for OS and contribute to clinical trials for new treatment modalities with targeted therapies to achieve improved clinical responses. In conclusion, the present study added new information on genetic aspects contributing for OS development, especially in pediatric patients. Genomic testing for OS will represent the opportunity to identify potential cancer driver genes to aid clinical decision making. Therefore, genetic profiling of OS needs further investigation.

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**Availability of data and materials**

The NGS datasets generated and/or analyzed during the current study are available in Figshare (https://doi.org/10.6084/m9.figshare.26015227.v1). Sanger sequencing datasets for *TP53* (exons 4-8) in the current study are available in Figshare (https://doi.org/10.6084/m9.figshare.27115351.v1).

**Authors' contributions**

MJ contributed to the conception of the present study, performed the experiments, analyzed the molecular data and wrote the original and final draft of the manuscript. RS and TC contributed significantly to the NGS experiments and analysis. EL and RP contributed to the acquisition and writing of the clinical data. AC contributed to the acquisition and writing of the histopathological data. AL and WM analyzed all the clinical data. GA and MO reviewed the study and confirmed the authenticity of all the raw data. All the authors read and approved the final version of the manuscript.

**Ethics approval and consent to participate**

The present study was approved by the Ethics and Research Committee of the National Institute of Traumatology and Orthopedics (approval no. CAAE: 60632822.4.0000.5273). Written informed consent was obtained from the patient and from the patient's legal guardian.

## Patient consent for publication

Written informed consent was obtained from the patient and from her legal guardian.

## Competing interests

The authors declare that they have no competing interests.

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