The Genomic and Immune Landscape of Osteosarcoma

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INTRODUCTION

Osteosarcoma (OS) is the most common primary bone malignancy and has one of the highest rates of genetic structural variation.

Additionally, several heritable genetic syndromes can predispose to the development of OS such as Li-Fraumeni syndrome (germline *TP53* mutation) and hereditary retinoblastoma (germline *RB1* mutation). Although germline alterations are frequently found in OS, genetic testing is not routinely offered.

Here, we evaluate tumor genomic alterations, incidental germline findings, and immune profiles of OS patients from one of the largest paired tumor/normal-matched (T/N) tissue datasets.

METHODS

- De-identified records of patients diagnosed with OS who underwent Tempus xT next-generation sequencing (NGS) were identified from the Tempus Database (n=126), including 55 tumor-only (44%) and 71 T/N-matched samples (56%).
- Pathogenic/likely pathogenic (P/LP) single-nucleotide variants (SNVs), insertions/deletions (indels), and copy number alterations (either loss or amplification) were evaluated.
- For each T/N-matched sample with detected SNVs/indels, the rate of incidental germline findings detected in 65 reportable hereditary cancer genes was calculated.
- Immune environment features including tumor mutational burden (TMB), PD-L1, microsatellite instability (MSI), neoantigen tumor burden, and immune cell composition were also measured from DNA-seq, RNA-seq, or IHC data.

Table 1. Cohort Overview

Characteristic	Prevalence or Median (n =126)
Age, median (IQR)	30 (16, 51), Range (1-87)
Sex, n (%)	Male: 77 (62%), Female: 47 (38%)
Race/Ethnicity, n (%)	White: 49 (72%), Asian: 8 (12%), Black/African American: 5 (7.4%), Other: 6 (8.9%)
Histology, n (%)	OS, NOS: 102 (81%), Chondroblastic OS: 15 (12%), Telangiectatic OS: 4 (3%), Fibroblastic OS: 2 (1.6%), Small cell OS: 2 (1.6%), Parosteal OS: 1 (0.8%)
Stage, n (%)	IV: 66 (86%), II: 7 (9.1%), III: 4 (5.2%)

% calculated from known. OS = Osteosarcoma.

SUMMARY

From T/N-matched NGS of a real-world OS cohort, the paired germline samples revealed a greater understanding of both somatic and germline findings than has been previously reported. Accordingly, T/N-matched sequencing in OS patients should be considered as results may have clinical implications for the patient, as well as at-risk family members.

RESULTS

Genomic and immune landscape of osteosarcoma

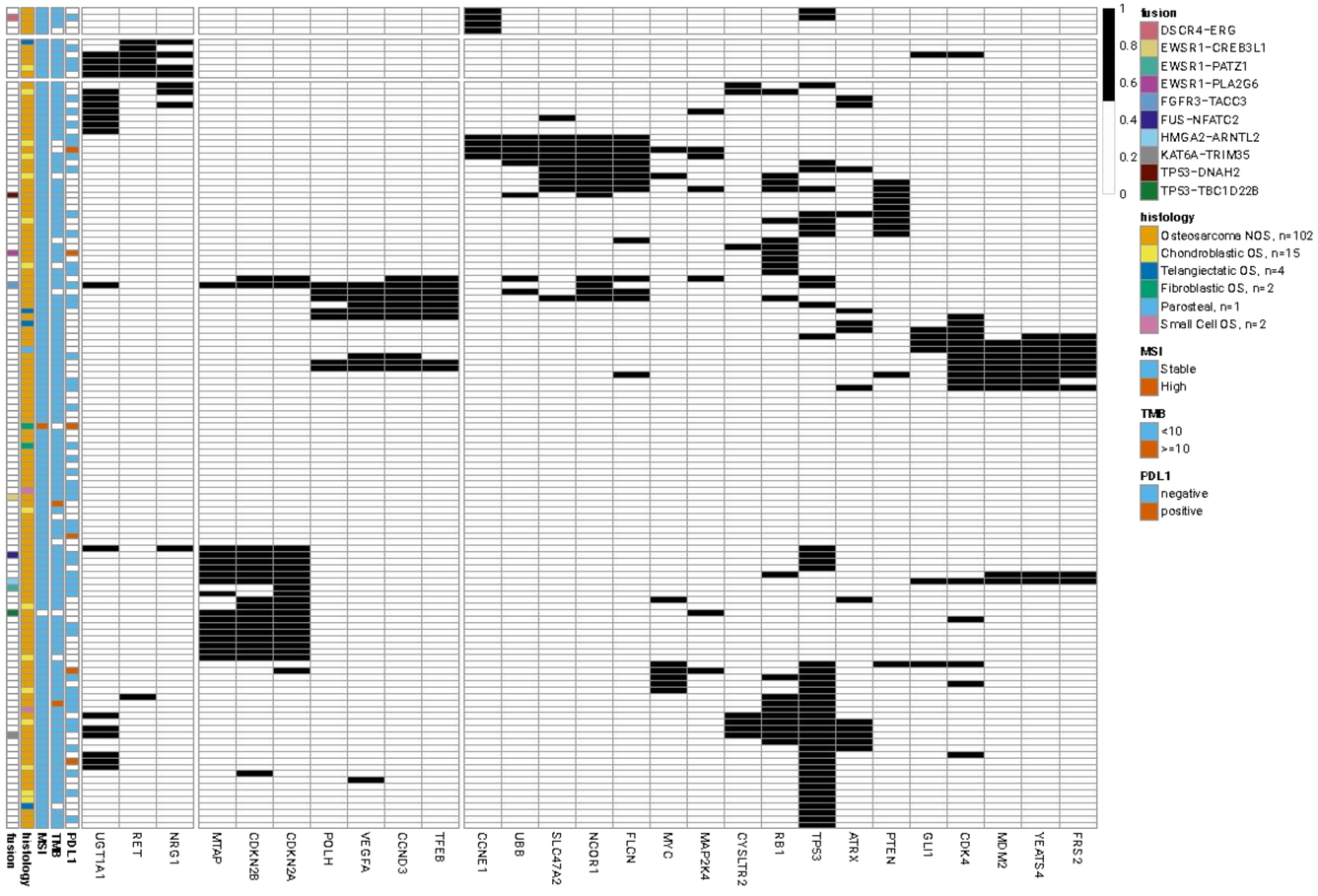

Figure 1. Each row corresponds to a different patient sample. Key findings are summarized below:

Incidental P/LP Germline Variants from T/N-Matched Sequencing

P/LP Variant	Prevalence, n (%)
MUTYH	2 (2.8%)
PMS2	2 (2.8%)
RB1	1 (1.4%)

Proportion of Macrophages in OS Immune Environment

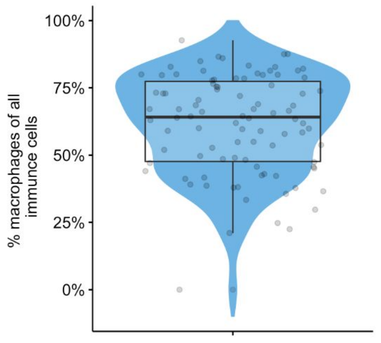

Figure 2. Immune cell infiltration assays by RNA-seq showed that macrophages (median=64% of immune cells) were the predominant immune infiltrating cell over B cells, CD4/8 T cells, and NK cells.

- Of the 126 samples, 117 (93%) had at least one P/LP mutation, CNA, or fusion.
- We identified frequent somatic mutations in *TP53* (n=44, 35%), *RB1* (n=22, 17%), *CDKN2A* (n=21, 17%), *CDKN2B* (n=19, 15%), *UTG1A1* (n=19, 15%), *CDK4* (n=18, 14%), and many others.
- We detected a total of ten distinct fusions out of 125 patients who had fusion data available (8%), three of which were detected via RNA only (*KAT6A-TRIM35*, *HMGA2-ARNTL2*, and *FUS-NFATC2*). Three of the fusions (*EWSR1-PATZ1*, *EWSR1-CREB3L1*, and *FUS-NFATC2*) are characteristic of Ewing variant sarcoma. Thus, we show that molecular data helped reclassify the pathological diagnosis of osteosarcoma in 2.4% of cases based on fusion data alone.
- Among the 71 T/N-matched samples, we detected P/LP germline variants in 5 OS patients (7%). The most prevalent P/LP germline variants were *MUTYH* (n=2, 3%), *PMS2* (n=2, 3%), and *RB1* (n=1, 1.4%). (Table 2)
- In the immune environment of OS, MSI was high in one patient out of 125 (0.8%), TMB was high in two out of 113 (2%), and PD-L1 positivity was observed in 6 out of 55 patients (11%).

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