Lists of supplementary files

Figure S1: The Kaplan-Meier curve of progression free survival (PFS) and overall survival (OS) between group A and group B. No statistical difference was found in PFS (A, *p*=0.33) or OS (B, *p*=0.06)

Figure S2: The gene signature of osteosarcoma patients. (A) The gene mutational signature of group A and group B. The y-axis represents the percentage of signature in the sample. (B) The gene mutational signature of paired primary and metastatic osteosarcoma. (C) The boxplot of mutational signature of paired primary and metastatic osteosarcoma. There were no statistically significant differences in all the 30 signatures. (D) The boxplot of mutational signature of group A and group B. A decomposition of mutation spectra showed that the signature 1 was statistical different between two groups, which mainly referred to ages(*p*=0.004).

Figure S3: The comparation of the age distribution between these two groups of patients with Mann-Whitney U test (*p*=0.06)

Figure S4: The phylogenetic trees of 11 representative osteosarcoma patients. 3/8 (37.5%) patients in group A (Patient 1、7、8) had a more linear evolutionary pattern, while 7/7 (100%) patients in group B (Patient 9、10、11、13、14、15、21) had a more parallel evolutionary pattern.

Figure S5: The evolutionary process of subclones in 12 representative osteosarcoma patients. The X-axis represents the time point, Y-axis represents clonal prevalence.

Figure S6: Primary lesions and metastatic lesions of paired osteosarcoma (OS) patients. (A) The barplot and boxplot of tumor mutation burden (TMB) of primary lesions and metastatic lesions of paired OS patients. (B) The barplot and boxplot of neoantigens of primary lesions and metastatic lesions of paired OS patients. (C) The profiling pathway of primary lesions and metastatic lesions of paired OS patients.

Figure S7: The PET/CT scan of one patient from group A with multiple bone metastasis without any pulmonary metastasis and receiving the therapy of programmed cell death 1 (PD-1) antibody. (A) The fusion images of maxilla and pelvis with high SUVmax before the therapy of PD-1 antibody. (B) The fusion images of maxilla and pelvis with low SUVmax after the therapy of PD-1 antibody.

Figure S8: The results of multiplex immunohistochemistry. (A) The density of macrophages (MΦ) between tumor and normal tissue. (B) The density of MI- and MII-like phenotype in all patients (left). And the density of MI- and MII-like phenotype among edge, margin and normal tissue. (C) The density of five different subtype of macrophages between tumor and normal tissue.

Table S1: Detailed clinical demographics of 38 patients with metastatic osteosarcoma

Table S2: The whole-exome sequencing data of 38 patients with osteosarcoma

Table S3: Scoring of various checkpoint molecule immunochemistry staining intensities for 6 patients

Table S4: The clinical information of Cancer Genome Atlas (TCGA) database

Table S5: The clinical information of Therapeutically Applicable Research To Generate Effective Treatments (TARGET) database