

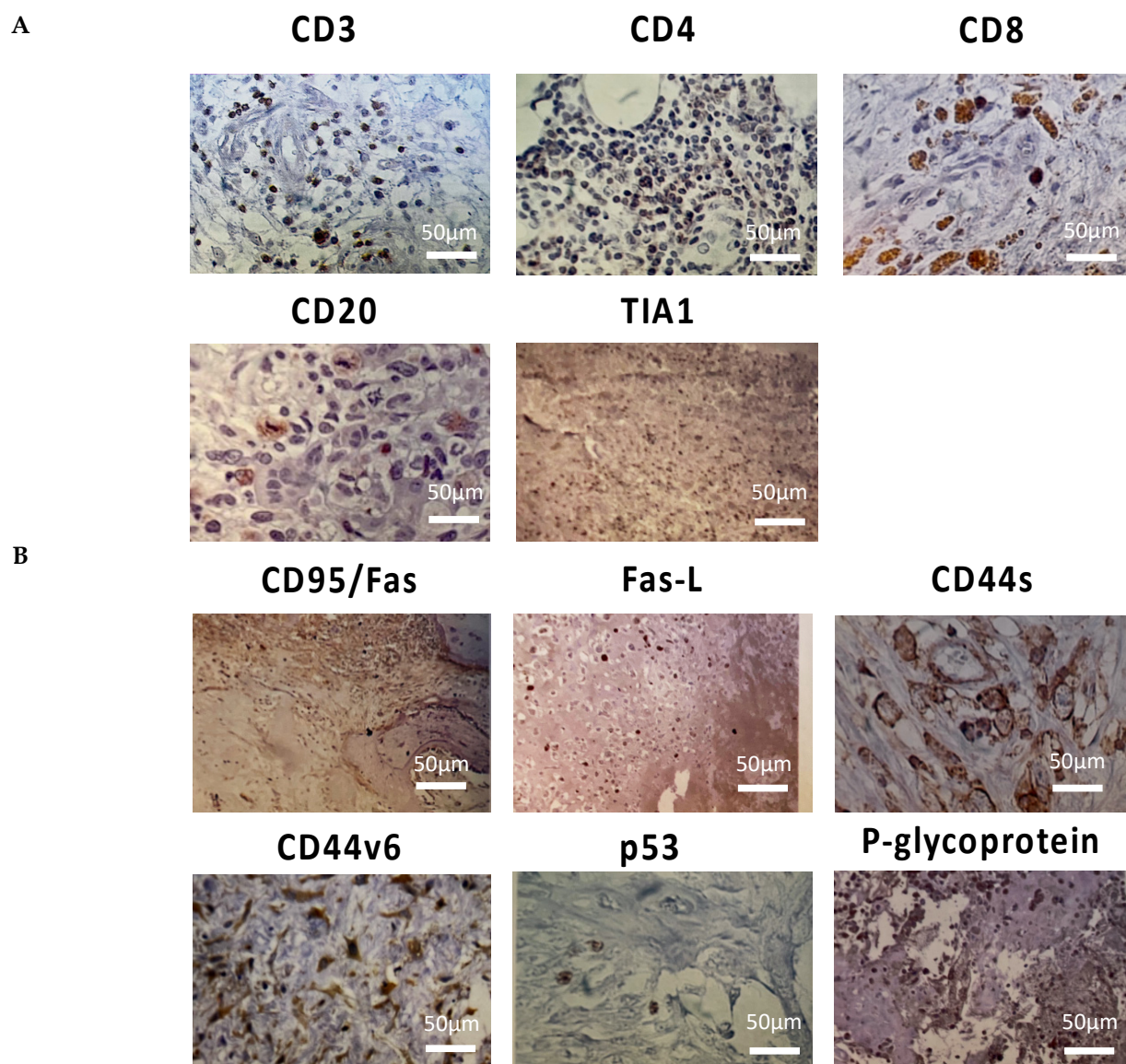
# Supplementary Materials: Tumor-infiltrating lymphocytes and cancer markers in osteosarcoma: influence on patient survival

José Manuel Casanova, Jani-Sofia Almeida, John David Reith, Luana Madalena Sousa, Ruben Fonseca, Paulo Freitas-Tavares, Manuel Santos-Rosa, Paulo Rodrigues-Santos

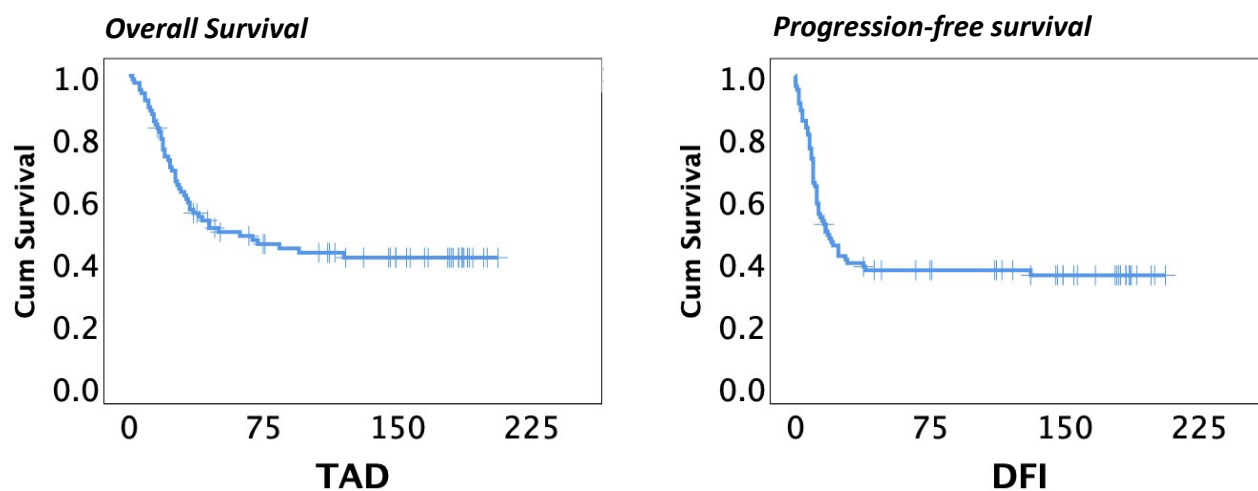
**Table S1.** Primary specifications of: antibodies, staining, and laboratorial techniques applied to the tumor tissue samples from OST patients.

| Antibody                  | Class | Target                        | Isotype     | Clone   | Reference   | Brand  | Antigenic recovery         | Staining | Ventana Kit                          | Control tissue                                 | Dil.  | Staining                   |
|---------------------------|-------|-------------------------------|-------------|---------|-------------|--|----------------------------|----------|--------------------------------------|--|-------|----------------------------|
| Anti-human CD3            | Mono  | T cells.                      | Rabbit      |         | A 452       | DAKO corporation; CA, USA                            | Micro-wave, citrate buffer | Ventana  | DAB detection Kit                    | Lymph node                                     | 01:50 | Membrane                   |
| Anti-human CD4            | Mono  | CD4 T cells                   | Rat / IgG1  | 1F6     | NCI-CD4-IF6 | Novocastra Laboratories Ltd; NCL, UK                 | Micro-wave, EDTA solution  | Ventana  | DAB high Intensity amplification Kit | Lymph node                                     | 01:20 | Membrane                   |
| Anti-human CD8            | Mono  | CD8 T cells                   | Rat / IgG1  | C8/144B | M-7103      | DAKO corporation; CA, USA                            | Micro-wave, citrate buffer | Ventana  | Amplification Kit                    | Lymph node                                     | 01:20 | Membrane                   |
| Anti-human TIA1           | Mono  | NK and CD8 T cells            | Rat / IgG1  | TIA-1   | PN 6604593  | Coulter Corporation Ltd; CA, USA                     | Micro-wave, citrate buffer | Ventana  | Amplification Kit                    | Lymph node                                     | 0.146 | Membrane (granules)        |
| Anti-human CD20           | Mono  | B cells                       | Rat / IgG2a | L26     | M 755       | DAKO corporation; CA, USA                            | Micro-wave, citrate buffer | Ventana  | DAB detection Kit                    | Lymph node                                     | 0.111 | Membrane                   |
| Anti-human CD95/Fas       | Mono  | Apoptosis mediator            | Rat / IgG1  | APO-1   | M3553       | DAKO corporation; CA, USA                            | Protease 2                 | Manual   |                                      | Lymph node / tonsil                            | 01:10 | Membrane or cytoplasm      |
| Anti-human Fas-L          | Poly  | Apoptosis mediator.           | Rabbit      | N-20    | sc-834      | Santa Cruz Biotechnology; CA, USA                    | Micro-wave, citrate buffer | Ventana  | DAB detection Kit                    | Colon adenocarcinoma                           | 01:25 | Membrane or cytoplasm      |
| Anti-human CD44s          | Mono  | Migration and cell signaling. | Rat / IgG1  | DF 1485 | M7082       | DAKO corporation; CA, USA                            | Micro-wave, citrate buffer | Manual   |                                      | Lymph node / tonsil                            | 0.097 | Membrane                   |
| Anti-human CD44v6         | Mono  | Metastatic processes.         | Rat / IgG1  | VVF7    | NCL-CD44v6  | Novocastra Laboratories Ltd; NCL, UK                 | Micro-wave, citrate buffer | Ventana  | DAB high Intensity amplification Kit | Colon adenocarcinoma with lymphatic metastases | 01:50 | Membrane                   |
| Anti-human p53            | Mono  | Apoptosis and cell cycle.     | Rat / IgG2b | DO-7    | M7001       | DAKO corporation; CA, USA                            | Micro-wave, citrate buffer | Ventana  | Amplification Kit                    | Breast adenocarcinoma                          | 01:50 | Nuclear                    |
| Anti-human P-glycoprotein | Mono  | Drug resistance.              | Cow / IgG1  | JBS-1   | YM-9001     | Accurate Chemical & Scientific Corporation; NY, USA. |                            | Ventana  | Amplification Kit                    | Kidney tissue                                  | 01:50 | Membrane and Golgi complex |

Legend: Mono – Monoclonal; Poly – Polyclonal; Dil. – Dilution.

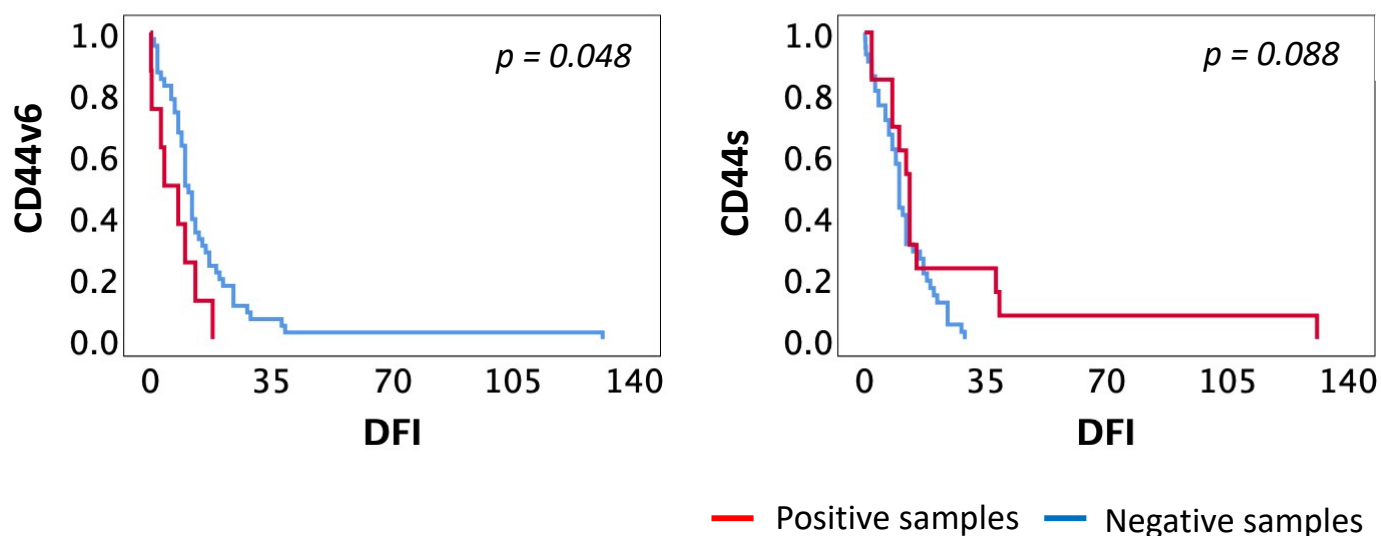


**Figure S1.** Immunohistochemistry representative images (400× amplification) of positive samples from OST patients for TIL infiltration and tumor markers expression. Scale bar, 50 µm.

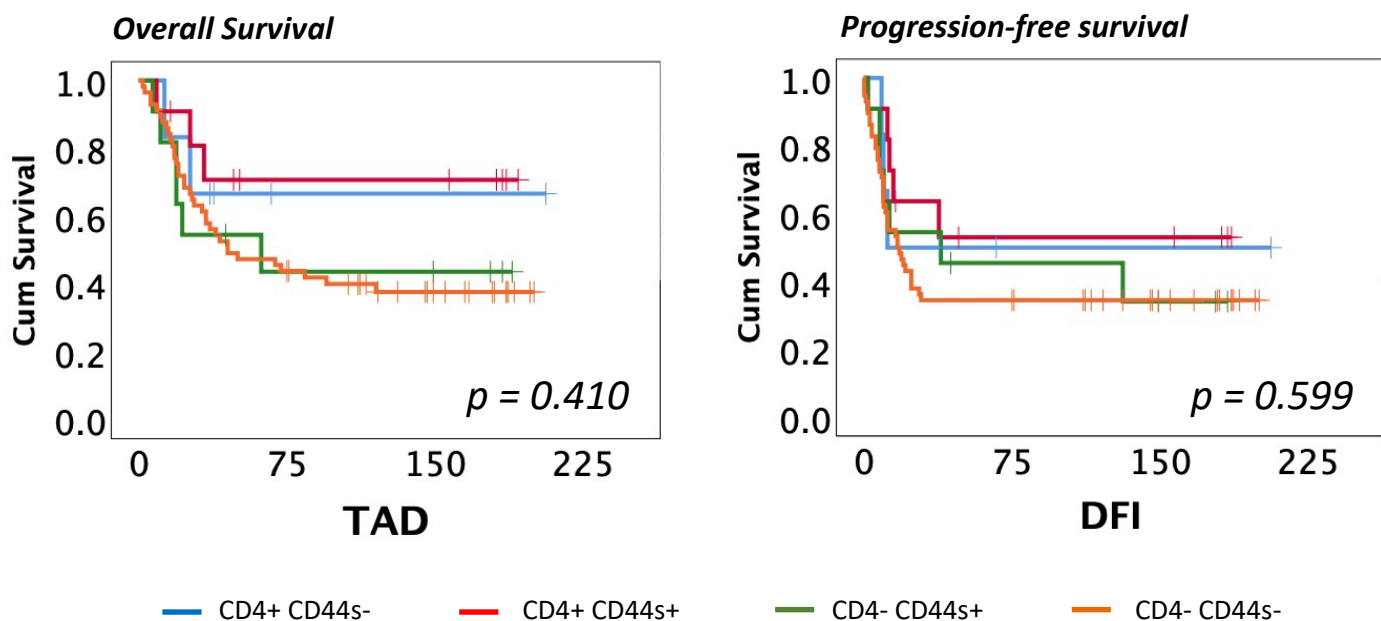


**Figure S2.** Overall survival and progression-free survival functions calculated for the OST patients enrolled in the study. TAD – Time after diagnosis (months); DFI – Disease-free interval (months).

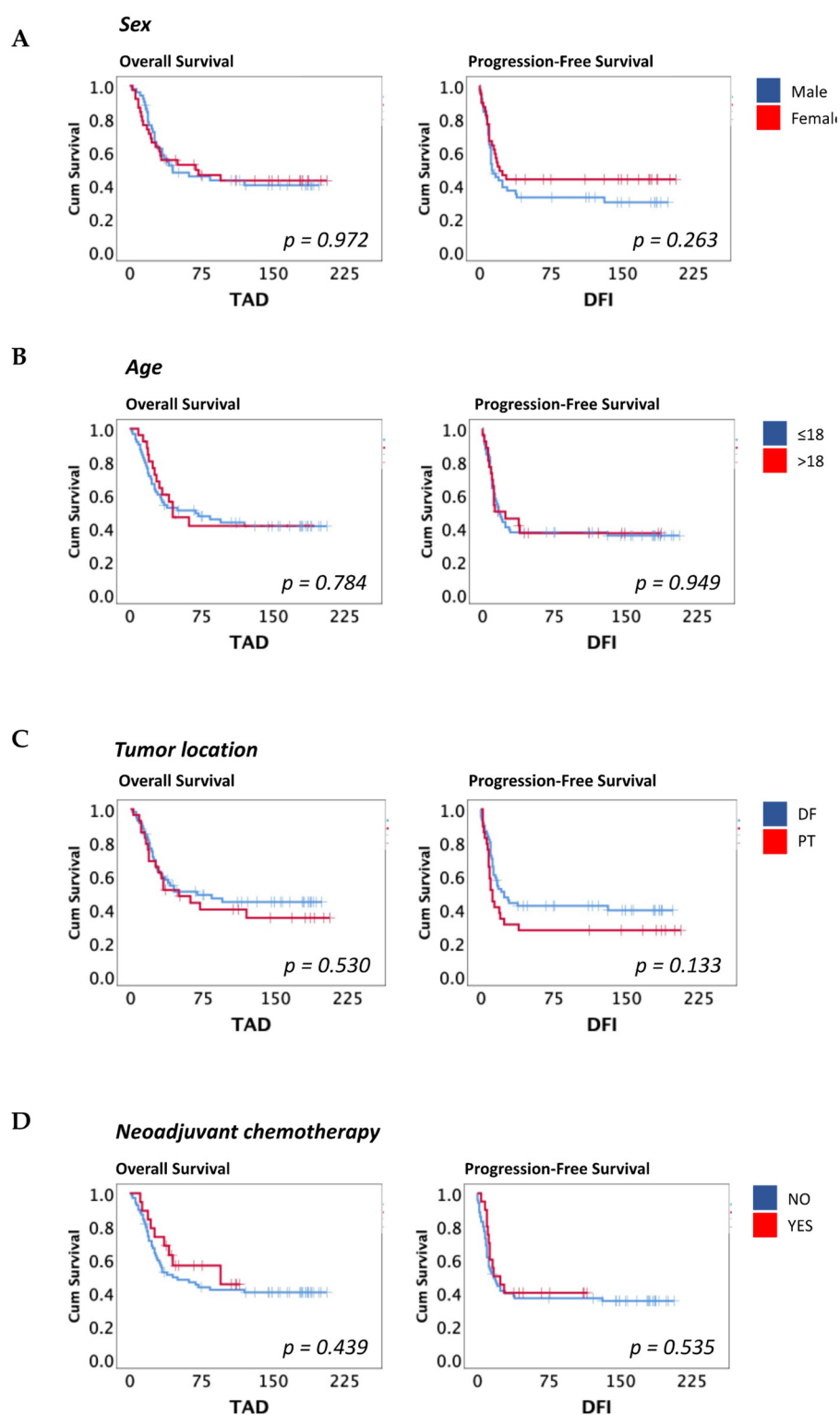
### Time to occurrence of metastases



**Figure S3.** Time to the occurrence of metastasis regarding CD44v6 and CD44s expression calculated for the OST patients enrolled in the study. DFI – Disease-free interval (months).



**Figure S4.** Overall and progression-free survival regarding the combination of positive and negative samples for CD4+ cells infiltration and CD44s expression calculated for the OST patients enrolled in the study. TAD – Time after diagnosis (months); DFI – Disease-free interval (months).



**Figure S5.** Overall survival and progression-free survival calculated for OST patients according to sex, age, tumor location and the use of neoadjuvant chemotherapy. TAD – Time after diagnosis (months); DFI – Disease-free interval (months).