Immune Cell Composition and Chronic Inflammation Association in Metastatic High-Grade Osteosarcoma

Maithily Diaz, MSci[1], Grant Eilers MD PhD[2], Antoine Bougoüin[3], Melissa Zimel MD[5], Varun Monga MD[4], Rosanna Wustrack MD[5], Kiauntee Murray MD[5]

1. School of Medicine, University of California, San Francisco, CA
2. Department of Pathology, University of California San Francisco
3. Centre de Recherche des Cordeliers, Paris, France
4. Department of Hematology and Oncology, University of California San Francisco
5. Department of Orthopedic Surgery, University of California San Francisco

Significance: Immune checkpoint inhibitors (ICI), are a promising cancer treatment across various malignancies but have limited effectiveness in osteosarcoma. Chronic inflammation is associated with tumor progression and metastasis, including specific macrophage-related activity in osteosarcoma. Previous research has shown tertiary lymphoid structures (TLS), organized aggregates of B and T cells, as predictive biomarkers for improved ICI therapy response in select soft tissue sarcomas however their presence and relevance in osteosarcoma remains poorly described. We sought to characterize immune cell composition (ICC) and chronic inflammatory state in metastatic osteosarcoma. We hypothesized TLS presence would be sparse and associated with increased ICC, potentially serving as a marker for therapy response.

Innovation: This study integrates multiplex immunofluorescence and clinicopathologic data to evaluate TLS presence and immune cell composition in primary high-grade osteosarcoma, with correlation to prior inflammatory conditions, metastatic outcomes and treatment response.

Approach: We retrospectively analyzed 79 formalin-fixed, paraffin-embedded (FFPE) resection specimens from 77 patients 0-65 years old with high-grade extremity osteosarcoma treated with neoadjuvant chemotherapy at a single center between 2014–2024. Immunofluorescence staining quantified ICC, including TLS, B cells, T cells, and macrophages; PD-L1 expression was assessed by immunohistochemistry. Clinical data included prior chronic inflammatory conditions (autoimmune or infection), metastasis, mortality, and chemotherapy response.

Results: In 61 patients evaluated, no patients had a prior history of inflammatory conditions. From 63 FFPE specimens, TLS was confirmed in two samples; one patient was a good responder to chemotherapy, but both patients experienced lung metastasis and are undergoing treatment. Overall, metastasis occurred in 26% of the cohort, and 19% were poor responders to chemotherapy. ICC was variable, with a trend toward increased B cells, cytotoxic T cells, and PD-L1 expression in TLS-positive patients compared with TLS-negative patients. Metastasis was associated with increased macrophage infiltration, but insignificant. In conclusion, neither clinical outcome nor therapy response was associated with TLS presence.

Discussion: This study showed that among a cohort of 61 patients with high-grade osteosarcoma, TLS was rare, and the rate of metastasis or response to immunotherapy was not significantly associated with TLS status or immune markers. We were unable to evaluate for prior chronic inflammatory conditions. Continued research will evaluate ICC, chronic immune conditions, and related outcomes, including metastasis and therapy response, in a larger cohort to further identify why salvage ICIs are ineffective in osteosarcoma.

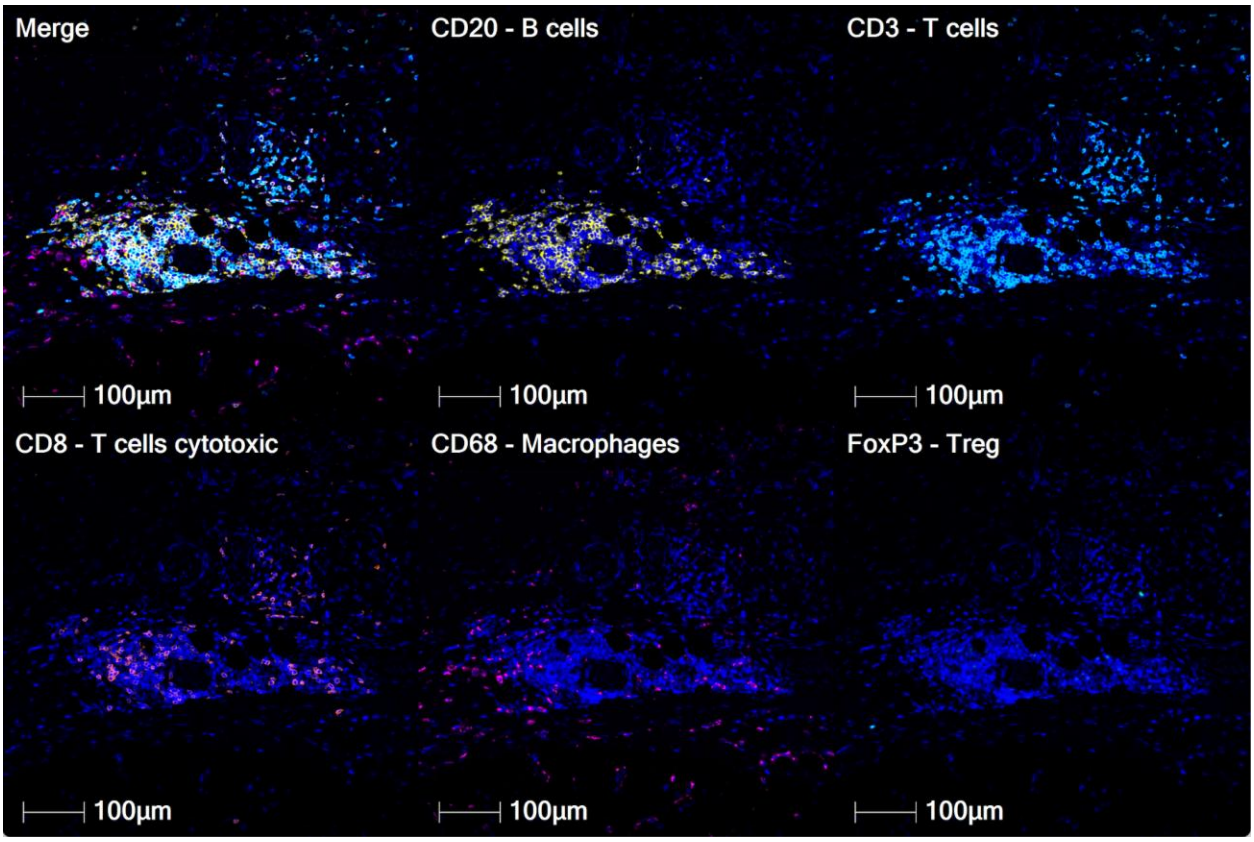

Figure 1: Tertiary lymphoid structures (TLS) are identified as an aggregate of CD20+ B cells and CD3+ T cells.