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Opinion

Two-Layer Precision Immunotherapy for Bone and Soft-Tissue Sarcomas

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Abstract

Bone and soft-tissue sarcomas resist single-agent checkpoint blockade because their microenvironment is myeloid-dominated and T-cell poor. Over the past two years the immunotherapy arsenal has expanded faster than the tools used to allocate it. We propose selecting patients through two complementary layers: molecular stratification to narrow candidate modalities, then patient-derived organoid testing of those modalities *ex vivo*. We review the 2024-2026 evidence and confront its central obstacle, a T-cell-poor organoid compartment. Whether functional prediction adds value beyond molecular biomarkers is a question a co-clinical trial could settle.

Keywords: Sarcoma, Tumor immune microenvironment, Immunotherapy, Patient-derived organoid, Precision oncology

Abbreviations: TCR-T: T-Cell Receptor-Engineered T Cell; CAR-T: Chimeric Antigen Receptor T-Cell; TLS: Tertiary Lymphoid Structures

The Problem

Sarcomas are heterogeneous mesenchymal cancers with poor advanced-stage outcomes. Their microenvironment is frequently myeloid-dominated and T-cell poor, and single-agent checkpoint blockade elicits responses in only a few histologies [1]. Spatial profiling backs this up. Immune-cold tumor regions track with worse survival [2], and lymph-node metastases actively rewire the immune tissue around them [3]. The treatment menu, though, looks nothing like it did two years ago. Afamitresgene autoleucel won approval in synovial sarcoma [4], the first approved T-cell receptor-engineered T cell (TCR-T) product for any solid tumor; NY-ESO-1-directed TCR-T reported comparable activity [5]. B7-H3 and GD2 chimeric antigen receptor T-cell (CAR-T) cells have entered sarcoma trials, with manageable toxicity but modest responses [6, 7]. Options are no longer the limiting factor. Matching is the problem: deciding which patient should get which option, a call physicians still base on one static molecular snapshot.

Two Complementary Layers

Our proposal stacks two layers (Figure 1). Layer 1 sorts tumors by what a biopsy can measure, a molecular-spatial stratification: histology and subtype, antigen and HLA status, and microenvironmental signatures. That triage shortens the list of candidate modalities. Layer 2 is the functional test. A patient-derived organoid is grown and challenged with each shortlisted modality ex vivo, and the readout is simply how much tumor is killed. Neither layer suffices alone. A molecular biomarker scales to every patient but only estimates a probability. An organoid

measures the real tumor-immune outcome in that patient's own cells, though slowly and one case at a time. We run them in this order for a practical reason: Layer 1 keeps the organoid workload small, and Layer 2 turns population-level odds into a decision for the individual patient.

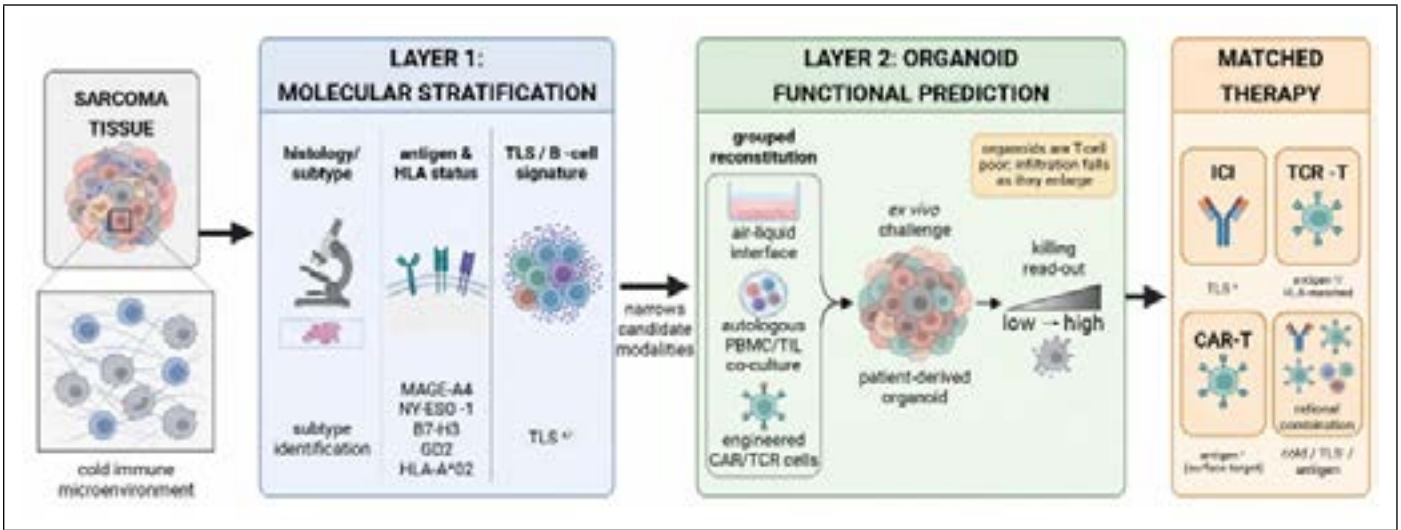

Figure 1: A two-layer strategy for allocating immunotherapy in bone and soft-tissue sarcoma.

Most sarcomas present a myeloid-dominated, T-cell-poor tumor immune microenvironment (left). In Layer 1 (molecular stratification), histology and subtype, antigen and HLA status (e.g., MAGE-A4, NY-ESO-1, B7-H3, GD2; HLA-A*02), and tertiary lymphoid structure (TLS)/B-cell signatures narrow the plausible modalities. In Layer 2 (organoid functional prediction), a patient-derived organoid, after reconstitution of an effector compartment by air-liquid interface culture or co-culture with autologous lymphocytes, expanded tumor-infiltrating lymphocytes, or engineered CAR/TCR cells, is challenged ex vivo, and the magnitude of organoid killing confirms and ranks the candidates. The bottleneck (amber) is that sarcoma organoids are T-cell poor and lose immune infiltration as they enlarge, which most constrains checkpoint-inhibitor prediction. The layers route tumors to matched therapy (right): TLS-positive tumors toward immune checkpoint inhibition; antigen- and HLA-matched tumors toward TCR-T; antigen-positive tumors toward CAR-T, which recognizes surface antigen independently of HLA; and cold, TLS-negative, antigen-negative tumors toward microenvironment-remodeling combinations ranked ex vivo. Created in BioRender. Liu, H. (https://BioRender.com/vixm9p1) is licensed under CC BY 4.0.

Evidence, and the Bottleneck

Within Layer 1, one predictor outperforms the rest, and it is architectural rather than molecular: Tertiary Lymphoid Structures (TLS). When PEMBROSARC enrolled only TLS-positive sarcomas, about 30% responded, against roughly 2% in an unselected population [8]. The idea traces back to the B-cell-based immune classes described earlier [9]. Antigen and HLA status, by contrast, acts as a near-deterministic eligibility gate for engineered cells [4-7]. Tumor mutational burden, microsatellite instability and PD-L1 add little in sarcoma [10]. For Layer 2, sarcoma organoid biobanks now span the histological breadth of the disease (126 patients, 24 subtypes) [11]. They predict CAR-T sensitivity from 44 lines [12] and reconstruct T-cell infiltration in an osteosarcoma xenograft-organoid system [13]. The obstacle is specific: sarcoma organoids are T-cell poor and lose immune infiltration as they enlarge [13]. Predicting checkpoint response, unlike engineered-cell killing, then depends on reconstituting an effector compartment by air-liquid interface culture or immune co-culture.

Matching, and a Testable Claim

The layers combine into a decision logic that routes tumors by phenotype. TLS-positive tumors point to checkpoint blockade; antigen- and HLA-matched tumors settle eligibility for engineered cells, with organoids confirming killing. The commonest and hardest case is the cold, TLS-negative, antigen-negative tumor, which leaves Layer 1 uninformative. There, a functional assay contributes most, empirically ranking microenvironment-remodeling combinations such as regorafenib plus avelumab [14]. The framework is falsifiable: a prospective co-clinical trial can stratify patients molecularly and assay their organoids in parallel, then measure whether functional prediction adds value beyond molecular selection. If it does not improve on molecular selection, it should be dropped; if it does, it should be standardized and scaled.

Box 1. Outstanding Questions

a) Can sarcoma organoids reconstitute a functional T-cell compartment well enough to predict checkpoint-inhibitor, not only engineered-cell, response?

b) In a prospective trial, does organoid functional read-out add predictive value beyond TLS and antigen/HLA status?

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Author Contributions

S.L., B.Y., and Z.L. conceptualized the article, performed the literature search, and wrote the original draft. S.Y. conceived and supervised the work, critically revised the manuscript, and provided final approval. All authors read and approved the final version.

Declaration of Interests

None.

Conflict of Interest

None.

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