Collaborative Understanding of the Range and Evolution of Osteosarcoma Single-cells
MIB AGENTS OSTEOSARCOMA ALLIANCE

Prospective Data Collection Plan
WORKING DRAFT

Purpose:

This document provides a consensus-based "wishlist" for biospecimen and clinical data collection in osteosarcoma clinical trials. It is intended as a practical guide to ensure that, as new trials are designed, they capture the essential data and biospecimens that will maximize downstream research value, support biomarker validation, and accelerate clinical translation of single-cell and multi-omic discoveries. A particular focus is the acquisition of serial, longitudinal data that will allow better understanding of disease evolution, including on therapy, as well as multi-modal data types to support the integration of evolving AI technologies in learning from real-world evidence.

This working plan reflects discussions from the MIB FACTOR 2025 CURE-OS breakout session and incorporates insights from investigators, clinicians, and patient advocates. It outlines core elements, priority data types, and collection timepoints to define a "Gold Standard Dataset" for osteosarcoma research.

Key Principles:

1. **Feasibility + Aspirational Vision:** Recommendations balance what is currently achievable across cooperative group and institutional settings with aspirational elements to guide future trial design and leverage novel technologies.
2. **Minimal Metadata Standards:** Each biospecimen must be annotated with harmonized clinical metadata (diagnosis, treatment regimen, imaging, outcomes).
3. **Temporal Depth:** Longitudinal sampling is critical to understand osteosarcoma biology, treatment response, and evolution.
4. **Integration:** Link biospecimens to clinical, imaging, pathology, and molecular data for comprehensive analysis.
5. **Transparency & Standardization:** Protocols should be shared across sites, with centralized curation to reduce siloing of cohorts and data.

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Recommended Timepoints & Data Types:

Timepoint	Clinical Data	Biospecimens	Pathology / Imaging	Molecular / Multi-omic Studies	Notes
Baseline/Diagnosis	Demographics, detailed clinical history, baseline labs	Core biopsy or surgical specimen (flash-frozen, FFPE, optional viably frozen); baseline blood (plasma, serum, PBMCs); germline DNA	H&E slides; digital pathology; MRI of primary site, CT chest, PET/CT or bone scan	Bulk DNA/RNA (ideal WGS/RNA-seq); ctDNA; optional: single-cell RNA-seq; spatial transcriptomics; proteomics; epigenetics (methylation)	Define baseline biology and risk stratification
Post neo-adjuvant treatment (e.g., post 2 cycles of MAP at time of surgery/local control)	Interim clinical response, key lab trends (LDH, Alk Phos), toxicity Operative details, pathology report, % necrosis, adverse events	Blood (serial plasma/serum for ctDNA, CTCs) including early on treatment; Resection specimen (tumor margins, adjacent tissue); blood (peri-operative)	Repeat MRI/CT chest/PET; radiographic response H&E slides; Digital pathology; gross + microscopic sections	Bulk DNA/RNA; ctDNA kinetics; protein biomarkers. Optional: single-cell / spatial profiling of residual viable tumor; proteomics	Capture treatment effect on tumor/TME; critical for response biomarkers. Identify resistant subclones; inform mechanisms of persistence.
On treatment residual disease (e.g., thoracotomies, metastasectomy during therapy)	Surgical details (site, number of nodules, margins, perioperative course); ongoing systemic therapy at time of surgery; treatment modifications	Fresh tumor tissue from resected nodules (flash-frozen, FFPE, optional viably frozen); paired blood (peri-operative plasma/serum)	H&E slides; Digital pathology. Radiology correlation (pre-op CT chest; CXRs; other imaging modalities)	Bulk DNA/RNA; single-cell RNA-seq; spatial profiling of residual lesions; ctDNA at peri-operative timepoints; proteomics	Biology of disease persisting under systemic therapy to define resistant clones, metastatic seeding biology, interaction with therapy.
End of therapy / Remission	Disease status, labs, adverse events, chemotherapy received with detailed timing/doses	Blood (plasma/serum, PBMCs)	MRI/CT chest/PET	ctDNA assessment; CTCs;	Enable surveillance biomarker development and MRD studies
Relapse / Progression	Progression type (local, distant), treatment at relapse, resectability, labs	Relapsed tumor biopsy or resection; blood (plasma/serum, PBMCs)	MRI/CT/PET imaging H&E slides; Digital pathology; gross + microscopic sections	Bulk DNA/RNA; optional: single-cell and spatial profiling, proteomics; ctDNA	Understand mechanisms of metastasis and resistance.
Long-term Follow-Up	Outcomes, survival (disease-free, overall), late toxicities, QOL metrics	Blood (annual)	Surveillance imaging; serial blood	ctDNA longitudinal tracking	Ensure durability of biomarkers and links to survivorship.

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Prioritized Data Types:

1. **Essential**
    a. Clinical annotations (treatment regimens, outcome, relapse details)
    b. Baseline tumor tissue (frozen + FFPE)
    c. Serial blood for ctDNA
    d. Digital pathology + imaging at baseline and longitudinally
2. **Strongly Recommended**
    a. Longitudinal tumor samples (surgery, relapse; frozen + FFPE)
    b. Bulk DNA/RNA (WGS/RNA-seq)
    c. Germline DNA
3. **Aspirational**
    a. Single-cell and spatial profiling at key timepoints; proteomics
    b. AI-ready integrated imaging, pathology, and omics datasets

Implementation Roadmap:

- **Consensus Protocols:** Align with cooperative groups to standardize biospecimen collection and metadata annotation.
- **Centralized Repository:** Link with CURE-OS single-cell data platform (Tab 2 deliverable) for data hosting and integration.
- **Collaborative Governance:** Define oversight, consent, and data-sharing frameworks
- **White Paper:** Draft and circulate a CURE-OS publication outlining the “Gold Standard Dataset” and rationale and use cases. What question can we answer and what technologies can we leverage if we have the right data?

Next Steps:

This working draft represents the first interaction of a prospective data collection framework. We invite input on feasibility, prioritization, and missing elements. Feedback will inform the next version, including a roadmap for pilot implementation.