Executive summary

This longitudinal osteosarcoma dataset spans four clinical timepoints from a single patient:

- **T0 (2022-11, initial resection)** – two regions
- **T1 (2024-06, re-resection)**
- **T2 (2025-01, biopsy)**
- **T3 (2025-04, resection)** – two regions

The most striking finding is a **transition from a tumour-dominated ecosystem at T0 to a myeloid-dominated ecosystem at T3**, with T2 representing an intermediate state. This suggests substantial remodelling of the tumour microenvironment (TME) during disease progression and/or treatment. All observations below are derived from osteosarc-multiqc_report.txt. [osteosarc-...iqc_report | Txt]

---

1. Major shifts in cellular composition

T0: Tumour-rich microenvironment

Tumour cells comprise approximately:

Sample	Tumour fraction
T0-0078011	47%
T0-0078018	60%

with myeloid cells representing only ~31–45% of cells. [osteosarc-...iqc_report | Txt]

This indicates a highly tumour-centric architecture with relatively limited immune infiltration.

T1: Tumour bottleneck

T1 becomes even more tumour-enriched:

Cell type	Fraction
Tumour	68%
Myeloid	20%
Lymphoid	<1%

[osteosarc-...iqc_report | Txt]

The near absence of lymphoid cells is notable and suggests an **immune-excluded state**.

T2: Immune infiltration emerges

At T2:

Cell type	Fraction
Tumour	45%
Myeloid	33%
Lymphoid	4.5%
Non-immune	11%

[osteosarc-...igc_report | Txt]

Compared with T1:

- tumour burden decreases,
- lymphoid compartment expands ~7–10 fold,
- myeloid populations increase substantially.

This appears to be the beginning of a transition toward a more inflamed microenvironment.

T3: Myeloid-dominated relapse

Both T3 regions are remarkably similar:

Sample	Tumour	Myeloid
T3-0103076	16%	74%
T3-0103350	21%	66%

[osteosarc-...igc_report | Txt]

The dominant feature of T3 is the overwhelming expansion of the myeloid compartment, which becomes the major cellular population.

This is consistent with a **myeloid-driven suppressive TME** rather than a tumour-cell-dominated niche.

---

2. Evolution of spatial organisation

Tumour neighbourhoods become less self-contained

Tumour self-neighbourhood counts fall dramatically:

Timepoint	Tumour-Tumour neighbours
T0-0078018	863,088
T0-0078011	570,268
T1	96,288
T2	194,834

T3-0103076	114,284
T3-0103350	123,294

[osteosarc-...igc_report | Txt]

T0 therefore contains large contiguous tumour masses, whereas later samples exhibit less extensive tumour clustering.

Increasing tumour–myeloid adjacency

Tumour neighbourhoods increasingly involve myeloid cells over time:

Sample	Tumour–Myeloid neighbours
T0-0078011	24,021
T0-0078018	71,481
T1	8,846
T2	23,743
T3-0103076	22,363
T3-0103350	21,865

[osteosarc-...igc_report | Txt]

Normalised to tumour abundance, T3 displays substantially greater myeloid engagement with tumour cells than T0.

This supports a model in which tumour cells become progressively embedded within a myeloid-rich niche.

---

3. Network centrality reveals changing ecological dominance

Tumour centrality peaks at T1

Tumour closeness centrality:

Sample	Tumour closeness
T0-0078011	0.60
T0-0078018	0.69
T1	**1.32**
T2	0.33
T3-0103076	0.46
T3-0103350	0.63

[osteosarc-...igc_report | Txt]

T1 is unusually tumour-centric, with tumour cells occupying the most connected and influential position in the cellular interaction network.

Myeloid influence increases later

Myeloid closeness centrality:

Sample	Myeloid closeness
T0-0078011	0.33
T0-0078018	0.31
T1	0.50
T2	0.43
T3-0103076	0.47
T3-0103350	0.54

[osteosarc-...igc_report | Txt]

By T3, myeloid cells have become among the most central populations within the tissue graph.

Lymphoid cells become progressively integrated

Lymphoid closeness centrality rises steadily:

Sample	Lymphoid closeness
T0-0078018	0.07
T0-0078011	0.09
T1	0.16
T2	0.27
T3-0103076	0.56
T3-0103350	0.47

[osteosarc-...igc_report | Txt]

Although lymphoid cells remain numerically minor, they become increasingly embedded and connected within the TME.

---

4. Cell–cell communication rewiring

The ligand–receptor analysis reveals distinct temporal programs.

Early (T0/T1): Matrix and stromal signalling

Dominant interactions include:

- SPARC–FGFR1
- DCN–KDR
- DCN–TGFB1
- DCN–MET
- DCN–EGFR

These are heavily associated with:

- extracellular matrix remodelling,
- osteogenic programs,
- stromal–tumour communication,
- angiogenic signalling.

[osteosarc-...igc_report | Txt]

Notably, T1 shows the strongest overall LR scores across many interactions, suggesting a highly active signalling state.

T2/T3: Antigen presentation and immune signalling

Later samples show stronger representation of:

- HLA-DRA–CD81
- HLA-DPA1–CD4
- HLA-DPA1–LAG3
- CCL5–CXCR4
- CCL5–CCR1
- IGF1–FLT1

[osteosarc-...igc_report | Txt]

This indicates increasing:

- myeloid antigen presentation,
- lymphoid recruitment,
- immune checkpoint-associated interactions,
- inflammatory signalling.

The appearance of **HLA-DPA1–LAG3** interactions is particularly interesting because it may indicate emergence of exhausted or suppressed T-cell states.

---

5. Moran's I indicates transition from osteogenic tumour programs to inflammatory programs

T0

Spatially structured genes include:

- RUNX2
- ALPL
- SATB2
- SP7
- COL1A1
- COL1A2
- DCN
- LUM

[osteosarc-...iqc_report | Txt]

These are classic osteoblastic/osteogenic osteosarcoma markers.

T2/T3

Strongly spatial genes include:

- CXCL9
- CXCL10
- CXCL11
- STAT1
- IFIT2
- IFIT3
- HLA-DPA1
- HLA-DRA

[osteosarc-...iqc_report | Txt]

Together these suggest increasing:

- interferon activity,
- antigen presentation,
- adaptive immune engagement.

The late TME appears much more immunologically active than the initial resection.

Biological interpretation

A plausible model is:

T0

Large osteogenic tumour masses with relatively limited immune involvement.

↓

T1

Tumour-dominant recurrence exhibiting maximal tumour centrality and strong stromal signalling, but profound lymphoid exclusion.

↓

T2

Transition state marked by recruitment of myeloid and lymphoid cells and increasing inflammatory signalling.

↓

T3

A heavily remodelled microenvironment characterised by:

- massive myeloid expansion,
- enhanced antigen-presentation programmes,
- stronger immune-cell connectivity,
- reduced tumour dominance,
- persistence of TGFB1/DCN-mediated stromal interactions.

[osteosarc-...iqc_report | Txt]

Key hypothesis for follow-up

The data are most consistent with evolution from an **osteogenic tumour-dominated ecosystem (T0/T1)** toward a **myeloid-rich, immunologically active but potentially suppressive ecosystem (T3)**. The combination of:

- high myeloid abundance,
- TGFB1-related signalling,
- IL10-associated interactions,
- HLA-DPA1–LAG3 communication,

suggests that the late TME may represent an **inflamed yet immune-suppressed state**, which could be highly relevant for understanding resistance mechanisms and therapeutic vulnerabilities. [osteosarc-...iqc_report | Txt]