Supplementary Material

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Tumor morphology and location associate with immune cell composition in pleomorphic sarcoma

Supplementary Material includes 3 figures and 5 tables.
Supplementary Figure 1

A

Superficial (N=4)  
Deep (N=6)

B

Fraction of Total

C

S = superficial  
D = Deep

Effectors S  
Effectors D  
45RO+ S  
45RO+ D  
Memory S  
Memory D  
PD1+ S  
PD1+ D  
CTLA4+ S  
CTLA4+ D  
Ki67+ S  
Ki67+ D  
CD38+ S  
CD38+ D  
ICOS+ S  
ICOS+ D

Frequency of CD8 subtypes

S = superficial  
D = Deep

Legend:
- Ungated
- Lymphocytes
- Neutrophils
- Eosinophils
- TAMs
- CD16+ Monocytes
- BDCA1+ DCs
- BDCA3+ DCs
Supplemental Figure 1. Impact of UPS tumor depth on tumor infiltrating immune cell subsets.

A) t-SNE plot analysis demonstrating immune populations in superficial and deep UPS tumors. B) Stacked bar graph demonstrating the fraction of immune cell populations in the entire UPS cohort, compared to superficial and deep UPS tumors. C) Bar graph demonstrating the relative frequency of CD8 subtypes in superficial and deep UPS tumors.
Supplementary Figure 2

% CD8+ cells

N=26
N=10

P=0.55

No neoadjuvant chemotherapy
Neoadjuvant chemotherapy
Supplementary Figure 2. % CD8+ T-Cells in UPS tumors derived from patients either treated or not treated with neoadjuvant chemotherapy.
Supplementary Figure 3

A

Percentage of CD45+ cells

\% B-Cell population

\( p = 0.54 \)

<8cm >8cm

B

Percentage of CD45+ cells

\% B-Cell population

\( p = 0.92 \)

Superficial Deep

C

Overall survival

\( P = 0.21 \)

CD20 Low

CD20 High

Months
Supplementary Figure 3. B-Cell tumor infiltration and association with tumor size, depth, and survival.

% B-Cell (HLADR+/CD3-/CD19+) population in prospectively profiled UPS tumors < or > 8cm (A) and superficial or deep (B) location. C) Overall survival of UPS patients (retrospective cohort) with tumors expressing either high (N=20) or low (N=16) CD20 (B-Cell marker) positive B-Cells.
Supplementary Table 1. Demographics and neoadjuvant treatment N=15.

| Patient | Gender | Age | Stage | Neoadjuvant Chemotherapy | Neoadjuvant radiation |
|---------|--------|-----|-------|--------------------------|------------------------|
| C001    | F      | 76  | III   | No                       | No                     |
| C004    | F      | 84  | II    | No                       | No                     |
| C012    | F      | 48  | III   | Yes                      | Yes                    |
| C014    | M      | 67  | IV    | No                       | No                     |
| C016    | M      | 70  | III   | No                       | No                     |
| C019    | M      | 62  | III   | No                       | No                     |
| C022    | F      | 57  | II    | No                       | No                     |
| C023    | M      | 70  | II    | No                       | No                     |
| C026    | M      | 76  | III   | No                       | No                     |
| C028    | F      | 64  | IV    | No                       | No                     |
| C031    | M      | 72  | III   | No                       | No                     |
| C034    | M      | 85  | IV    | No                       | No                     |
| C036    | F      | 66  | III   | No                       | No                     |
| C039    | M      | 71  | III   | Yes                      | No                     |
| 12519   | M      | 75  | II    | No                       | No                     |
Supplementary Table 2. Tumor Characteristics (N=15).

| Patient | Tumor Size (cm) | Tumor Depth | Tumor Grade | Necrosis points 0-3 | Mitotic Figures / 10 HPF |
|---------|-----------------|-------------|-------------|---------------------|-------------------------|
| C001    | 5.3             | Deep        | 3           | 1                   | 22                      |
| C004    | 3               | Superficial | 3           | 0                   | 35                      |
| C012    | 21.5            | Deep        | 2           | 0                   | 1                       |
| C014    | 9               | Deep        | 3           | 1                   | 50                      |
| C016    | 5.3             | Deep        | 3           | 1                   | 62                      |
| C019    | 34              | Deep        | 3           | 1                   | 41                      |
| C022    | 3               | Superficial | 3           | 1                   | 33                      |
| C023    | 6.3             | Superficial | 3           | 1                   | 21                      |
| C026    | 11.7            | Deep        | 3           | 1                   | 30                      |
| C028    | 8.8             | Superficial | 3           | 1                   | 30                      |
| C031    | 6.9             | Deep        | 3           | 1                   | 22                      |
| C034    | 13.3            | Deep        | 3           | 1                   | 45                      |
| C036    | 6.5             | Deep        | 3           | 0                   | 40                      |
| C039    | 6.2             | Deep        | 3           | 1                   | 32                      |
| 12519   | 6.5             | Superficial | 3           | 1                   | 25                      |
Supplementary Table 3. Clinical characteristics and follow-up (N=15).

| Patient | FU (mo) | Adj. CTX | Adj. RT | LR | MR | Time to LR/MR (mo) | Death | Current Status |
|---------|---------|----------|---------|----|----|-------------------|-------|---------------|
| C001    | 43      | No       | Yes     | No | Yes| 8                 | No    | AWD           |
| C004    | 46      | No       | Yes     | No | No |                   | No    | NED           |
| C012    | 13      | No       | Yes     | No | No |                   | No    | NED           |
| C014    | 9       | No       | Yes     | No | Yes| 1                 | Yes   | DOD           |
| C016    | 31      | No       | Yes     | No | No |                   | No    | NED           |
| C019    | 14      | No       | Yes     | Yes| No | 27                | Yes   | DWD           |
| C022    | 27      | No       | No      | No | No |                   | No    | NED           |
| C023    | 16      | No       | Yes     | No | No |                   | No    | NED           |
| C026    | 22      | No       | Yes     | No | No |                   | No    | NED           |
| C028    | 21      | No       | Yes     | No | No |                   | No    | NED           |
| C031    | 18      | No       | Yes     | No | No |                   | No    | NED           |
| C034    | 4       | No       | Yes     | No | No |                   | Yes   | DOD           |
| C036    | 14      | No       | Yes     | No | No |                   | No    | NED           |
| C039    | 15      | Yes      | No      | No | Yes| 4                 | No    | AWD           |
| 12519   | 12      | No       | Yes     | No | No |                   | No    | NED           |

FU = follow-up; mo = months; Adj. = adjuvant; CTX = chemotherapy; RT = radiation therapy; LR = local recurrence; MR = metastatic recurrence; AWD = alive with disease; NED = no evidence of disease; DOD = died of disease; DWD = died with disease.
**Supplementary Table 4. Immune cell markers**

| Panel       | Population Name           | Corresponding Markers                                                                 |
|-------------|---------------------------|--------------------------------------------------------------------------------------|
| T-cell      | CD8 T Cell                | CD45+/CD3e+/CD8+                                                                     |
| T-cell      | PD-1+ CD8                 | CD45+/CD3e+/CD8+/PD-1<sup>high</sup>                                                |
| T-cell      | Ki67+ CD8                 | CD45+/CD3e+/CD8+/Ki67<sup>high</sup>                                                |
| T-cell      | CTLA4+ CD8                | CD45+/CD3e+/CD8+/CTLA4<sup>high</sup>                                               |
| T-cell      | ICOS+ CD8                 | CD45+/CD3e+/CD8+/ICOS<sup>high</sup>                                                |
| T-cell      | CD8 Effector              | CD45+/CD3e+/CD8+/CD127-/CD25-                                                       |
| T-cell      | CD8 Memory                | CD45+/CD3e+/CD8+/CD127+/CD25-/CD45RO+                                               |
| T-cell      | CD8 Naive                 | CD45+/CD3e+/CD8+/CD127+/CD25-/CD45RO-                                               |
| T-cell      | CD4 Thelper               | CD45+/CD3e+/CD4+/CD25-/FoxP3-                                                       |
| T-cell      | PD-1+ CD4 Thelper         | CD45+/CD3e+/CD4+/CD25-/FoxP3-/PD-1+                                                 |
| T-cell      | Ki67+ CD4 Thelper         | CD45+/CD3e+/CD4+/CD25-/FoxP3-/Ki67+                                                 |
| T-cell      | CTLA4+ CD4 Thelper        | CD45+/CD3e+/CD4+/CD25-/FoxP3-/CTLA4+                                                |
| T-cell      | CD38+ CD4 Thelper         | CD45+/CD3e+/CD4+/CD25-/FoxP3-/CD38+                                                 |
| T-cell      | ICOS+ CD4 Thelper         | CD45+/CD3e+/CD4+/CD25-/FoxP3-/ICOS+                                                 |
| T-cell      | CD4 Effector              | CD45+/CD3e+/CD4+/CD25-/FoxP3-/CD127-                                                |
| T-cell      | CD4 Memory                | CD45+/CD3e+/CD4+/CD25-/FoxP3-/CD127-/CD45RO+                                         |
| T-cell      | CD4 Naive                 | CD45+/CD3e+/CD4+/CD25-/FoxP3-/CD127-/CD45RO-                                         |
| T-cell      | T-regulatory              | CD45+/CD3e+/CD4+/CD25+/FoxP3+                                                       |
| T-cell      | NK Cells                  | CD45+/CD3e-/HLADR-/CD56+                                                            |
| T-cell      | Myeloids                  | CD45+/CD3e-/HLADR+                                                                 |
| Myeloid     | Neutrophils               | CD45low/SSC-A<sub>high</sub>/CD16<sub>high</sub>                                      |
| Myeloid     | Eosinophils               | CD45low/SSC-A<sub>high</sub>/CD16<sub>low</sub>                                       |
| Myeloid     | Lymphocytes               | CD45+/CD56,CD19,CD20,CD3e) + (Dump Channel)                                        |
| Myeloid     | CD16+ Monocytes           | CD45+/CD56,CD19,CD20,CD3e)/HLADR+/CD16+                                            |
| Myeloid     | Tumor Associated Macrophages (TAMs) | CD45+/CD56,CD19,CD20,CD3e)/HLADR+/CD16-/CD11c+/CD14+                              |
| Myeloid     | Dendritic Cells (DCs)     | CD45+/CD56,CD19,CD20,CD3e)/HLADR+/CD16-/CD14-/CD11c+                              |
| Myeloid     | BDCA3+ DCs                | CD45+/CD56,CD19,CD20,CD3e)/HLADR+/CD16-/CD14-/CD11c+/BDCA-3+                      |
| Myeloid     | BDCA1+ DCs                | CD45+/CD56,CD19,CD20,CD3e)/HLADR+/CD16-/CD14-/CD11c+/BDCA-1+                      |
Supplementary Table 5. Neutrophil counts, lymphocyte counts and Neutrophil-to-lymphocyte ratio (NLR).

|               | All (counts) | <8cm (counts) | >=8cm (counts) |
|---------------|--------------|---------------|----------------|
| Neutrophils   | 12790        | 3524          | 9266           |
| Lymphocytes   | 63880        | 35116         | 28764          |
| NLR           | 0.20         | 0.10          | 0.32           |