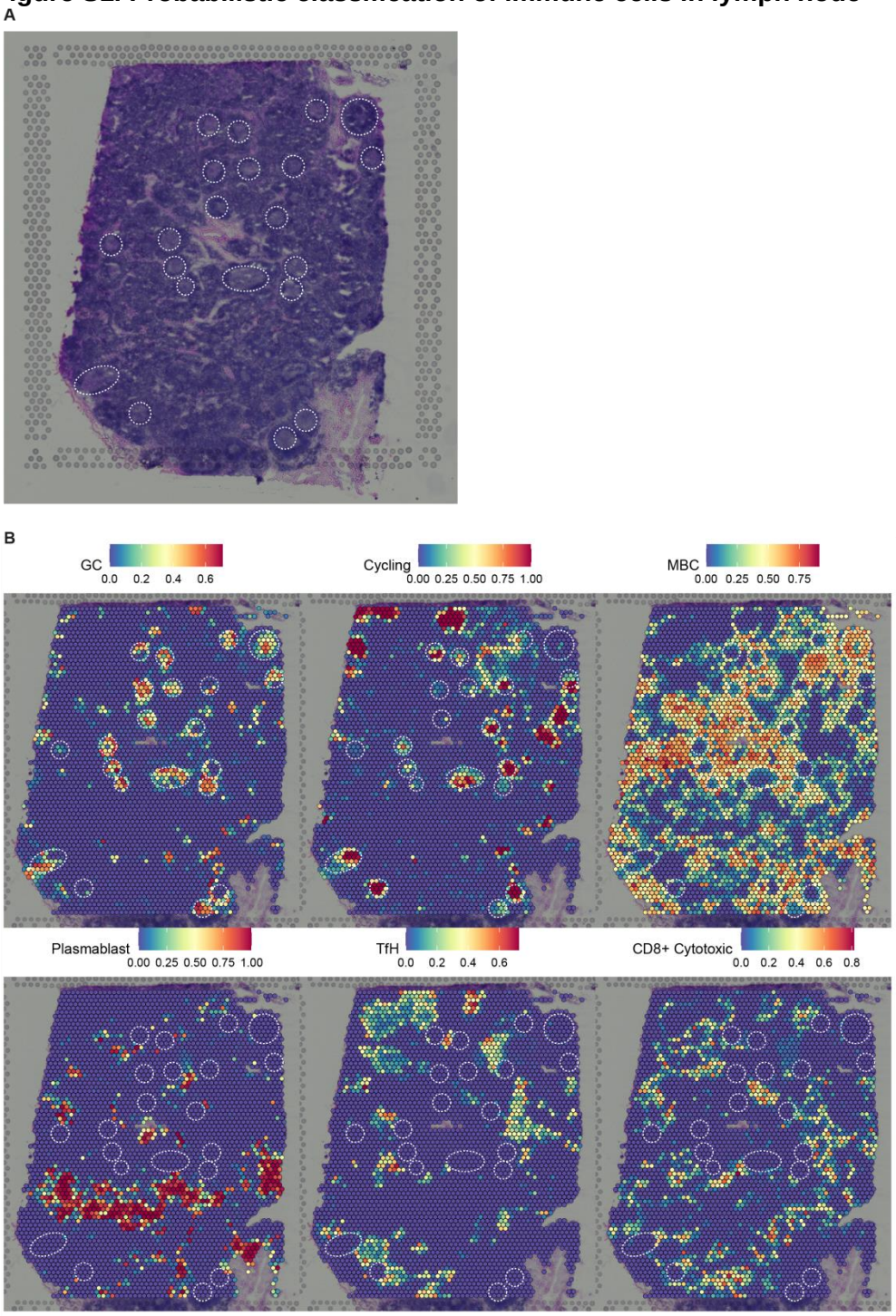
*Horeweg et al.*

# Tertiary lymphoid structures critical for prognosis in endometrial cancer patients - a TransPORTEC study

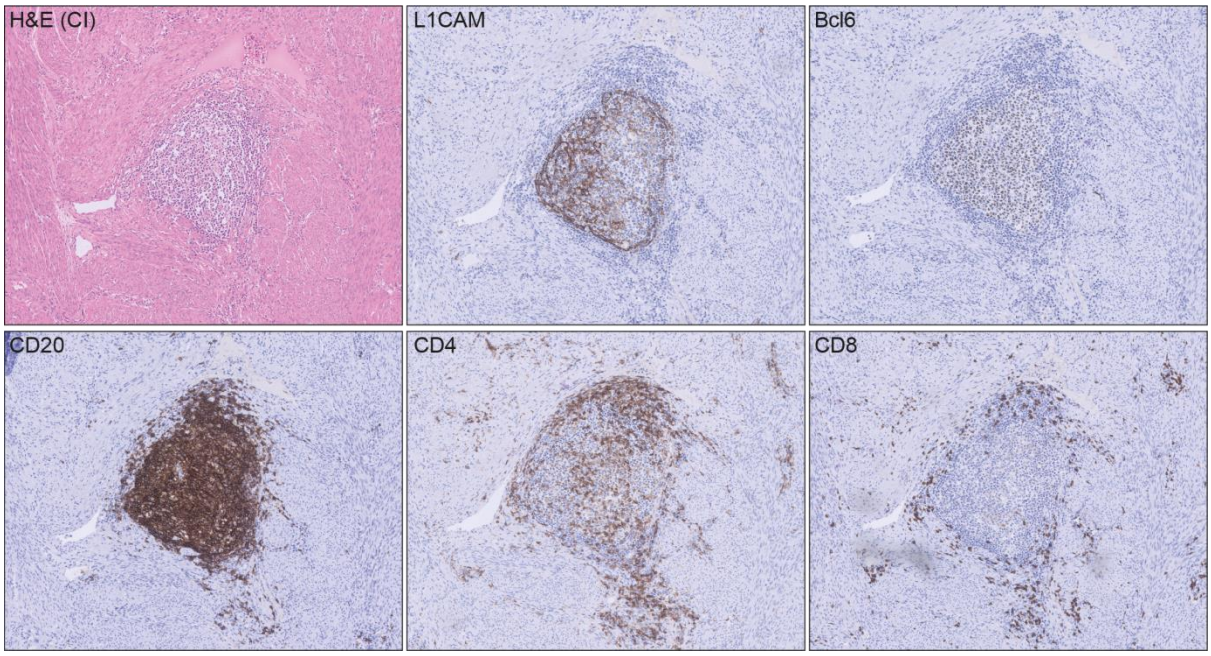
## Supplemental data

### Figure S1. Probabilistic classification of immune cells in lymph node



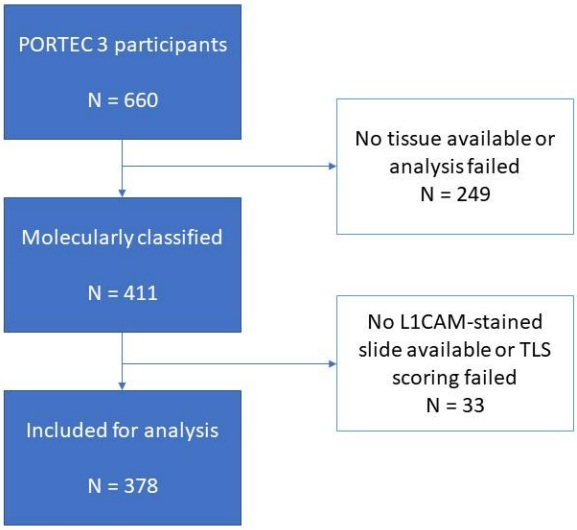

Panel A: Germinal centers manually assigned on H&E of Visium lymph node data. Panel B: probabilistic classification for annotated immune cells in spatial transcriptomic data of a human lymph node.

### Figure S2. Hallmarks of mature TLS in L1CAM-positive TLS




Hallmark features of TLS maturation in L1CAM-positive TLS as determined by Bcl6, CD20, CD4 and CD8 immunohistochemistry

**Figure S3. CONSORT flowchart PORTEC-3**




Definition of abbreviations: PORTEC-3 = adjuvant chemoradiotherapy versus radiotherapy alone in women with high-risk endometrial cancer trial; TLS = tertiary lymphoid structure.

## Table S1. Genes differentially expressed between TLS-positive and TLS-negative UCEC TCGA cases

Provided separately in the excel file ‘Table S1. Genes differentially expressed between TLS-positive and TLS-negative UCEC TCGA cases’

## Table S2. Overview of included and excluded PORTEC-3 participants

| Characteristics | Total N (%) | Included N (%) | Excluded N (%) | Difference p-value |
|---|---|---|---|---|
| No. of patients | 660 (100.0) | 378 (57.3) | 282 (42.7) | |
| Received treatment | | | | |
| external beam radiotherapy | 333 (50.5) | 187 (49.5) | 146 (51.8) | 0.56 |
| chemoradiotherapy | 327 (49.5) | 191 (50.5) | 136 (48.2) | |
| Age - median (IQR) | 62 (12) | 62 (12) | 63 (12) | 0.21 |
| Histotype and grade | | | | |
| endometrioid grade 1-2 | 255 (38.6) | 153 (40.5) | 102 (36.2) | 0.52 |
| endometrioid grade 3 | 185 (28.0) | 104 (27.5) | 81 (28.7) | |
| non-endometrioid | 220 (33.3) | 121 (32.0) | 99 (35.1) | |
| Stage | | | | |
| IA | 78 (11.8) | 50 (13.3) | 28 (9.9) | 0.53 |
| IB | 117 (17.7) | 65 (17.2) | 52 (18.4) | |
| II | 170 (25.8) | 96 (25.4) | 74 (26.2) | |
| III | 295 (44.8) | 167 (44.3) | 128 (45.2) | |
| Myometrial invasion | | | | |
| ≤50% | 239 (36.2) | 136 (36.0) | 103 (36.5) | 0.90 |
| >50% | 418 (63.3) | 240 (63.5) | 178 (63.1) | |
| unknown | 3 (0.5) | 2 (0.5) | 1 (0.4) | |
| LVSI | | | | |
| no | 272 (41.2) | 139 (36.8) | 133 (47.2) | 0.007 |
| yes | 388 (58.8) | 239 (63.2) | 149 (52.8) | |

Definition of abbreviations: IQR = interquartile range; LVSI = lymphovascular space invasion

**Table S3. Association of TLS with multiple classifying molecular features in high risk endometrial cancer**

| | Total | TLS: none | TLS: ≥1 | Number of TLS | |
|---|---|---|---|---|---|
| **Molecular features** | **n (%)** | **n (%)** | **n (%)** | **mean** | **range** |
| *POLE*mut single classifier | 31 (100) | 15 (48.4) | 16 (51.6) | 1.48 | 0-14 |
| MMRd single classifier | 117 (100) | 94 (80.3) | 23 (19.7) | 0.74 | 0-10 |
| p53abn single classifier | 83 (100) | 78 (94.0) | 5 (6.0) | 0.19 | 0-6 |
| NSMP | 121 (100) | 108 (89.3) | 13 (10.7) | 0.21 | 0-5 |
| *POLE*mut, MMRd and p53abn | 2 (100) | 0 (0.0) | 2 (100) | 10.50 | 0-20 |
| *POLE*mut and MMRd | 9 (100) | 5 (55.6) | 4 (44.4) | 3.00 | 0-19 |
| *POLE*mut and p53abn | 5 (100) | 3 (60.0) | 2 (40.0) | 2.60 | 0-9 |
| MMRd and p53abn | 10 (100) | 4 (40.0) | 6 (60.0) | 1.00 | 0-2 |

According to Léon-Castillo et al. (J Pathol 2020) endometrial cancer with multiple classifying features are assigned to the *POLE*mut group if a pathological *POLE* mutation is present and to the MMRd subgroup in case of MMRd and p53abn.

Among the tumors assigned to the *POLE*mut molecular subgroup (n=47), 31 (66.0%) had a single classifying feature and 16 (34.0%) had multiple classifying features. Among the 31 POLE endometrial cancers with a single classifying feature, 16 (51.6%) had any TLS, among the 16 POLEs with multiple classifying features 8 (50.0%) had any TLS (Fisher exact p = 1.00). Also, the numbers of TLS between these two groups were not significantly different (Mann-Whitney U p = 0.58).

Among the 127 tumors assigned to the MMRd molecular subgroup, TLS were present among 23 of the 117 (19.7%) MMRd endometrial cancer with a single classifying feature; versus TLS presence in 6 out of 10 (60.0%) of those with multiple classifying features (Fisher exact p = 0.009). The numbers of TLS were significantly higher among MMRd/p53abn than MMRd single classifiers (Mann-Whitney U p = 0.013).

Definitions of abbreviations: *POLE*mut = polymerase epsilon mutated endometrial cancer; MMRd = mismatch repair deficient endometrial cancer; p53abn = p53 abnormal endometrial cancer; NSMP = no specific molecular profile endometrial cancer.

**Table S4. Association of TLS with clinicopathological features and immune cell densities in high risk endometrial cancer**

| Characteristics | Total | TLS: none | TLS: ≥1 | p value |
|---|---|---|---|---|
| **All patients** | **378 (100.0)** | **307 (81.2)** | **71 (18.8)** | |
| Age - median (IQR) | 62 (12) | 62 (12) | 59 (11) | 0.13 |
| Histotype and grade | | | | |
| endometrioid grade 1-2 | 153 (40.5) | 135 (44.0) | 18 (25.4) | 0.003 |
| endometrioid grade 3 | 104 (27.5) | 74 (24.1) | 30 (42.3) | |
| non-endometrioid | 121 (32.0) | 98 (31.9) | 23 (32.4) | |
| Stage | | | | |
| IA | 50 (13.2) | 37 (12.1) | 13 (18.3) | 0.15 |
| IB | 65 (17.2) | 49 (16.0) | 16 (22.5) | |
| II | 96 (25.4) | 83 (27.0) | 13 (18.3) | |
| III | 167 (44.2) | 138 (45.0) | 29 (40.8) | |
| Myometrial invasion | | | | |
| ≤50% | 136 (36.2) | 111 (36.4) | 25 (35.2) | 0.85 |
| >50% | 240 (63.8) | 194 (63.6) | 46 (64.8) | |
| LVSI | | | | |
| no | 139 (36.8) | 114 (37.1) | 25 (35.2) | 0.76 |
| yes | 239 (63.2) | 193 (62.9) | 46 (64.8) | |
| Molecular group | | | | |
| *POLE*mut | 47 (12.4) | 23 (7.5) | 24 (33.8) | <0.0001 |
| MMRd | 127 (33.6) | 98 (31.9) | 29 (40.8) | |
| p53abn | 83 (22.0) | 78 (25.4) | 5 (7.0) | |
| NSMP | 121 (32.0) | 108 (35.2) | 13 (18.3) | |
| CD8 densities (log2 transformed) | | | | |
| intraepithelial - median (IQR) | 5.5 (3.3) | 5.3 (3.1) | 7.3 (3.0) | <0.0001 |
| intrastromal - median (IQR) | 5.9 (2.9) | 5.8 (2.9) | 6.9 (2.8) | <0.0001 |
| intratumoral - median (IQR) | 6.0 (2.5) | 5.8 (2.4) | 7.2 (2.8) | <0.0001 |
| CD20 densities (log2 transformed) | | | | |
| intraepithelial - median (IQR) | 3.2 (3.5) | 2.9 (3.9) | 3.6 (2.7) | 0.12 |
| intrastromal - median (IQR) | 5.2 (3.9) | 4.5 (4.3) | 6.4 (3.8) | 0.007 |
| intratumoral - median (IQR) | 4.2 (3.6) | 3.8 (3.7) | 5.3 (4.2) | 0.024 |

Definitions of abbreviations: TLS = tertiary lymphoid structures; IQR = interquartile range; LVSI = lymphovascular space invasion; *POLE*mut = polymerase epsilon mutated endometrial cancer; MMRd = mismatch repair deficient

endometrial cancer; p53abn = p53 abnormal endometrial cancer; NSMP = no specific molecular profile endometrial cancer.

**Table S5. Association of CD8 densities with clinicopathological features in high risk endometrial cancer**

| Characteristics | Intraepithelial | | Intrastromal | | Intratumoral | |
|---|---|---|---|---|---|---|
| | median (IQR) | p value | median (IQR) | p value | median (IQR) | p value |
| Histotype and grade | | | | | | |
| endometrioid gr 1-2 | 5.3 (2.6) | 0.13 | 5.6 (2.5) | 0.001 | 5.7 (2.1) | 0.002 |
| endometrioid gr 3 | 6.1 (3.4) | | 6.8 (3.2) | | 6.6 (3.0) | |
| non-endometrioid | 6.0 (4.1) | | 6.4 (3.5) | | 6.5 (3.2) | |
| Stage | | | | | | |
| IA | 5.8 (4.4) | 0.32 | 6.9 (4.1) | 0.088 | 6.7 (5.2) | 0.087 |
| IB | 6.2 (3.0) | | 6.5 (3.6) | | 6.8 (3.1) | |
| II | 5.2 (3.3) | | 5.5 (3.2) | | 5.6 (2.9) | |
| III | 5.7 (3.2) | | 5.9 (2.5) | | 6.0 (2.2) | |
| Myometrial invasion | | | | | | |
| ≤50% | 5.5 (3.4) | 0.59 | 5.9 (3.0) | 0.64 | 5.9 (3.0) | 0.55 |
| >50% | 5.7 (3.3) | | 6.0 (2.8) | | 6.1 (2.4) | |
| LVSI | | | | | | |
| no | 5.5 (3.0) | 0.54 | 5.9 (3.3) | 0.43 | 5.8 (2.7) | 0.30 |
| yes | 5.6 (3.3) | | 6.0 (2.7) | | 6.2 (2.4) | |
| Molecular group | | | | | | |
| *POLE*mut | 7.2 (3.1) | <0.0001 | 7.4 (2.8) | <0.0001 | 7.5 (2.5) | <0.0001 |
| MMRd | 6.7 (2.7) | | 6.6 (2.5) | | 6.8 (2.7) | |
| p53abn | 4.5 (3.5) | | 5.0 (3.3) | | 5.4 (3.2) | |
| NSMP | 4.9 (2.2) | | 5.3 (2.6) | | 5.5 (2.0) | |

Definitions of abbreviations: IQR = interquartile range; LVSI = lymphovascular space invasion; corr = correlation; *POLE*mut = polymerase epsilon mutated endometrial cancer; MMRd = mismatch repair deficient endometrial cancer; p53abn = p53 abnormal endometrial cancer; NSMP = no specific molecular profile endometrial cancer.

**Table S6. Association of CD20 densities with clinicopathological features in high risk endometrial cancer**

| Characteristics | Intraepithelial median (IQR) | p value | Intrastromal median (IQR) | p value | Intratumoral median (IQR) | p value |
|---|---|---|---|---|---|---|
| Histotype and grade | | | | | | |
| endometrioid gr 1-2 | 2.7 (3.6) | 0.12 | 4.3 (3.6) | .002 | 3.6 (3.2) | 0.007 |
| endometrioid gr 3 | 3.5 (4.0) | | 6.0 (3.9) | | 4.9 (4.2) | |
| non-endometrioid | 3.1 (4.2) | | 5.5 (6.0) | | 4.1 (5.0) | |
| Stage | | | | | | |
| IA | 3.4 (3.2) | 0.77 | 5.8 (4.6) | 0.15 | 4.4 (4.1) | 0.48 |
| IB | 3.2 (4.0) | | 5.4 (4.8) | | 4.0 (4.5) | |
| II | 3.0 (4.2) | | 4.8 (4.3) | | 4.0 (3.7) | |
| III | 3.1 (3.8) | | 4.5 (4.4) | | 3.8 (3.8) | |
| Myometrial invasion | | | | | | |
| ≤50% | 3.2 (3.9) | 0.69 | 5.4 (5.1) | 0.95 | 4.2 (3.9) | 0.89 |
| >50% | 3.1 (3.7) | | 4.8 (4.3) | | 3.9 (3.7) | |
| LVSI | | | | | | |
| no | 3.0 (4.2) | 0.67 | 5.2 (4.7) | 0.48 | 3.9 (4.0) | 0.92 |
| yes | 3.2 (3.7) | | 4.6 (4.2) | | 4.0 (3.8) | |
| Molecular group | | | | | | |
| *POLE*mut | 4.0 (3.6) | <0.009 | 6.8 (3.3) | <0.0001 | 5.7 (4.6) | <0.0001 |
| MMRd | 3.6 (3.2) | | 6.1 (3.7) | | 5.3 (3.6) | |
| p53abn | 2.3 (4.0) | | 4.0 (5.3) | | 3.2 (4.5) | |
| NSMP | 2.5 (3.5) | | 3.8 (3.5) | | 3.2 (2.9) | |

Definitions of abbreviations: IQR = interquartile range; LVSI = lymphovascular space invasion; corr = correlation; *POLE*mut = polymerase epsilon mutated endometrial cancer; MMRd = mismatch repair deficient endometrial cancer; p53abn = p53 abnormal endometrial cancer; NSMP = no specific molecular profile endometrial cancer.

**Table S7. Factors associated with TLS presence in high-risk endometrial cancer**

| | Univariable analysis | | | Multivariable analysis | | |
|---|---|---|---|---|---|---|
| | OR | 95% CI | p value | OR | 95% CI | p value |
| Age | 0.99 | 0.96-1.02 | 0.35 | 1.01 | 0.97-1.06 | 0.57 |
| Histograde | | | | | | |
| EEC grade 1-2 | reference | | | reference | | |
| EEC grade 3 | 3.04 | 1.59-5.82 | 0.001 | 2.01 | 0.78-5.18 | 0.15 |
| non-EEC | 1.76 | 0.90-3.44 | 0.098 | 1.59 | 0.55-4.58 | 0.39 |
| Stage (I-II vs. III) | 0.85 | 0.50-1.43 | 0.53 | 1.22 | 0.56-2.66 | 0.61 |
| LVSI (no vs. yes) | 1.09 | 0.63-1.86 | 0.76 | 0.81 | 0.37-1.77 | 0.60 |
| Molecular group | | | | | | |
| NSMP | reference | | | reference | | |
| POLEmut | 8.67 | 3.85-19.51 | <0.0001 | 4.94 | 1.43-17.05 | 0.011 |
| MMRd | 2.46 | 1.21-5.00 | 0.013 | 1.27 | 0.49-3.29 | 0.63 |
| p53abn | 0.53 | 0.18-1.56 | 0.25 | 0.31 | 0.07-1.40 | 0.13 |
| CD8 density | | | | | | |
| intraepithelial | 1.38 | 1.18-1.62 | <0.0001 | | | |
| intrastromal | 1.35 | 1.15-1.58 | <0.0001 | | | |
| intratumoral | 1.47 | 1.23-1.75 | <0.0001 | 1.30 | 1.04-1.62 | 0.020 |
| CD20 density | | | | | | |
| intraepithelial | 1.10 | 0.98-1.24 | 0.12 | | | |
| intrastromal | 1.12 | 1.02-1.23 | 0.023 | 0.97 | 0.84-1.15 | 0.86 |
| intratumoral | 1.12 | 1.01-1.24 | 0.033 | | | |

Univariable and multivariable regression analysis using Cox proportional hazards models show the association of clinicopathological factors, molecular class and cytotoxic T-cell (CD8) and B-cell (CD20) with presence of tertiary lymphoid structures detected using L1CAM. Covariates were pre-specified according to Léon-Castillo et al. (2020). In the multivariable model, the CD8 and CD20 marker with the strongest association was entered in the multivariable model. -2 Log likelihood = 207.369, Nagelkerke R = 0.239.

**Table S8. Impact of TLS and immune cell densities on time to recurrence in high-risk endometrial cancer**

| | Univariable analysis | | | | |
|---|---|---|---|---|---|
| | HR | 95% CI | p value | C-index (se) | AIC |
| *Recurrence (n=252, 75 events)* | | | | | |
| CD8 density | | | | | |
| intraepithelial | 0.85 | 0.78-0.93 | 0.00049 | 0.608 (0.034) | 780.171 |
| intrastromal | 0.87 | 0.80-0.95 | 0.0046 | 0.585 (0.034) | 784.093 |
| intratumoral | 0.83 | 0.74-0.93 | 0.0011 | 0.597 (0.034) | 781.202 |
| CD20 density | | | | | |
| intraepithelial | 0.93 | 0.84-1.03 | 0.18 | 0.546 (0.034) | 790.026 |
| intrastromal | 0.92 | 0.85-1.00 | 0.048 | 0.560 (0.033) | 787.936 |
| intratumoral | 0.92 | 0.84-1.01 | 0.073 | 0.552 (0.034) | 788.614 |
| TLS | | | | | |
| number of TLS | 0.62 | 0.42-0.92 | 0.017 | 0.576 (0.018) | 778.315 |
| none vs. ≥1 | 0.25 | 0.10-0.62 | 0.0028 | 0.575 (0.019) | 778.397 |

Univariable Cox proportional hazards models show impact of log2-transformed T-cell (CD8) and B-cell (CD20) densities in the intraepithelial, intrastromal and the intratumoral (intraepithelial + intrastromal) compartments and the presence of tertiary lymphoid structures on time to endometrial recurrence. Definition of abbreviations: HR = hazard ratio; CI = confidence interval; C-index - concordance index; se = standard error; AIC = Akaike's Information Criterion; TLS = tertiary lymphoid structure.

**Table S9. Correlation between immune cell densities and TLS in high-risk endometrial cancer**

| | | CD8 | | | CD20 | | | TLS | |
|---|---|---|---|---|---|---|---|---|---|
| | | intra-epithelial | intra-stromal | intra-tumoral | intra-epithelial | intra-stromal | intra-tumoral | number | none vs. ≥1 |
| $CD8^+$ | jntraepithelial | | | | | | | | |
| | intrastromal | 0.848** | | | | | | | |
| | intratumoral | 0.920** | 0.955** | | | | | | |
| $CD20^+$ | jntraepithelial | 0.390** | 0.398** | 0.408** | | | | | |
| | intrastromal | 0.507** | 0.554** | 0.541** | 0.695** | | | | |
| | intratumoral | 0.493** | 0.522** | 0.524** | 0.797** | 0.947** | | | |
| TLS | number | 0.276** | 0.240** | 0.272** | 0.144* | 0.209** | 0.191** | | |
| | none vs. ≥1 | 0.282** | 0.246** | 0.281** | 0.133* | 0.198** | 0.179** | 0.993** | |

Spearman's correlation coefficients. Definition of abbreviation: TLS = tertiary lymphoid structures.

* Significance at the <0.05 level (2-tailed)

** Significance at the <0.01 level (2-tailed)

**Table S10. Prognostic factors for endometrial cancer-specific survival in high-risk endometrial cancer**

| | Pathologic model | | | Molecular model | | | Molecular-immune model | | |
|---|---|---|---|---|---|---|---|---|---|
| | HR | 95% CI | p value | HR | 95% CI | p value | HR | 95% CI | p value |
| *Endometrial cancer-specific survival (n=376, 75 events)* | | | | | | | | | |
| Age | 1.08 | 1.04-1.11 | $4.21 \times 10^{-6}$ | 1.06 | 1.02-1.10 | 0.00099 | 1.06 | 1.03-1.10 | 0.00075 |
| Adjuvant treatment | | | | | | | | | |
| RT | reference | | | reference | | | reference | | |
| CTRT | 0.68 | 0.43-1.08 | 0.11 | 0.68 | 0.43-1.07 | 0.10 | 0.83 | 0.54-1.28 | 0.13 |
| Histograde | | | | | | | | | |
| EEC grade 1-2 | reference | | | reference | | | reference | | |
| EEC grade 3 | 2.09 | 1.11-3.92 | 0.022 | 1.65 | 0.82-3.31 | 0.16 | 1.95 | 0.97-3.92 | 0.061 |
| non-EEC | 2.39 | 1.32-4.35 | 0.0043 | 1.22 | 0.59-2.52 | 0.59 | 1.37 | 0.67-2.82 | 0.39 |
| Stage (I-II vs. III) | 1.79 | 1.10-2.93 | 0.020 | 1.73 | 1.06-2.80 | 0.027 | 1.81 | 1.11-2.94 | 0.017 |
| LVSI | 1.29 | 0.75-2.22 | 0.35 | 1.29 | 0.75-2.24 | 0.36 | 1.24 | 0.71-2.17 | 0.44 |
| Molecular group | | | | | | | | | |
| NSMP | | | | reference | | | reference | | |
| POLEmut | | | | no events | | | no events | | |
| MMRd | | | | 1.41 | 0.70-2.84 | 0.34 | 1.65 | 0.82-3.31 | 0.16 |
| p53abn | | | | 3.99 | 1.89-8.40 | 0.00027 | 3.52 | 1.69-7.34 | 0.00077 |
| TLS | | | | | | | 0.15 | 0.04-0.61 | 0.0085 |

Three Cox proportional hazards models show impact of respectively clinicopathological factors, clinicopathological + molecular factors, and clinicopathological + molecular factors + presence of tertiary lymphoid structures on endometrial cancer-specific survival. Covariates were pre-specified according to Léon-Castillo et al. (2020). The addition of the molecular classifier to the pathologic model was associated with an improvement in model fit evidenced by: (i) reduction in Akaike's information criterion (AIC) 828.372 vs. 797.269, (ii) increase in model concordance (C index 0.719 vs. 0.777 and (iii) likelihood ratio test for comparison of nested models $p = 4.38 \times 10^{-8}$. Likewise, the addition of TLS presence improved model fit: (i) AIC 797.269 vs. 786.669, (ii) C index 0.777 vs. 0.796 (iii) likelihood ratio test for nested models p = 0.00039. Definition of abbreviations: HR = hazard ratio; CI = confidence interval; RT = external beam radiotherapy; CTRT = chemoradiotherapy; EEC = endometrioid endometrial cancer; LVSI = lymphovascular space invasion; NSMP = no specific molecular profile; *POLE*mut = pathogenic polymerase epsilon mutation; MMRd = mismatch repair deficient; p53abn = p53 abnormal; TLS = tertiary lymphoid structure

**Table S10. Sensitivity analysis: prognostic impact of immuno-biomarkers for recurrence of high-risk endometrial cancer**

| | TLS (none vs. ≥1) | | | Intraepithelial $CD8^+$ density | | | Intrastromal $CD20^+$ density | | |
|---|---|---|---|---|---|---|---|---|---|
| | HR | 95% CI | p value | HR | 95% CI | p value | HR | 95% CI | p value |
| *Recurrence (n=252, 75 events)** | | | | | | | | | |
| Age | 1.03 | 1.00-1.06 | 0.045 | 1.03 | 1.0-1.06 | 0.059 | 1.03 | 1.00-1.07 | 0.042 |
| Adjuvant treatment | | | | | | | | | |
| RT | reference | | | reference | | | reference | | |
| CTRT | 0.54 | 0.34-0.86 | 0.010 | 0.52 | 0.33-0.99 | 0.0082 | 0.54 | 0.34-0.87 | 0.011 |
| Histograde | | | | | | | | | |
| EEC grade 1-2 | reference | | | reference | | | reference | | |
| EEC grade 3 | 0.84 | 0.40-1.76 | 0.64 | 0.90 | 0.44-1.86 | 0.77 | 0.80 | 0.38-1.70 | 0.57 |
| non-EEC | 0.60 | 0.28-1.32 | 0.21 | 0.60 | 0.27-1.36 | 0.22 | 0.57 | 1.08-3.02 | 0.17 |
| Stage (I-II vs. III) | 1.82 | 1.09-3.04 | 0.022 | 1.79 | 1.07-3.00 | 0.027 | 1.81 | 1.08-3.02 | 0.023 |
| LVSI | 1.10 | 0.63-1.90 | 0.74 | 1.13 | 0.66-1.95 | 0.66 | 1.12 | 0.64-1.94 | 0.69 |
| Molecular group | | | | | | | | | |
| NSMP | reference | | | reference | | | reference | | |
| POLEmut | no events | | | no events | | | no events | | |
| MMRd | 1.32 | 0.72-2.40 | 0.37 | 1.50 | 0.80-2.82 | 0.21 | 1.25 | 0.68-2.29 | 0.48 |
| p53abn | 0.47 | 2.08-10.61 | 0.00020 | 5.60 | 2.42-12.96 | $5.90 \times 10^{-5}$ | 5.06 | 2.21-11.57 | 0.00012 |
| Immunological factor | 0.39 | 0.16-0.99 | 0.048 | 0.89 | 0.80-0.99 | 0.029 | 0.98 | 0.91-1.07 | 0.69 |
| C-index (se) | | 0.745 (0.026) | | | 0.736 (0.027) | | | 0.731 (0.027) | |
| AIC | | 747.391 | | | 747.731 | | | 752.272 | |

Three multivariable Cox proportional hazards models show impact of clinicopathological factors, molecular class and 3 immunological factors on time to recurrence. Covariates were pre-specified according to Léon-Castillo et al. (2020).

* Only cases with complete data on TLS, CD8 and CD20 were included for analysis.

Definition of abbreviations: HR = hazard ratio; CI = confidence interval; RT = external beam radiotherapy; CTRT = chemoradiotherapy; EEC = endometrioid endometrial cancer; LVSI = lymphovascular space invasion; NSMP = no specific molecular profile; *POLE*mut = pathogenic polymerase epsilon mutation; MMRd = mismatch repair deficient; p53abn = p53 abnormal; TLS = tertiary lymphoid structure

**Table S11. Biomarker analysis performed and reported in this study**

| Analysis | Objective | Endpoint | Population | Methods | Reported |
|---|---|---|---|---|---|
| Prognostic impact TLS | Primary | TTR, CCS | PORTEC-3 | KM analysis log rank test, MV regression using Cox proportional hazards models | Main text, Table 1, Figure 3B-C, Figure 4, Table S8 |
| Relation TLS with molecular subgroups and CD8 | Secondary | Incidence | PORTEC-3 | Quantification of TLS on L1CAm stained whole slides, descriptive statistics, supervised hierarchical cluster analysis | Main text, Figure 3A, Tables S3-7 |
| Prognostic impact CD8 and CD20 | Sensitivity | TTR | PORTEC-3 | MV regression using Cox proportional hazards models | Main text, Table S10 |
| Prognostic impact TLS by molecular group | Exploratory | TTR | PORTEC-3 | KM analysis, log rank test | Main text, Figure 3D-E |