Clonality and antigen-specific responses shape the prognostic effects of tumor-infiltrating T cells in ovarian cancer

SUPPLEMENTARY MATERIALS

Statistical methods

Examining the distribution of clone frequencies, we used Fisher’s model for unseen species assuming an exponential model (shape = 1) to calculate the sequencing coverage; we estimated that 78% of tumor samples have at least 90% coverage and the median coverage is 83%. Low coverage was not associated with any of the repertoire statistics.

Supplementary Figure 1 and Supplementary Table 2 contain a spread of statistics meant to capture different features of the repertoire. We report on clonality as defined above as well as the TOPX statistics (the % of the repertoire captured by the most common X reads), the NX statistics (the number of clones required to capture X% of the repertoire) and the RX statistics (the % of the PBMC repertoire that maps to the corresponding clones identified by NX).

Patient characteristics and survival data

Study outcomes included overall survival (OS), the time until death; and progression-free survival (PFS), the time to recurrence or progression, both measured from the time of definitive surgery. Progression or recurrence was defined by time to radiologically confirmed relapse of disease. If no imaging data were available, progression was determined by increase in CA125 to twice the upper limit of normal or two times the nadir value if CA125 was never normal during primary treatment.

Sequencing variation and TCR repertoire statistics depend on degree of infiltration

Each patient’s TIL TCR repertoire contained between 224 and 94,770 (1Q: 2,918; 3Q 15,190; median: 11,590) unique AA sequences (Supplementary Table 2). The number of reads in the tumor specimens (17,978–13,368,648) varied over three orders of magnitude and was associated with density of CD3⁺ T cell infiltration measured by IHC (Spearman’s rho = 0.340, p = 0.001). Between 65.3% (1st quartile) and 84.0% (3rd quartile) of reads are captured by the most common 1000 clones implying that most samples have a large number of rare reads.

To leverage the deep TCR sequence and frequency information in our 99 samples, we first analyzed the variety of TCR sequences across all patients. We obtained 992,791 unique TIL TCR sequences in 289,963,375 normalized reads in combined 99 patients. In matched PBMC samples, there were 6,275,193 unique clones in 400,143,279 normalized reads. We found that, on average, two patients share 2.6% of PBMC clones (range: 0.5–4.7%) which is lower than previously reported results.

Noting that there were similar overall levels of reads, the overall TIL repertoire appeared more restricted than the PBMC repertoire. The most shared TIL clone was found in only 41 of the 99 patients while the most shared PBMC clones appeared in more than 90 of the patients. Again these clones did not comprise an unusually large fraction of the reads.

With respect to the usage of specific clones in the repertoire, the average rate of reads to their unique TCR clone may describe the evenness. For example, the mean ovarian cancer TIL repertoire had one unique clone for every 364 reads (median 1 per 199 reads). The range varied from 1 per 2714 reads (strongly restricted) to 1 per 25 reads, which was close to the noise threshold at 20 reads.

Individual TIL repertoires

We describe each TIL repertoire by plotting the relative frequency of each clone in order from most common to least using the log-rank to emphasize the most common clone and to de-emphasize the long tail of low abundance clones. All the plots in Supplementary Figure 2 have the same x-axis so the longer the right tail, the more unique clones in the repertoire. Additionally, we have split the cohort into roughly equal sized bins based on the TOP1 statistic. Therefore the more monoclonal cases and the more polyclonal cases are grouped together and the set of putatively oligoclonal cases are in between. Repertoire specific statistics are given for each patient.
Supplementary Figure 1: Prognostic effect of T cell infiltration and CT antigen levels in TCGA cohort. (A) mRNA expression of T cell markers (CD3, CD8, GZMB, IL2) and 91 unselected CT antigens used to form an antigen burden score. Median expression levels used to stratify into 4 groups based on CD3 levels and CT antigen burden. Prognostic effect of CD3 infiltration and CT antigen burden for (B) PFS and (C) OS. (D) Volcano plot of gene levels in CD3^hi CT antigen^hi category. Upregulated genes in blue include CXCL9/10, IFI6/35 and ISG15 (interferon induced). (E) Composite analysis of CT antigen levels based on overall CD3 T cell infiltration.
Supplementary Figure 2: Overview of TCR sequencing results. (A) 4.91 million clones from 99 ovarian cancer patients show a wide variety of clone frequency between the tumor infiltrating (TIL) and peripheral repertoires (PBMC) across stage and degree of infiltration. (B) Clonality and diversity summarize variation in the TCR repertoire; depth is a measure of sequencing quality and observable TCR sequences in samples.
Supplementary Figure 3: Individual patient TCR repertoire composition. TCR repertoire can be stratified into three categories: (A) polyclonal, (B) oligoclonal, or (C) monoclonal.
Supplementary Figure 4: TCR clones common to multiple repertoires. Clones sharing and frequency in PBMC samples (A) and across TIL repertoires versus their average frequency (B) or depth of infiltration (C). Note that the scale of the y-axes differs between plots. Clone subsets are highlighted if they are common to multiple repertoires (blue), relatively frequent (green) or have a high level of absolute infiltration (red).

Supplementary Figure 5: Clone overlap between TIL and PBMC repertoires. Overlap between patient repertoires is tissue specific; TIL repertoires are more exclusive than PBMC repertoires.
Supplementary Figure 6: Restricted mean survival (RMS) overall survival and progression-free survival estimates for the effect of exclusivity and clone-level overlap between TIL and PBMC repertoires. Cox model-based RMS estimates for PBMC exclusivity stratified by NY-ESO-1 serology for OS (A) and PFS (B); clone overlap stratified on NY-ESO-1 serology (C, D).
### Supplementary Table 1: Prognostic features of TCR repertoires across studies

|          | Overall Survival |                  | Progression Free Survival |                  |
|----------|------------------|-------------------|---------------------------|-------------------|
|          | HR               | p-value           | HR                        | p-value           |
| TCGA-TIL |                  |                   |                           |                   |
| Entropy  | 0.76 (0.64–0.91) | 0.003209          | 0.86 (0.74–1.01)          | 0.05778           |
| Clonality| 1.07 (0.89–1.30) | 0.462697          | 1.08 (0.92–1.26)          | 0.37364           |
| Infiltration| 0.78 (0.65–0.94)   | 0.008096          | 0.86 (0.74–1.01)          | 0.05753           |
| Diversity| 0.77 (0.64–0.92) | 0.003699          | 0.87 (0.75–1.01)          | 0.05925           |
| TOP1     | 1.36 (1.14–1.64) | 0.000807          | 1.19 (1.02–1.38)          | 0.02547           |
| N25      | 0.72 (0.58–0.90) | 0.002949          | 0.80 (0.68–0.95)          | 0.00932           |
| RPCI-TIL |                  |                   |                           |                   |
| Entropy  | 0.78 (0.59–1.04) | 0.0932            | 0.95 (0.75–1.21)          | 0.699             |
| Clonality| 1.21 (0.90–1.62) | 0.2032            | 0.99 (0.76–1.30)          | 0.9528            |
| Infiltration| 0.91 (0.68–1.21)   | 0.5065            | 0.81 (0.64–1.03)          | 0.0901            |
| Diversity| 0.82 (0.62–1.09) | 0.1766            | 0.97 (0.75–1.23)          | 0.7763            |
| TOP1     | 1.13 (0.84–1.51) | 0.4149            | 1.04 (0.80–1.35)          | 0.7765            |
| N25      | 0.81 (0.54–1.22) | 0.3182            | 0.84 (0.6–1.19)           | 0.3299            |
| RPCI-PBMC|                  |                   |                           |                   |
| Entropy  | 0.79 (0.61–1.03) | 0.081             | 0.87 (0.68–1.11)          | 0.2686            |
| Clonality| 1.20 (0.92–1.58) | 0.1821            | 1.09 (0.84–1.43)          | 0.513             |
| Infiltration| 0.84 (0.65–1.09)   | 0.194             | 0.84 (0.69–1.02)          | 0.0802            |
| Diversity| 0.80 (0.62–1.03) | 0.0832            | 0.84 (0.67–1.06)          | 0.14              |
| TOP1     | 1.19 (0.92–1.52) | 0.1804            | 1.02 (0.78–1.35)          | 0.8711            |
| N25      | 1.11 (0.87–1.42) | 0.4135            | 0.97 (0.78–1.22)          | 0.8216            |

### Supplementary Table 2: TCR sequencing on matched TIL and PBMC samples from RPCI ovarian cancer cohort. See Supplementary Table 2
### Supplementary Table 3: TCR clones and read processing

| TIL                      | Total Clones | Unique Clones | Observed Reads | Normalized Reads |
|--------------------------|--------------|---------------|----------------|------------------|
| Observed NT sequences    | 1,454,072    | 1,443,896     | 305,389,678    | 347,052,958      |
| Productive NT sequences  | 1,194,008    | 1,184,830     | 259,152,997    | 289,963,375      |
| Unique AA sequences      | 1,136,217    | 992,791       | 259,152,997    | 289,963,375      |
| Sequence found in PBMC   | 243,510      | 213,072       | 141,510,702    | 152,888,787      |
| % TIL Exclusive          | 78.56%       | 78.53%        | 45.39%         | 47.27%           |

| PBMC                     | Total Clones | Unique Clones | Observed Reads | Normalized Reads |
|--------------------------|--------------|---------------|----------------|------------------|
| Observed                 | 10,487,218   | 10,337,157    | 505,261,517    | 479,220,568      |
| Productive               | 8,785,710    | 8,637,309     | 400,143,279    | 432,017,588      |
| AA Unique                | 8,213,806    | 6,275,193     | 432,017,588    | 400,143,279      |

NT: Nucleotide, AA: Amino Acid.

### Supplementary Table 4: Association between the top 18 common clones and PFS

| Clone                  | Frequency of clone | Log frequency of clone | Frequency Rank clone |
|------------------------|--------------------|------------------------|----------------------|
|                        | subjects with clone| log HR | p-value | Bonferroni p | log HR | p-value | Bonferroni p | log HR | p-value | Bonferroni p |
| CASSLGETQYF            | 42                 | 0.015 | 0.893 | 1.000 | -0.033 | 0.784 | 1.000 | -0.035 | 0.771 | 1.000 |
| CASSLGETQYF            | 34                 | -0.210 | 0.231 | 1.000 | -0.150 | 0.222 | 1.000 | -0.151 | 0.222 | 1.000 |
| CASSLGGNTEAFF          | 34                 | 0.038 | 0.722 | 1.000 | -0.016 | 0.893 | 1.000 | -0.017 | 0.885 | 1.000 |
| CASSLTDQYF             | 31                 | 0.428 | 0.003 | 0.046 | 0.050 | 0.668 | 1.000 | 0.036 | 0.757 | 1.000 |
| CASSLSTDTQYF           | 31                 | -0.104 | 0.360 | 1.000 | -0.128 | 0.278 | 1.000 | -0.131 | 0.273 | 1.000 |
| CASSFQETQYF            | 30                 | -0.152 | 0.385 | 1.000 | -0.087 | 0.472 | 1.000 | -0.085 | 0.484 | 1.000 |
| CASSLGYEQYF            | 30                 | -0.077 | 0.496 | 1.000 | -0.010 | 0.928 | 1.000 | -0.151 | 0.222 | 1.000 |
| CASSQETQYF             | 29                 | -0.074 | 0.517 | 1.000 | -0.028 | 0.810 | 1.000 | -0.029 | 0.802 | 1.000 |
| CASSLGEAFF             | 28                 | 0.008 | 0.946 | 1.000 | -0.097 | 0.416 | 1.000 | -0.102 | 0.393 | 1.000 |
| CASSSTDQYF             | 28                 | -0.240 | 0.290 | 1.000 | 0.035 | 0.760 | 1.000 | 0.030 | 0.795 | 1.000 |
| CASSPSTDQYF            | 27                 | -0.059 | 0.568 | 1.000 | -0.101 | 0.389 | 1.000 | -0.095 | 0.421 | 1.000 |
| CASSLNTEAFF            | 27                 | -0.075 | 0.684 | 1.000 | 0.169 | 0.145 | 1.000 | 0.178 | 0.126 | 1.000 |
| CASSLSYEQYF            | 27                 | 0.250 | 0.036 | 0.649 | 0.114 | 0.326 | 1.000 | 0.103 | 0.368 | 1.000 |
| CASSLGPNEAFF           | 27                 | 0.136 | 0.161 | 1.000 | 0.230 | 0.049 | 0.873 | 0.225 | 0.054 | 0.975 |
| CASSSSTDQYF            | 26                 | 0.151 | 0.169 | 1.000 | 0.105 | 0.368 | 1.000 | 0.099 | 0.393 | 1.000 |
| CASSLRTEQYF            | 26                 | 0.027 | 0.787 | 1.000 | 0.157 | 0.164 | 1.000 | 0.163 | 0.152 | 1.000 |
| CASSLPQNYGTF           | 26                 | -0.107 | 0.449 | 1.000 | -0.077 | 0.510 | 1.000 | -0.067 | 0.568 | 1.000 |
| CASSLRQNEAFF           | 25                 | -0.438 | 0.687 | 1.000 | 0.079 | 0.502 | 1.000 | 0.099 | 0.399 | 1.000 |

← Only Bonferroni significant clone.
### Supplementary Table 5: Cox model regression stratified by stage

| PBMC Exclusivity (Stage Stratified) | Overall Survival | Progression-Free Survival |
|------------------------------------|------------------|---------------------------|
| HR (95%CI), score test p-value      | HR (95%CI), score test p-value |
| Exclusivity*                       | 0.06 (0.00–0.61), 0.017 | 0.70 (0.47–1.03), 0.068 |
| NY-ESO-1 Status                    | 1.68 (0.94–2.99), 0.078 | 1.83 (1.08–3.10), 0.026 |
| Log10 CD3 level*                   | 0.68 (0.52–0.88), 0.003 | 0.79 (0.34–0.99), 0.037 |
| Overlap vs NYESO-1                 | 1.91 (1.08–3.36), 0.025 | 2.17 (1.18–3.99), 0.013 |
| Overall model fit                  | Wald Test p = 0.003 | Wald Test p = 0.011 |
| Deviance test (Interaction)        | p = 0.022          | p = 0.011          |

| TIL/PBMC Overlap (Stage Stratified) | Overall Survival | Progression-Free Survival |
|------------------------------------|------------------|---------------------------|
| HR (95%CI), score test p-value      | HR (95%CI), score test p-value |
| Overlap*                           | 1.51 (1.08–2.12), 0.015 | 1.39 (0.99–1.96), 0.058 |
| NY-ESO-1 Status                    | 1.67 (0.95–2.95), 0.078 | 1.78 (1.06–3.00), 0.030 |
| Log10 CD3 level*                   | 0.66 (0.51–0.86), 0.002 | 0.77 (0.62–0.96), 0.021 |
| Overlap vs NYESO-1                 | 0.49 (0.27–0.87), 0.016 | 0.44 (0.25–0.76), 0.004 |
| Overall model fit                  | Wald Test p = 0.003 | Wald Test p = 0.004 |
| Deviance test (Interaction)        | p = 0.016          | p = 0.004          |

*Scaled to unit std. deviation.