

# Peripheral T-cell expansion predicts immunotherapy response

Immune checkpoint blockade has been widely presumed to rescue chronically stimulated, tumor-resident, exhausted T cells. However, researchers at Genentech (South San Francisco, CA, USA) found evidence for an alternate mechanism. Using single cell immune profiling, they observed that clonal expansion of effector-like T cells in patient tumors was reflected in normal adjacent tissue and peripheral blood. Further analysis suggested that, in patients responsive to anti-PDL1 therapy, intratumoral T cells are replenished with non-exhausted cells from outside the tumor. TD Wu et al., *Nature*. (2020).

Snapshot	10x Genomics product
<p><b>Research area:</b> Therapeutic development</p> <p><b>Organism:</b> Human</p> <p><b>Sample type:</b> Flow-sorted CD3<sup>+</sup> or CD45<sup>+</sup> T cells from tissue and blood</p> <p><b>Research question:</b> What mechanisms account for different patient responses to immunotherapy?</p>	<p><b>Chromium Single Cell Immune Profiling Solution</b></p> <ul style="list-style-type: none"> <li>Chromium Single Cell 5' Library and Gel Bead Kit</li> <li>Chromium Single Cell V(D)J Enrichment Kit, Human T Cell</li> <li>Chromium Single Cell A Chip Kit</li> <li>Chromium i7 Multiplex Kit</li> <li>Cell Ranger analysis pipelines</li> </ul>

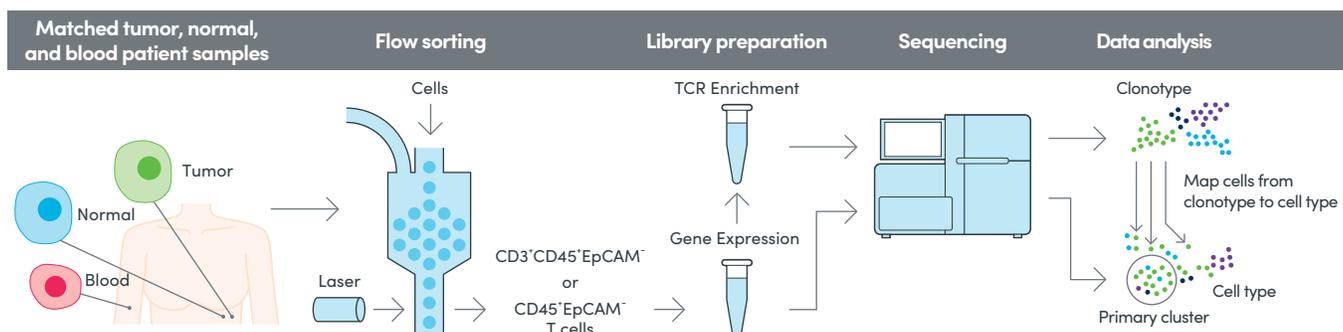
## Experiment overview

### Isolation and sequencing of T cells from cancer patients

- Collection of fresh surgical samples for paired tumor and normal adjacent tissue from 14 patients with different tumor types, along with blood from 4 patients
- Flow sorting of T cells based on expression of CD3, CD45, and EpCAM
- Single cell whole transcriptome profiling of 200,626 immune cells, and full-length, paired T-cell receptor sequencing for 141,623 T cells using the Chromium Single Cell Immune Profiling Solution

### Mapping clonotype to T-cell phenotype

- Data from samples for each patient were aggregated, and then clonotypes identified across samples based on consensus CDR3 nucleotide sequence for  $\alpha$ - and  $\beta$ -chains
- Clustering of aggregate immune cells based on whole transcriptome sequencing followed by annotation using reference gene signatures in Seurat
- Single cells from each clonotype were mapped to primary cell phenotypes by clustering cells with similar transcriptional profiles



## Why single cell?

Clonal expansion can be directly measured by single cell sequencing of paired T-cell receptors since the output is the number of cells expressing each clonotype. By sequencing hundreds of thousands of immune cells, emergent patterns in clonal expansion can be identified. Single cell sequencing is required to definitively determine which clones are expanding, as T-cell receptor transcripts are mapped to individual cells. In combination with single cell gene expression, gene signatures for dual-expanded clones were identified, providing the groundwork for analysis of clinical data.

## Computational analysis

### Identification of dual-expanded clones

Full-length, paired  $\alpha$ - and  $\beta$ -chain T-cell receptor sequences were generated by Cell Ranger for each cell, along with identification of clonotypes within each sample. Researchers used a custom script [[ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE139555](https://ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE139555)] to compare consensus CDR3 regions across samples and identified shared clonotypes between tumor and normal adjacent tissue for each patient. Dual-expanded clonotypes had at least one cell in both tumor and normal adjacent tissue (Figure 1) and were frequently detected in blood, suggesting peripheral infiltration of clonal T cells in tumor and normal tissue.

## Results

### Clonotypes mapped to multiple cell-type clusters

Individual cells from each clonotype were mapped to cell-type clusters. Most clones were derived primarily from a single cluster, though other clones were more heterogeneous. Dual-expanded clones were typically  $CD8^+$  T cells, while  $CD4^+$  T cells were often singletons (Figure 2). Blood-expanded clones were largely T effector cells, presumed to infiltrate both tumor and normal tissue, resulting in correlated dual expansion.

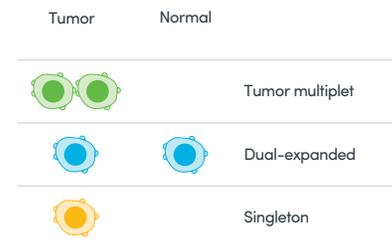
### Peripheral expansion associated with improved cancer outcome

Analysis of published single cell and bulk sequencing clinical data revealed a correlation between peripheral T-cell recruitment and response to anti-PDL1 therapy. After immunotherapy, new clones were detected in tumor samples, many of which appeared to be derived from peripheral  $CD8^+$  T cells. Such clones were more likely to be non-exhausted and had greater clone size than clones not detected in blood. In three randomized trials of anti-PDL1 immunotherapy, patients with gene signatures suggestive of dual-expanded clones showed improved survival compared to patients without clonal expansion.

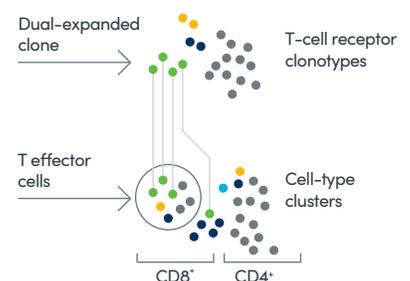
## References

1. TD Wu et al., Peripheral T cell expansion predicts tumour infiltration and clinical response. *Nature*. 579, 274–278 (2020).

For a full list of publications visit [10xgenomics.com/publications](https://10xgenomics.com/publications)



**Figure 1. Identification of dual-expanded clones.** Using full-length, paired T-cell receptor sequences, clonotypes were compared across samples per patient. If more than one cell from a clone was observed in the tumor, that clonotype was considered a tumor multiplet. If at least one cell was seen in both tumor and normal tissue, clonotypes were termed dual-expanded. Singleton refers to a clone with only one cell in either tumor or normal tissue.



**Figure 2. Mapping clonotype to cell type.** While clonotype is determined by T-cell receptor chains and CDR3 sequence, cell-type clustering and annotation depends on gene expression. Mapping cell-by-cell from clonotype to cell type demonstrated that many representatives of dual-expanded clones were  $CD8^+$  T effector cells. Most  $CD4^+$  T cells were singletons.