

# Quantifying the Body's Immune Response to Tumors

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The adaptive immune system is geared towards fighting off foreign invaders. This system can also be activated against the body's own cells, however, such as those that start becoming abnormal on the road to cancer. These renegade cells often present aberrant proteins on their surface that are recognized as different, thus triggering an immune response that includes attack by tumor-infiltrating lymphocytes (TILs). While TILs have been shown to directly attack tumor cells, and the presence of TILs is reported to improve cancer survival, their presence in tissue samples have been difficult to accurately quantify with existing methods. In a paper in *Science Translational Medicine*, Drs. Harlan Robins and Jason Bielas, along with other colleagues in the Public Health Sciences, Clinical Research, and Human Biology Divisions, describe a new method for precisely measuring TILs, and demonstrate its translational potential for clinical use.

This method, termed QuanTILfy, is able to both count the absolute number of TILs and assess the T cell clonality within tissue samples, including tumors. This is done by measuring genetic rearrangements in the T-cell receptor loci of T cells using a digital droplet polymerase chain reaction approach, and comparing this to a control locus to allow for genome-normalized quantification. Essentially, "T cell clones have unique DNA sequences that are analogous to product barcodes on items at the grocery store," says senior author Bielas. "Our technology is comparable to a barcode scanner, which gives highly quantitative information on the total number of scanned products and the type of product." Importantly, this method is robust and standardizable, as opposed to previous methods that were more qualitative and difficult to compare between samples.

To both validate their method and demonstrate its potential translational utility, the researchers applied QuanTILfy to several clinical questions. In the first set of experiments, results from five T-cell acute lymphoblastic leukemia (T-ALL) patients indicated the expected clonal T cell expansion of a single clonal subgroup, while a healthy control showed an even distribution of the 8 QuanTILfy TIL subgroups. "We demonstrated that this method can be used to diagnose T cell acute lymphoblastic leukemia quickly and effectively from a blood draw," says Bielas.

Next, the researchers investigated the spatial distribution of TILs in primary ovarian tumors from three patients. Each tumor was systematically assessed, with punch biopsies taken at intervals both within the interior of the tumor and on the margins. Using QuanTILfy, the researchers were able to observe differences in the fraction of TILs (as a percentage of total cells) both between and within these tumors. Tumors 1 and 2 showed consistent TIL fractions across the biopsy locations, with tumor 1 showing lower TIL infiltrations than tumor 2 (<0.5% vs. 5-10%). Tumor 3, on the other hand, showed heterogeneity between its two halves (2-7% vs. 13-15%). This suggested these halves were generating different immune responses and may have different biologies, demonstrating the importance of accurately assessing the entire tumor. As such, some tumors may require more than one biopsy to avoid reporting potentially misleading TIL levels.

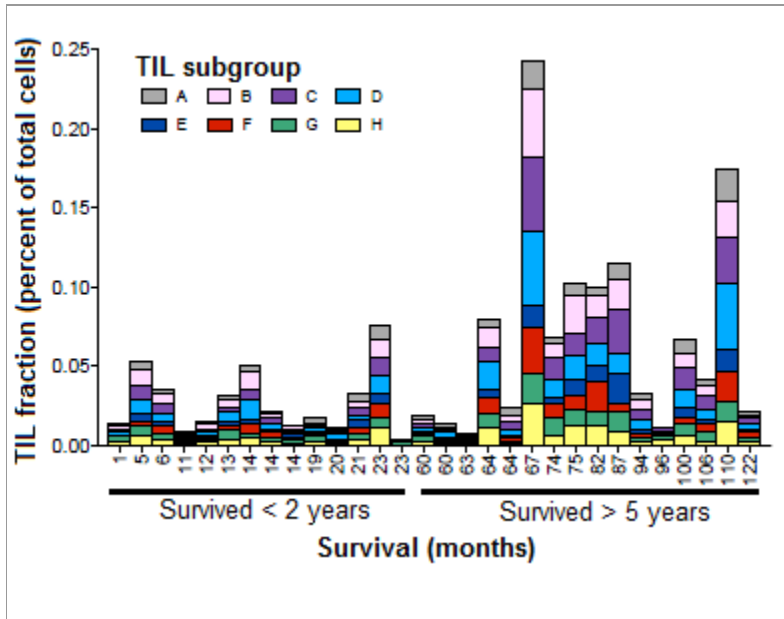
The researchers then compared TIL levels in matched primary and metastatic tumor tissues from 18 women with late-stage serous ovarian carcinoma. The metastases exhibited a higher TIL count in 14 of the women, with an average twofold higher TIL fraction in the metastases than the paired primary (10.7% vs. 5.2%). This suggests that, compared to the primary tumor, the cellular immune response is different and greater in metastatic tumors within the same individual.

Lastly, the researchers compared TIL fractions in primary tumors of 30 patients with stage III or IV ovarian cancer. The average TIL fraction was significantly higher in tumors from patients who survived longer than five years compared to those who survived less than two years (see figure). While exceptions were observed, this suggests that higher TIL levels are positively correlated with outcome, and is consistent with the hypothesis that T cells play an active role in suppressing tumorigenesis. Additionally, the researchers "were surprised to find so much T cell diversity in ovarian tumors," says Bielas, "indicating that the immune response to the tumor is multifaceted, rather than focused on one or a few aberrant tumor proteins."

Overall, this new method holds promise for translation into research and clinical care. Moving forward, says lead author Robins, the researchers are "engaged in large scale studies for multiple tumor types, designed to show that precisely-measured TIL counts and clonality, via QuanTILfy, are both prognostic of clinical outcome and predictive for treatment decisions." Adds Bielas, "the implementation of this assay in the clinic should improve cancer diagnostics and ultimately save lives."

Other FHCRC investigators contributing to this project were Drs. Nolan Ericson (PHS), Jamie Guenthoer (HB), Kathy O'Briant (PHS), Muneesh Tewari (PHS, HB, CRD), and Charles Drescher (PHS).

[Robins HS, Ericson NG, Guenthoer J, O'Briant KC, Tewari M, Drescher CW, Bielas JH. 2013. Digital genomic quantification of tumor-infiltrating lymphocytes. \*Sci Trans Med.\* 5\(214\):214ra169.](#)



(Image provided by Dr. Jason Bielas)

Tumor-infiltrating lymphocyte (TIL) fraction in tumor biopsies (y-axis) from 30 ovarian carcinoma patients with known survival outcomes (x-axis). No clonal expansion (TIL subgroups) was seen in any of the tumors. On average, the TIL fraction was 2.5-fold higher in those who survived longer than 5 years compared to those who survived less than two years ( $p=0.03$ ).