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**IDENTIFICATION OF DISTINCT IMMUNE LANDSCAPES OF INFILTRATING T CELLS IN COLON CANCER USING MULTIPLEX IMMUNOFLUORESCENCE STAINING**

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**Background** The immune system recognizes foreign microorganisms as "non-self" and reacts to destroy these disease-causing agents, playing a similar role in protecting the body from malignancies. The spatial distribution of T cell subsets in tumor tissues, like colon cancer, may provide information on the role of the immune system in tumor development. To gain novel insights into different types of T cells including cytotoxic T (Tc) cells, helper T (Th) cells, and regulatory T (Treg) cells, a seven-color multiplex immunofluorescence (mIF) panel was used to study the number and spatial distribution of these T cell subsets in a panel of colon tumors.

**Methods** We simultaneously assessed CD3, CD8, Foxp3, Ki67, Granzyme B and pan cytokeratin in ninety colon cancer cases using a tyramide signal amplification based mIF approach. Using dedicated image analysis software, we analyzed the multiple cell phenotypes and their spatial distribution inside tumor stromal and epithelial regions of interest. Moreover, in a previous study, next-generation sequencing was performed on the same samples, resulting in consensus clustering based on the immunologic constant of rejection (ICR) genes,<sup>1</sup> segregating colon cancer patients in three different groups: ICR low, medium and high. Statistical analyses were conducted to determine associations between the density of T cell subsets and their spatial location.

**Results** All T cell subtypes were more prevalent in the stromal fraction than in the epithelial fraction, but the proportion of Ki-67+ or Granzyme B+ T cells was significantly higher in the tumor epithelium than in the tumor stroma. In both tumor epithelium and tumor stroma, T cell densities were significantly higher in those with high ICR than in those with low ICR. Meanwhile, the median distance between immune cells and epithelial cells was significantly smaller in ICR-high than in ICR-low. Interestingly, patients with an ICR high/Th cells high experienced improved overall survival ( $p = 0.016$ ).

**Conclusions** In this study, we quantified the spatial distribution of T cell subsets and highlight the tumor infiltration of Th cells, which can improve the prognostic value of T cell immune signatures in colon cancer.

**REFERENCE**

1. Roelands J, Decock J, Boughorbel S, *et al.* A collection of annotated and harmonized human breast cancer transcriptome datasets, including immunologic classification. *F1000Research*. 2017;6:296.

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