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Multiplex Immunofluorescence in Colorectal Cancer: A Retrospective Analysis from SCOT and QUASAR 2 Trials

Jim Mason
Cancer Genetics and Epigenetics, MedSci Publisher, Richmond, BC, V7A 4Z5, Canada
Corresponding author email: jim.mason@sophiapublisher.com
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"The Lancet Oncology" published an article on February 1, 2024, titled "Multiplex analysis of intratumoural immune infiltrate and prognosis in patients with stage II-III colorectal cancer from the SCOT and QUASAR 2 trials: a retrospective analysis." The authors, Anja L. Frei, Anthony McGuigan, Ritik R.A.K Sinha, David N. Church, Viktor H. Koelzer, among others, are affiliated with the University of Zurich, Wellcome Centre for Human Genetics at the Nuffield Department of Medicine, University of Oxford, and the Cancer Research UK Glasgow Clinical Trials Unit at the University of Glasgow and other units. David N. Church is the corresponding author. This research utilizes data from the SCOT and QUASAR 2 clinical trials to deeply analyze the prognostic significance of intratumoural immune cell infiltrates in stage II-III colorectal cancer. Employing advanced multiplex immunofluorescence staining techniques, the study evaluates the density of various immune cells including CD8+ cytotoxic T cells, FoxP3+regulatory T cells, CD20+B cells, and CD68+macrophages within the tumor microenvironment. The primary goal of the research is to reveal the associations between these immune cells and the patients' recurrence-free interval and overall prognosis. This method aims to optimize risk stratification in colorectal cancer and provide guidance for personalized treatment strategies.

1 Interpretation Experimental Data

This study employed multiplex immunofluorescence staining to meticulously analyze immune cells and their densities within colorectal cancer tissues. Specific markers targeted included CD8+cytotoxic T cells, FoxP3+regulatory T cells, CD20+B cells, and CD68+macrophages. The process involved precise quantification of cell densities in both intraepithelial and interstitial sites. Immune markers were assessed using the HALO AI DenseNet v2 machine learning classifier, which is capable of accurately locating and quantifying immune cells in different tumor areas. The study focused on the correlation between immune cells and the patients' recurrence-free intervals, particularly highlighting the prognostic value of intraepithelial CD8+cells. It noted that including data from interstitial FoxP3+cells in the analysis provided more accurate prognostic insights, thereby enhancing the predictive power of disease prognosis.

Figure 2 further confirms the prognostic value of CD8IE and FoxP3IS densities in colorectal cancer from the QUASAR 2 trial. Multivariate linear regression analysis reveals independent predictors including age, gender, tumor staging, and molecular characteristics. Part B, through multivariate analysis, shows that the composite markers based on CD8IE and FoxP3IS densities are associated with the risk of cancer recurrence, with adjusted hazard ratios emphasizing the importance of these markers. Part C's Kaplan-Meier curves group patients based on marker densities, displaying recurrence-free intervals for different groups, confirming risk stratification, with higher density groups having a better prognosis.

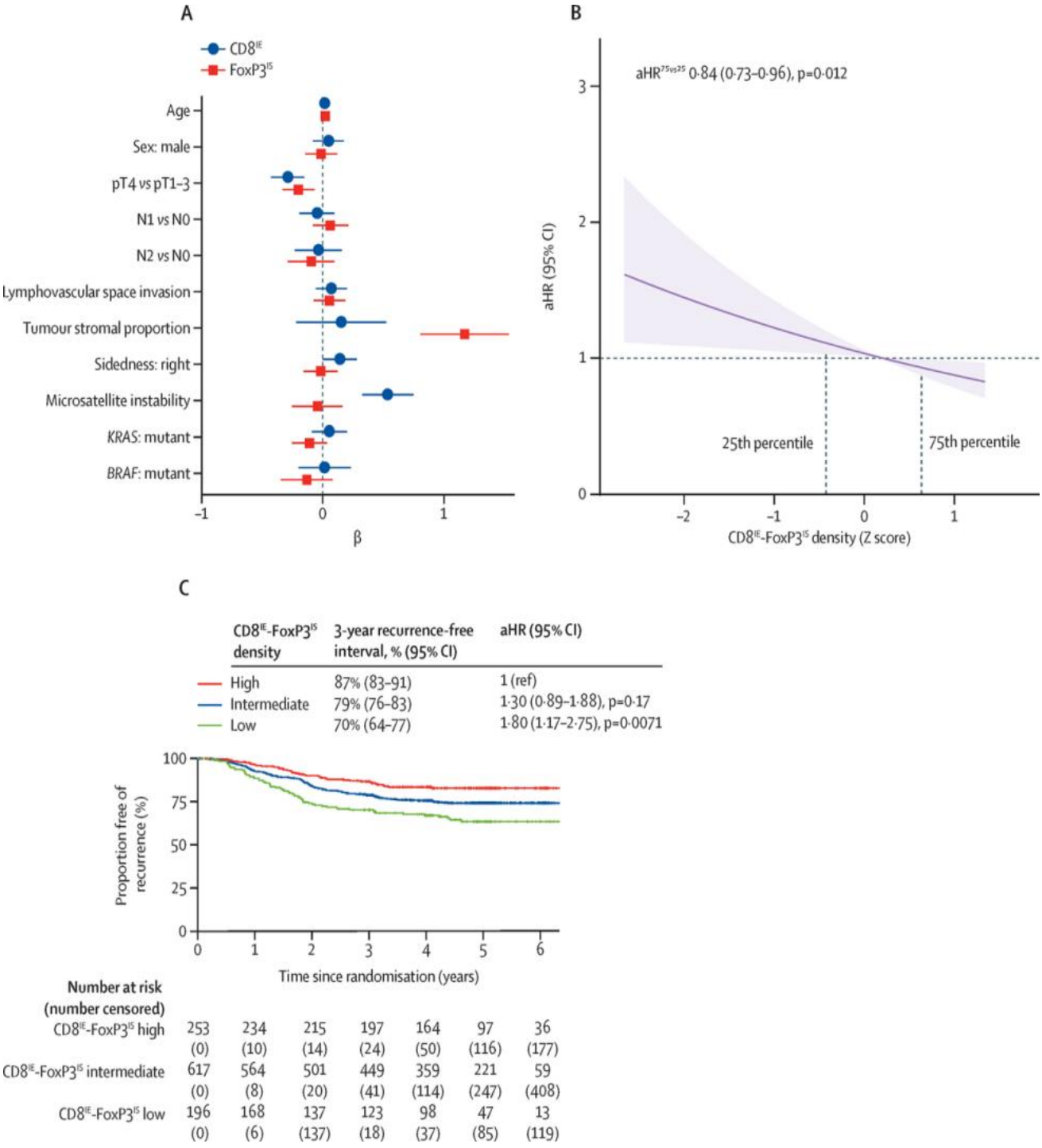

Figure 2 Validation of predictors and prognostic value of CD8IE and FoxP3IS in cases from the QUASAR 2 trial

Figure 4 displays the proportion of patients without recurrence across N0, N1, and N2 stages, categorized by different CD8IE-FoxP3IS density levels (high, medium, low). Multivariable analysis indicates that higher CD8IE-FoxP3IS densities are associated with improved prognosis across all N stages, particularly in the N0 high-density group, where the three-year recurrence-free interval reaches 94%. The pie charts show the importance of various factors in the multivariable regression, emphasizing the critical role of immune infiltration in adjusting tumor staging risks.

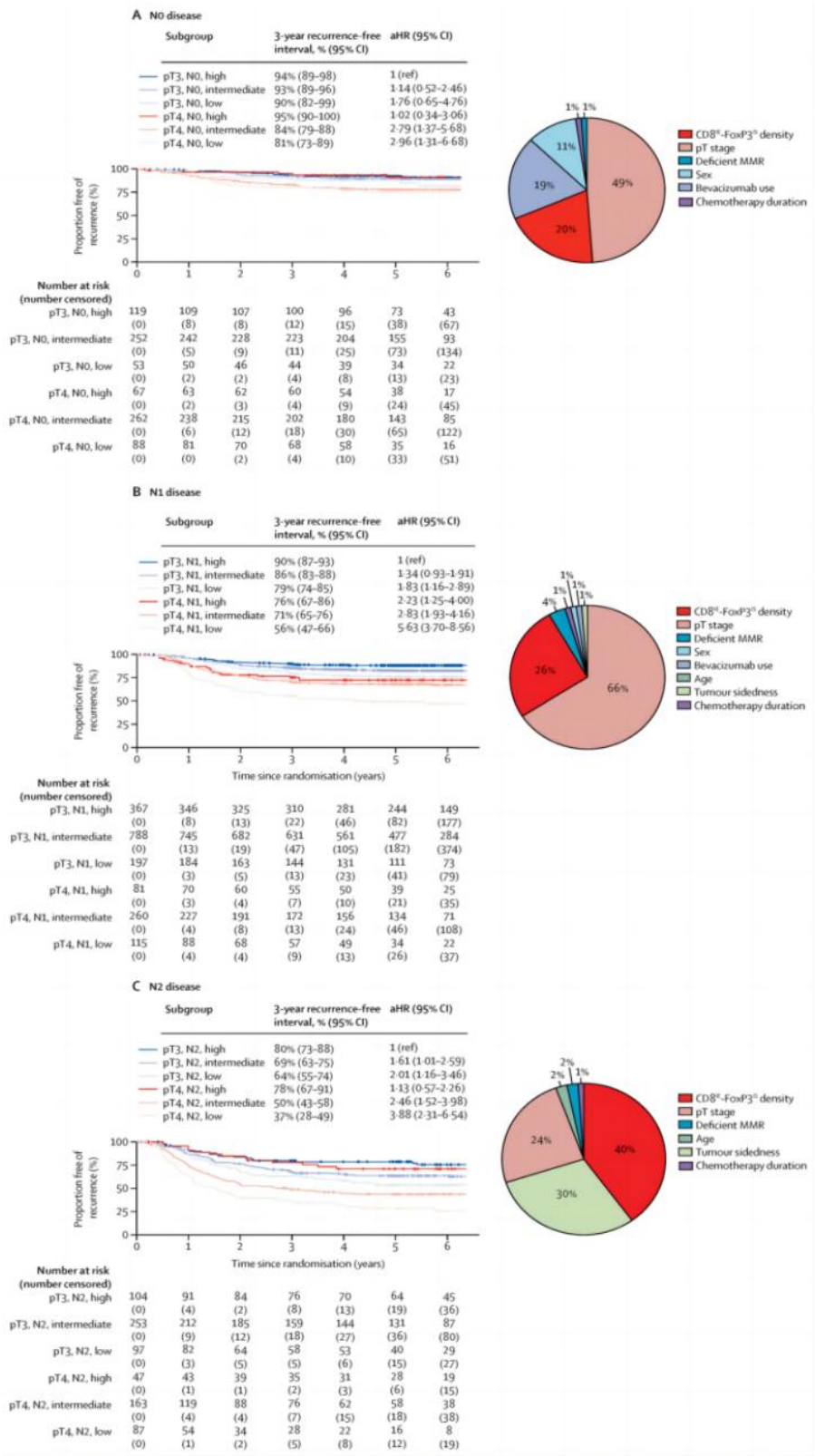

Figure 4 Stratification of risk by CD8IE-FoxP3IS density and N stage

2 Insights of Research Findings

In this study, the combined assessment of the density of CD8+T cells within the epithelial layer (CD8IE) and FoxP3+regulatory T cells in the stromal layer (FoxP3IS) revealed significant prognostic value. This composite

marker showed superior prognostic value over single immune cell types in both continuous variables and categorized density groups. In the SCOT trial, higher densities of CD8IE and FoxP3IS were positively correlated with longer recurrence-free intervals, suggesting a potential additive or synergistic effect when considering both cell types. These findings were reaffirmed in the QUASAR2 cohort validation, observing similar trends, thus confirming the robustness and practical utility of the immunotyping method in predicting the prognosis of colorectal cancer patients. The analysis strongly supports the use of combined CD8IE and FoxP3IS densities as a prognostic indicator superior to traditional single-marker methods, providing compelling evidence that integrated CD8IE and FoxP3IS densities offer significant advantages over traditional single-marker approaches for prognosis assessment.

3 Evaluation of the Research

This study is particularly noteworthy for the use of multiple immunofluorescence immunoassays in the field of colorectal cancer. The innovative approach of quantifying immune cells using a machine learning-based tissue classifier contributes to enhancing the accuracy and reliability of prognostic assessments. However, potential selection bias in choosing specific clinical trial cohorts and tissue microarrays may limit the general applicability of the findings. Additionally, the analysis is confined to pre-specified immune markers, potentially overlooking other important immune components. These factors suggest a need for further research involving broader and more diverse patient groups, along with an expansion of immune markers to fully validate and generalize these findings across different clinical settings.

4 Concluding Remarks

This study underscores the pivotal role of tumor immune environment analysis in predicting colorectal cancer prognosis, particularly emphasizing the importance of the integrated assessment of CD8IE and FoxP3IS. These findings support a more refined stratification of patient risk, revealing that the interaction between cytotoxic T cells and regulatory T cells within the tumor milieu significantly impacts prognosis. The combined marker of CD8IE and FoxP3IS not only enhances predictive accuracy but also holds promise for guiding personalized therapeutic interventions. If based on individual immune characteristics, this method could significantly improve precision and potentially have a profound impact on clinical decision-making. Future research should focus on validating these results, incorporating more case data to enhance the applicability and reliability of this prognostic tool in routine clinical practice.

5 Access Original Paper

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