1506 COMPREHENSIVE ASSESSMENT OF LYMPHOCYTE COUNTS IN COLORECTAL CANCER: DEFINING THE OPTIMAL PROGNOSTIC MARKER

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Background Intra-tumor lymphocyte counts can stratify colorectal cancer (CRC) prognosis and help select patients for and define response to immunotherapy. The internationally validated Immunoscore™ predicts CRC survival risk by averaging percentile scores of tumor CD3 and CD8 cell densities. However, arbitrary percentile cutoffs can distort non-normally distributed variables such as cell counts, while neglecting intra-tumoral heterogeneity and potentially confounding clinical, pathologic and genomic factors. We sought to better characterize the biological and prognostic significance of CD3 and CD8 quantification in CRC.

Methods Clinicopathologic and oncologic outcome variables were obtained for 200 CRC patients undergoing surgery in a single US center, and each tumor subjected to next-generation sequencing and quantitative CD3/CD8 immunohistochemistry, from which percentile cell counts were averaged (I-score) in a manner analogous to the Immunoscore™. I-score and exploratory endpoints, including CD3 and CD8 cell densities/percentiles, CD3-CD8 density/percentile differences, and CD3:CD8 density/percentile ratios were assessed for clinicopathologic and genomic (40 commonly mutated genes) correlates (linear regression), and for disease-specific survival (DSS; Cox proportional hazards models).

Results Excellent risk stratification by TNM stage and I-score was confirmed (figure 1). CD3 density among CRCs was bimodal and right-skewed, while CD8 density was Kurtotic and right-skewed (p<0.0001, figure 2). Density, percentile, and intra-tumoral variability for CD3 and CD8, as well as combination metrics including I-score, CD3-CD8 density/percentile differences, and CD3:CD8 density/percentile ratios defined distinct clinicopathologic and genomic subsets, suggesting that each may hold unique biological significance (table 1). CD3 density, CD8 density/percentile, I-score and CD3:CD8 percentile ratio showed univariate association with DSS. Only CD3:CD8 percentile ratio was TNM stage-independent on multivariate analysis, whereas I-score was not. CD3 density bimodality at 235/mm² generated two biologically and prognostically distinct subgroups, as did a shoulder (120/mm²) in the CD8 distribution (figure 2, table 1). Potential bimodality in the CD3:CD8 percentile ratio distribution (at 0.7) likewise defined distinct biological and prognostic subsets (figure 3, table 1).

Conclusions Composite percentile-based scores can distort or mask potentially important biologic subgroups. I-score underperformed prognostically in this series, being closely associated with confounding biologic variables such as male sex, active smoking, T/N/M stage, and mutations in BRAF, GNAS, POLE, RNF43, SMAD4 and MMR genes. We achieved increasingly precise and biologically relevant biomarkers by using data-driven CD3/CD8 density cutoffs and ratios, while controlling for important clinicopathologic and molecular variables in CRC. Independent validation and inclusion of other immune or stromal cell types will bring these findings closer to clinical utility in CRC.

Abstract 1506 Table 1 Established and candidate immune outcome variables in CRC tissue, with clinical, pathologic (disease-specific survival) value on univariate analysis and controlled for pT/N/M stages

Abstract 1506 Figure 1 Survival estimates of cohort by AJCC Stage and by I-score tertile (method analogous to Immunoscore™)

Abstract 1506 Figure 2 Histogram of CD3 and CD8 cell densities, with survival estimates dichotomized at specific breakpoints
Abstract Figure 3: Histogram of CD3:CD8 density percentile ratios, with survival estimates dichotomized at specified breakpoint.