Phenotyping TILs \textit{in situ}: Automated Enumeration of FOXP3+ and CD69+ T Cells in Follicular Lymphoma

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Background: In many cancers, tumor-infiltrating lymphocytes (TILs) indicate levels of tumor immunogenicity and predict survival. In particular, increased levels of regulatory T cells (Tregs) are associated with poorer prognosis, whilst CD69+ T-cells may also be prognostic. Visual TIL assessment cannot easily determine the type of lymphocyte \textit{in situ} and multimarker quantitation is difficult with standard methods. We present a multi-marker, computer-aided event-counting method for determining the phenotypes of lymphocytes in follicular lymphoma using a multispectral imaging (MSI) automated tissue segmentation and counting approach.

Material and methods: A tissue microarray containing follicular lymphoma (FL) cores from 70 patients was chromogenically immunostained for CD3, CD69 and FOXP3, counterstained with hematoxylin, of which 40 cores were informative for both triplex staining and clinical follow-up. Each core was imaged using MSI and the individual staining of each marker separated from each other using spectral unmixing. Images were analyzed using software trained to recognize different tissue compartments based on morphology, specifically based on CD3 rich (extra-follicular) and poor (intra-follicular) areas. The FOXP3 or CD69 status of each CD3+ TIL was then determined and number Treg (FOXP3+/CD3+) and CD69+ T-cells counted.

Results: The intra-follicular (CD3 poor) and extra-follicular (CD3 rich) regions were accurately recognized within each core, based on abundance of CD3 cells. MSI enabled the accurate quantitation of CD3, CD69 and FOXP3 without crosstalk. The number of FOXP3+/CD3+ Tregs and CD69+ T-cells were counted and used in Kaplan-Meier survival analysis, which demonstrated association of FOXP3+/CD3+ Tregs with favourable outcome in both the intra and extra-follicular
areas, plus CD69+ T-cells in intra-follicular areas; CD69+ T-cells were not prognostic in extra-follicular areas.

Conclusions: This study demonstrates use of an automated method for counting Tregs in follicular lymphoma, showing association of FOXP3+ Tregs with good outcome.