Aim: Successes with PD-L1 drugs and adoptive immunotherapy demonstrate the efficacy of leveraging the immune system to fight cancer. However, host-tumor interaction is complex and difficult to characterize with immunohistochemistry or flow cytometry. Capturing spatial relationships of immune phenotypes in and around tumor is enabled by multiplexed immunofluorescence labelling and multispectral imaging, potentially forming the basis of assays to guide therapy and monitor response. Here we present a multi-marker, computer-aided method for analysing the distributions of CD3/FOXP3 (Treg) and CD3/CD69 (Tact) T cells in follicular lymphoma sections using a multispectral imaging (MSI) and automated analysis approach.

Methods: A single section of a tissue microarray containing triplex follicular lymphoma cores from 40 subjects was stained for CD3, FOXP3, CD69 and hematoxylin. Each core was imaged using MSI and the individual staining of each marker separated from each other using spectral unmixing. CD3+ TILs were located using automated image analysis. The FOXP3 and CD69 status of each CD3+ TIL was then determined and the spatial distributions of each were used as input into the spatial distribution analysis.

Results: Kaplan-Meier analysis demonstrated favourable outcome with higher numbers of CD3+, CD3+/FOXP3+ and CD3+/CD69+ cells. HID analysis demonstrated the association of favourable outcome with high entropy, representative of a diffuse spatial pattern, of FOXP3+ and CD69+ positive T cells.

Conclusions: In this study we report that higher Treg cell counts in a diffuse pattern was associated with favorable prognosis. This supports the importance of Tregs in the tumour microenvironment. It is pertinent to mention that contradictory findings are routinely reported from studies investigating the role of Tregs in solid and haematological malignancies. This is due to the complex interactions between pro-/ anti-tumour immune factors present in the tumour microenvironment. The resultant effects are due to the summation of the activities of these factors. It is therefore even more relevant that a method such as exhibited here, capable of defining and measuring the effect on patient outcome of the spatial patterns of multiple cellular phenotypes in the tumor microenvironment is available.

Disclosure: R. Lloyd and J.R. Mansfield: Employee of PerkinElmer. All other authors have declared no conflicts of interest.