A MULTI-MODAL ANALYSIS APPROACH LEVERAGING MULTIPLEXED SPATIAL PHENOTYPING AND MULTI-OMICS ANALYSIS TO BETTER UNDERSTAND THE PROGNOSTIC VALUE OF TERTIARY LYMPHOID STRUCTURES IN NSCLC

¹Julie Berthe*, ¹Sriram Sridhar, ¹Felix Segerer, ¹Marco Testori, ¹Megha Saraiya, ¹Lorenz Roggioni, ¹Harald Hessel, ¹Alma Andoni, ¹Anatoliy Shumilov, ¹Andreas Spitzmüller, ¹Mari Heininen-Brown, ¹Jorge Blando, ¹Felicia Ng, ¹Emma Jones, ¹Sophie Willis, ¹Michael Surace, ²Rieneke van de Ven, ²Tanja de Gruij, ¹Helen Angell. ¹AstraZeneca, Cambridge, UK; ²Cancer Center Amsterdam, Amsterdam, Netherlands

Background Tertiary Lymphoid Structures (TLS) are highly organized ectopic lymphoid structures found in inflamed or tumor tissues, acting as sites of lymphoid recruitment and immune activation. A high TLS density within the tumor is commonly associated with an increased prognostic effect of TILs and with an improved disease free survival and overall survival for patients.¹ However, the existence of conflicting studies suggest that multiple TLS features should be taken into account when assessing their prognostic value, such as their location, cellular composition, maturation stage and spatial organisation, as those may affect their functionalities.²

Methods With the aim of gaining insights into TLS biology and evaluating the prognostic role of TLS in Non-Small Cell Lung Carcinoma according to their multiple features, we developed a TLS multiplex immunofluorescent (mIF) panel that includes T cells (CD3, CD8), B cells (CD20), Follicular Dendritic cells (CD21, CD23) and mature dendritic cells (DC-LAMP) markers. We deployed this panel across a cohort of primary tumors from NSCLC patients (n=408) and established a mIF image analysis workflow to assess the status and spatial location of each cell within the tissue. A H&E staining of the same tissue section was performed to evaluate mIF spatial data in relation to the tumor context. Additional multi-omics assessments were conducted across the same cohort including; whole exome sequencing, NanoString transcriptomics, and immunohistochemistry (e.g. PD-L1, FOXP3, Nkp46, LKB1, CTLA4). We have leveraged clinical metadata, including demographics (e.g. age, sex, smoking status) and clinical risk factors (e.g. stage, grade, Standard of Care treatment) with clinical follow up (e.g. OS, PFS) for prevalence analysis, novel biomarker identification, and survival association.

Results Assessment of the prevalence of each cell phenotype within the tumor tissue and TLS, the cell-cell interactions, the distance between each cell type, and the distance of non-TLS immune cells to the closest TLS will be described, demonstrating the different types of lymphoid aggregates and TLS and their functional status. An integrative analysis combining spatial biology data with multi-omics and clinical data will be presented evaluating the prognostic value of TLS composition, maturation status and spatial organization, in correlation with additional biomarkers and clinical characteristics.

Conclusions This exploratory study using cutting-edge technologies enables us to better understand how TLS orchestrate an organised anti-tumour response, defining TLS spatial biomarker signatures, TLS gene signatures, and TLS features associated with patient outcomes to evaluate in the clinic.

REFERENCES

1. Marie-Caroline Dieu-Nosjean, Jérémy Goc, Nicolas A Giraldo, Catherine Sautès-Fridman, Wolf Herman Fridman. Tertiary lymphoid structures in cancer and beyond. *Trends Immunol* 2014;**35**(11):571–580.
2. Catherine Sautès-Fridman, Florent Petitprez, Julien Calderaro, Wolf Herman Fridman. Tertiary lymphoid structures in the era of cancer immunotherapy. *Nat Rev Cancer* 2019;**19**(6):307–325.

Ethics Approval The study was approved by AstraZeneca.

<http://dx.doi.org/10.1136/jitc-2021-SITC2021.039>