579 **NOVEL DIGITAL IMAGE APPROACH OF MULTIPLEX IMMUNOFLUORESCENCE BASED PD-L1 EXPRESSION ENABLES THE STRATIFICATION OF ADVANCED NSCLC PATIENTS TREATED WITH DURVALUMAB**

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Background Pathologist-based scoring of PD-L1 expression on tumor cells using IHC[1] has shown clinical utility in predicting favorable overall survival in advanced non-small cell lung cancer (NSCLC) patients treated with anti-PD-(L)1 therapies including durvalumab.[2-3]Quantitative Continuous Scoring (QCS)[4] enables the continuous measurement of the PD-L1 expression on single cells and the selection of the PD-L1 expression cutoff that best stratifies anti-PD-L1-treated patients with respect to prevalence and log-rank test p-value.[5] We present here the extension of QCS to PD-L1 measured by multiplex immunofluorescence (mIF)[6] to evaluate its ability to optimize patient stratification.

Methods Pre-treatment tumor samples from advanced NSCLC patients enrolled in durvalumab nonrandomized phase 1/2 trial (CP1108/NCT01693562) [2], were stained by mIF panel containing PD-L1. [6] Similarly to IHC PD-L1 QCS, mIF PD-L1 QCS consists of two deep-learning models, first to segment epithelium regions and second to detect membrane, cytoplasm and nuclei of each epithelium cell, transferring for the second model annotations from IHC to mIF domain. [7] The mIF images are normalized based on batch statistics prior to image analysis. PD-L1 expression is measured for each epithelium cell as the average of the PD-L1 signal in the segmented membrane. Cells with expression higher than an expression threshold (T_{PDL1}) are considered positive. A slide is considered QCS-positive if it comprises a greater percentage of PD-L1 positive cells (QCS-score) than a cutoff value (CoV).

Results The QCS-scores are computed on 119 NSCLC patients treated with durvalumab. As a first proof of concept that QCS-scoring can replicate tumor proportion scoring (TPS), we optimize T_{PDL1} as to maximize the correlation between QCS and TPS scores (figure 1). Second, we estimate for different combinations of (T_{PDL1}, CoV) the log rank p-value associated with the stratification between patients with low and high QCS scores. A subregion of the parameter space was identified for which the stratification is significant ($p<0.01$) with more than 50% prevalence in the positive subgroup (figure 2). The p-value is minimized ($p=7.2\ 10^{-5}$) for ($T_{PD\text{-}L1}=37$, $CoV=0.75\%$), yielding a median OS of 5.58 months and 13.44 months in the QCS negative and positive subgroups respectively, similar to those of IHC PD-L1 manual scoring with 25% cutoff.

Conclusions The extension of QCS to mIF imaging provides opportunities to evaluate continuous PD-L1 expression of single tumor cells in relation to spatial distribution of other cells (e.g. PD1+ CD8+ T cells) and identify predictive biomarkers of tumor-immune cell interactions of anti-PD-(L)1 therapies.

Trial Registration CP1108/NCT01693562

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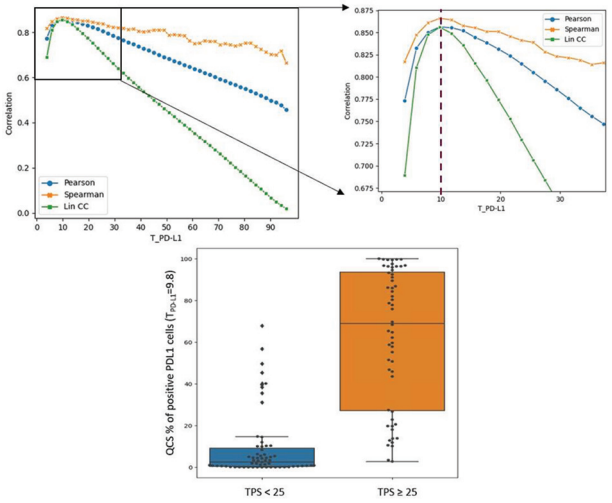
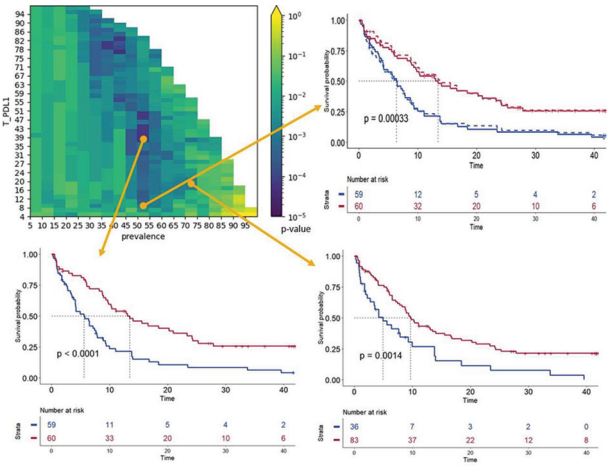


Abstract 579 Figure 1 Correlation to pathologist-based TPS score
Top: Lineplots of Pearson and Spearman correlations as well as of Lin correlation coefficient between the pathologist-based TPS scores and the QCS-based scores, computed for increasing expression threshold values (TPD-L1). The QCS shows maximum Pearson (0.856), Spearman (0.866) and Lin (0.856) correlations to the manual TPS score for TPD-L1 = 10. Bottom: QCS scores within the negative and positive patient subgroups as per pathologist assessment of IHC PD-L1 TPS.



Abstract 579 Figure 2 OS patient stratification
Log rank p-values for OS stratification obtained by spanning the parameter space associated to the QCS, the higher CoV the lower the prevalence of the positive patient subgroup. Top right: Kaplan Meier (KM) curves obtained with manual IHC PD-L1 TPS score at 25% cutoff

(dashed line) and with median split for the QCS expression cut-off (TPD-L1=10) maximizing the correlation to TPS (full line). Bottom: KM curves of the QCS-based stratification as to minimize the p-value (TPD-L1=37) for a minimum prevalence of 50% (left) and as to maximize the prevalence (TPD-L1=18) (right).

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