



# P2.077-248: Spatial immune profiling and integrated pathologic risk stratification in resected lung squamous cell carcinoma

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## INTRODUCTION

Postoperative risk stratification in resected lung squamous cell carcinoma (LSCC) still relies on pathologic stage, whereas histopathologic prognostic refinement remains limited. PD-L1 is clinically relevant, but its biological and prognostic significance may depend on the broader immune and morphologic context of the tumor microenvironment (TME). This study investigated whether compartment-specific immune profiling could integrate these features and improve prognostic stratification in this setting.

## METHODS

Forty-seven primary resected LSCCs were retrospectively analyzed. Brightfield multiplex immunohistochemistry combined with custom AI-based deep-learning whole-slide image analysis was used to quantify CD4+, CD8+, CD20+, and CD68+ immune cells in the tumor core and invasive tumor front. An Immunoscore-like model was built from CD4+, CD8+, and CD20+ compartment-based densities, generating five score classes grouped into cold, excluded, and hot immune phenotypes. Associations with clinicopathologic variables, PD-L1 expression, and worst pattern of invasion (WPOI) were assessed using MANOVA, Chi-squared, and Kruskal-Wallis tests. Relapse-free survival (RFS) and overall survival (OS) were analyzed using Kaplan-Meier estimates and log-rank tests.

## RESULTS

The cohort comprised 25 stage I, 13 stage II, and 9 stage III tumors; relapse occurred in 15/47 cases during follow-up. Immune phenotypes were distributed as cold in 21.3%, excluded in 53.2%, and hot in 25.5% of cases. PD-L1 expression showed a significant multivariate association with immune-cell composition across the TME ( $p=0.010$ ) and at the invasive tumor front ( $p=0.009$ ), suggesting that PD-L1 status is associated with distinct patterns of spatial immune organization. The combined PD-L1/WPOI variable was significantly associated with immune composition at the invasive tumor front ( $p=0.022$ ), supporting an interaction between TME and WPOI. RFS differed significantly according to AJCC stage ( $p=0.011$ ), AJCC stage/Immunoscore-like grouping ( $p<0.001$ ), and PD-L1/WPOI categories ( $p=0.007$ ). Among stage  $\geq$ II tumors, the Immunoscore-like model identified subgroups with distinct relapse patterns, while PD-L1-positive tumors with high WPOI defined the subgroup at highest risk of recurrence. No significant differences were observed for OS.

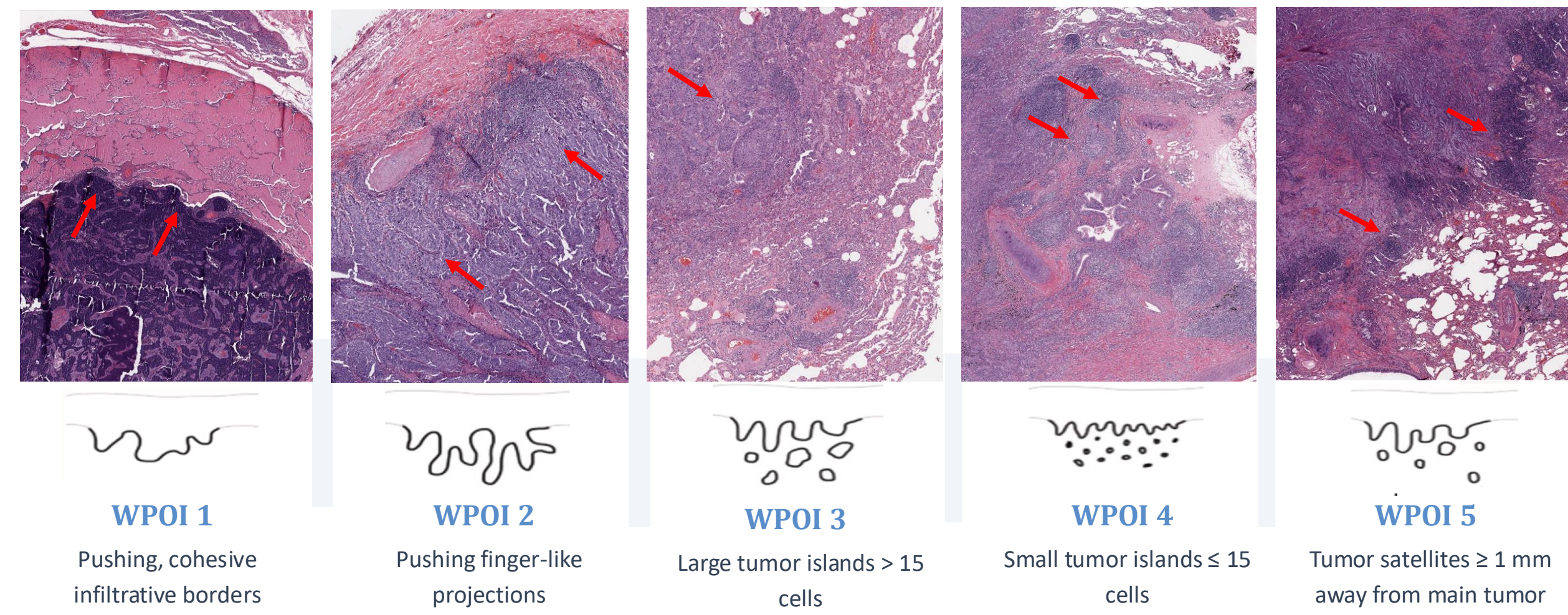
## CONCLUSIONS

In resected LSCC, spatial immune profiling provides prognostically relevant information beyond standard staging. PD-L1 appears to gain greater interpretive value when assessed within the spatial immune and morphologic context of the TME. Integrating AJCC stage with an Immunoscore-like model, and PD-L1 with WPOI, may refine postoperative risk stratification and identify biologically distinct LSCC subsets with distinct relapse risk.

## FIGURES AND GRAPHS

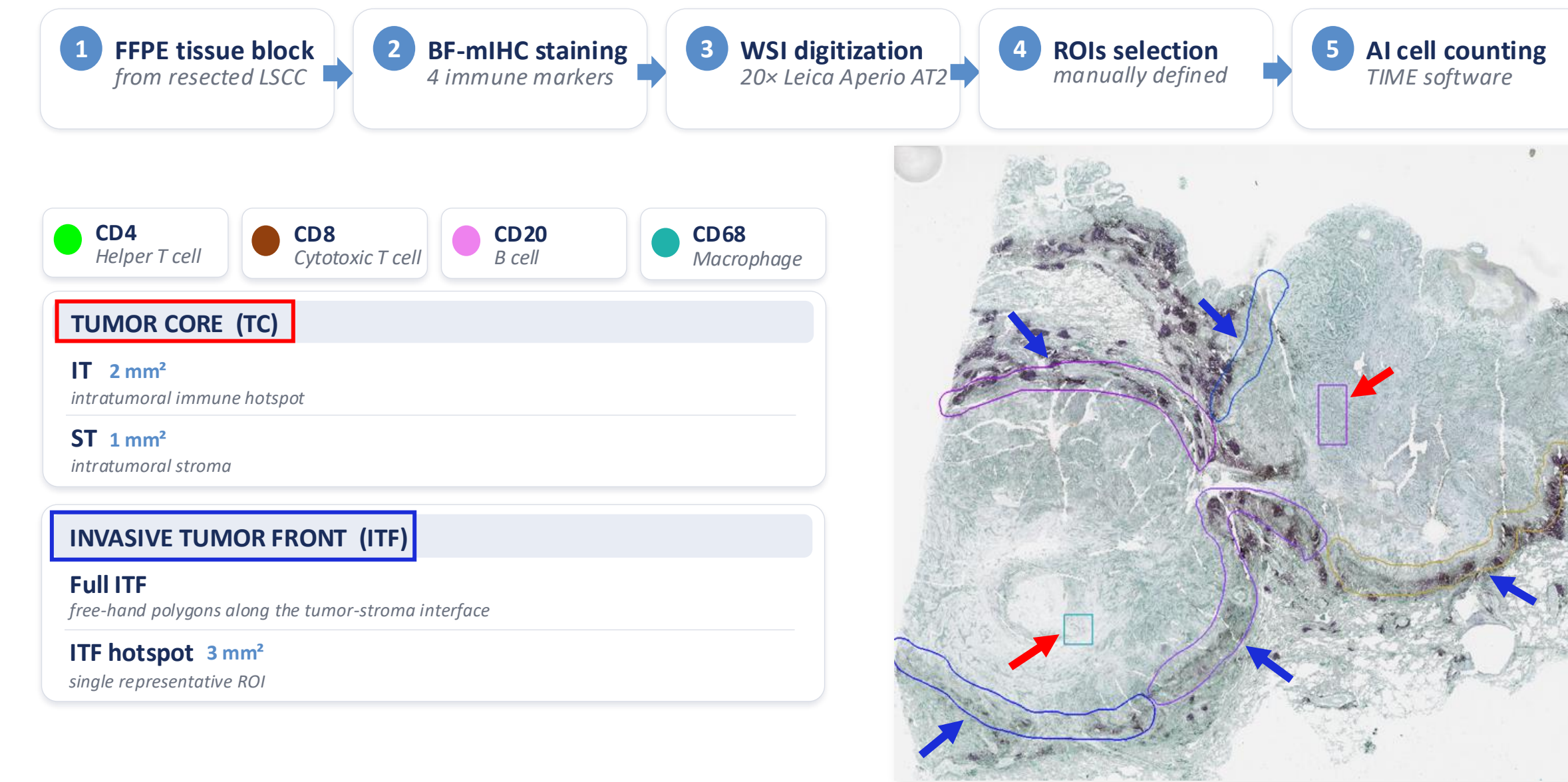
### Worst Pattern of Invasion (WPOI)

Originally described in oral squamous cell carcinoma by Brandwein-Gensler et al.



### BF-mIHC & AI-based whole-slide image analysis

Four immune markers and the manual definition of regions of interest

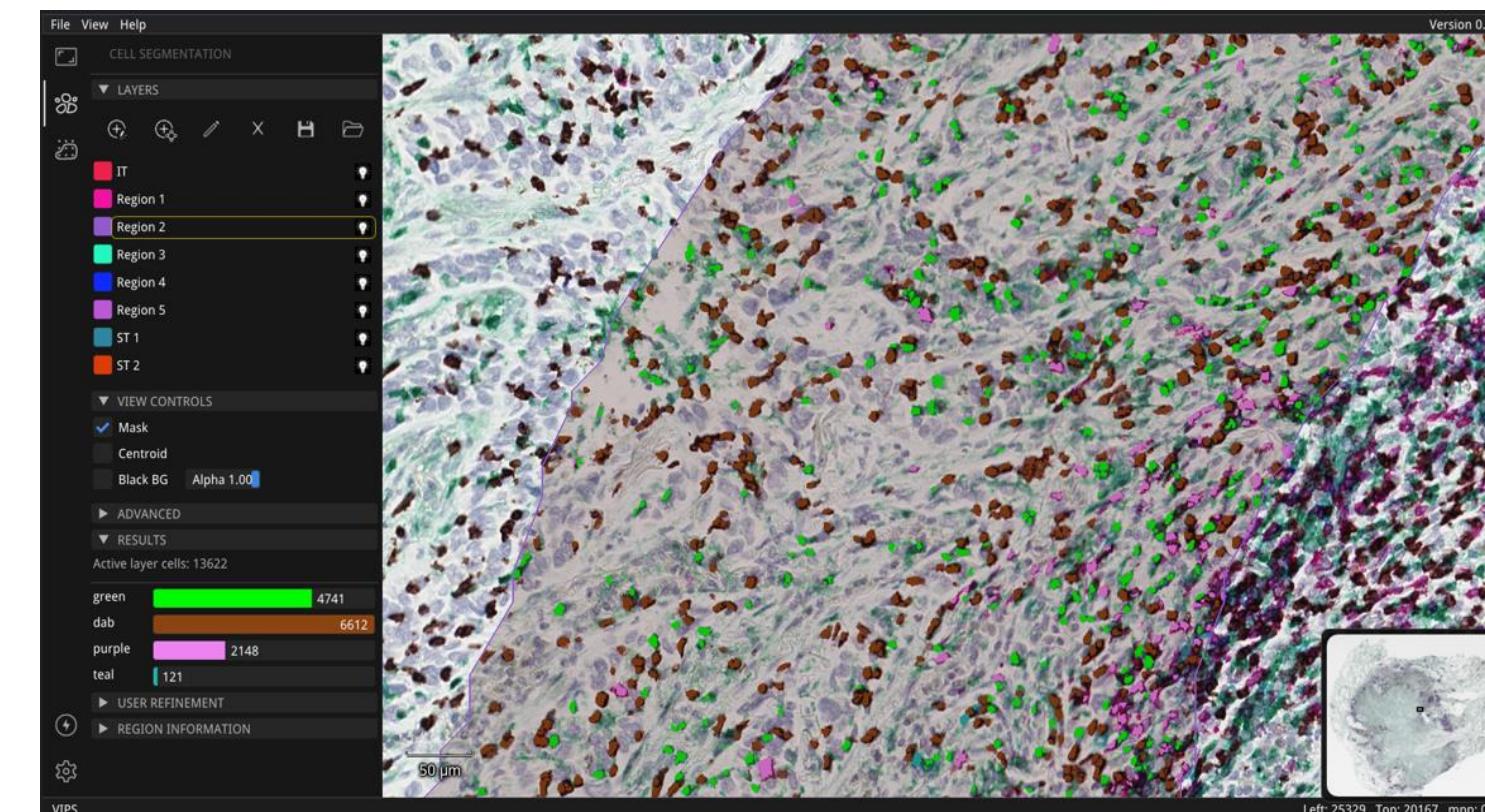


### BF-mIHC & AI-based whole-slide image analysis

AI-based automated cell counting with the TIME software



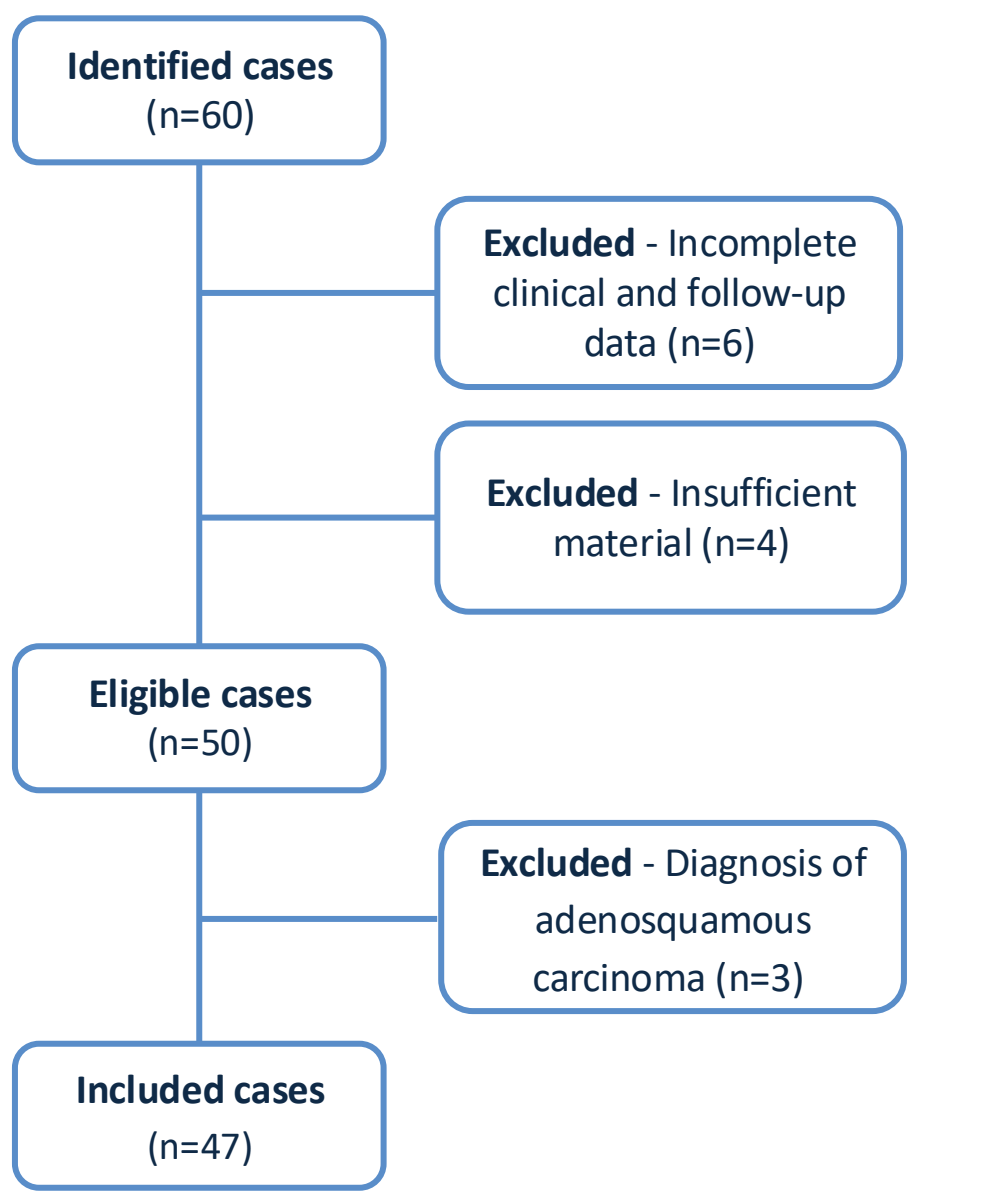
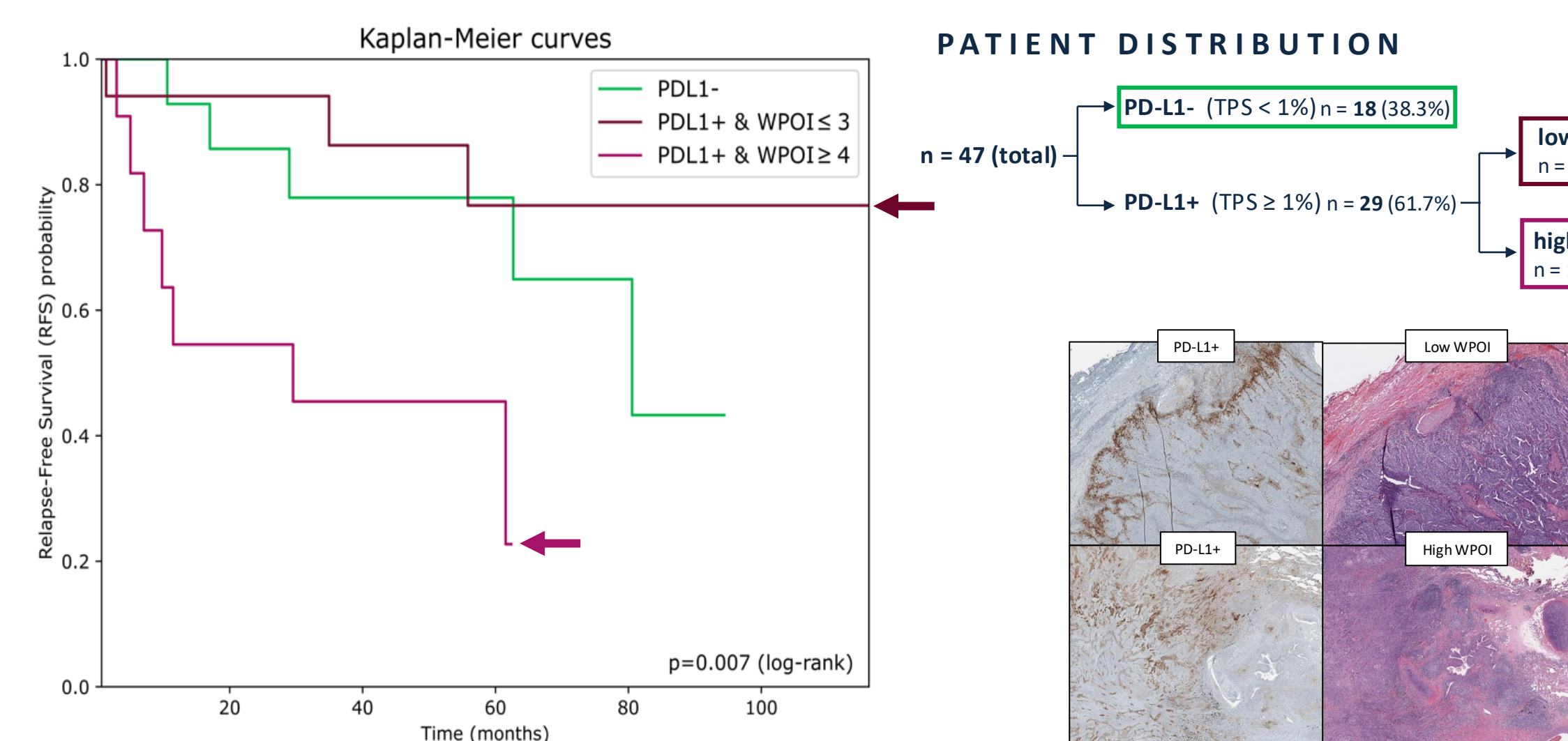
### TIME SOFTWARE INTERFACE



### KEY FEATURES

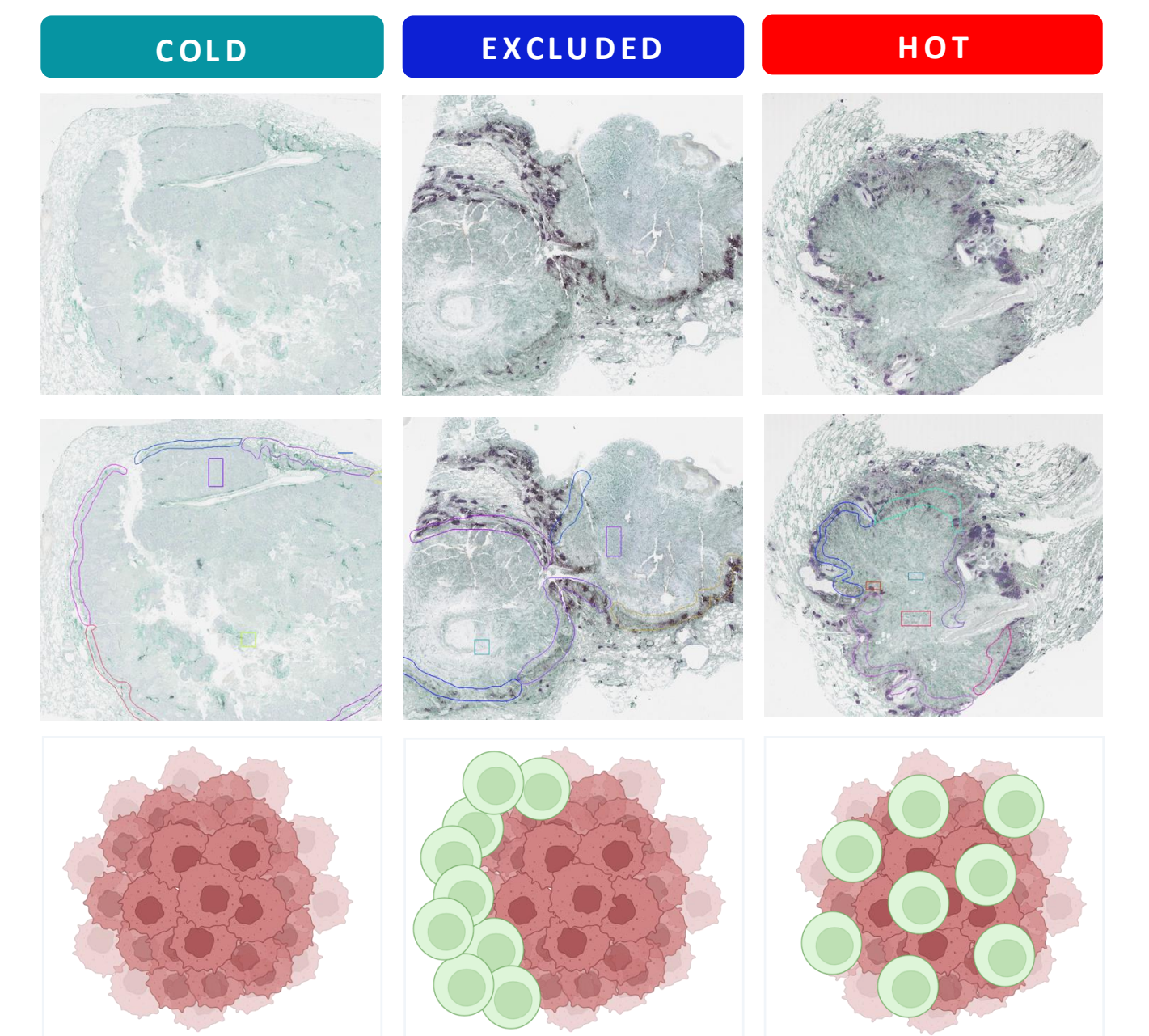
- Deep-learning segmentation**  
Deep learning multi-class model that detects and counts CD4<sup>+</sup>, CD8<sup>+</sup>, CD20<sup>+</sup>, CD68<sup>+</sup> cells
- Pre-trained & validated**  
On NSCLC and SCC of the penis cohorts
- Compartment-specific output**  
Cell counts per ROI enabling spatial profiling across ITF and TC
- Reproducibility**  
operator-independent, time-efficient, standardised across cases

### Prognostic role of PD-L1/WPOI



### Percentile IS class Phenotype

| Parameter  | ROI         | Markers                                                 | 0-10<br>IS 0<br>COLD | 10-25<br>IS 1<br>COLD | 25-70<br>IS 2<br>EXCLUDED | 70-95<br>IS 3<br>HOT | 95-100<br>IS 4<br>HOT |
|------------|-------------|---------------------------------------------------------|----------------------|-----------------------|---------------------------|----------------------|-----------------------|
| TILs / ITF | ITF hotspot | CD4 <sup>+</sup> + CD8 <sup>+</sup> + CD20 <sup>+</sup> |                      |                       |                           |                      |                       |
| TILs / TC  | IT + ST     | CD4 <sup>+</sup> + CD8 <sup>+</sup> + CD20 <sup>+</sup> |                      |                       |                           |                      |                       |
| CD8 / ITF  | ITF hotspot | CD8 <sup>+</sup>                                        |                      |                       |                           |                      |                       |
| CD8 / TC   | IT + ST     | CD8 <sup>+</sup>                                        |                      |                       |                           |                      |                       |



### Prognostic role of the Immunoscore-like model

