

**– Online Supplement –**

**Immune cell composition and functional marker dynamics from multiplexed immunohistochemistry to predict response to neoadjuvant chemotherapy in the WSG-ADAPT-TN trial**

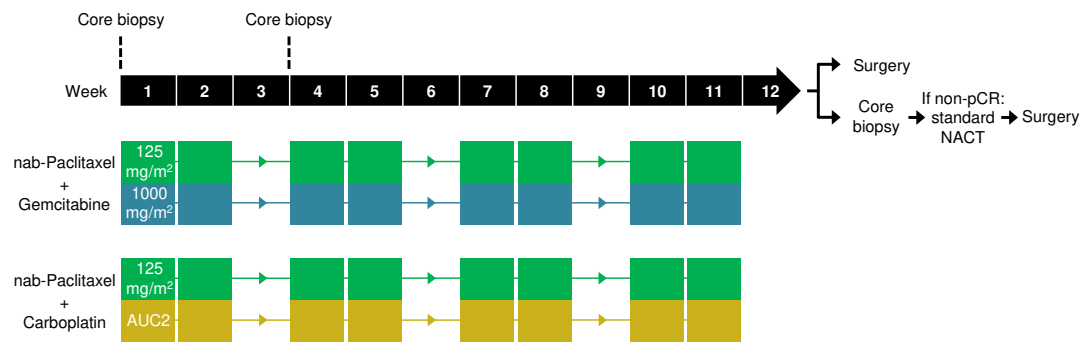
**Supplementary Tables**

Supplemental Table 1: Association between pCR and cellular markers at baseline

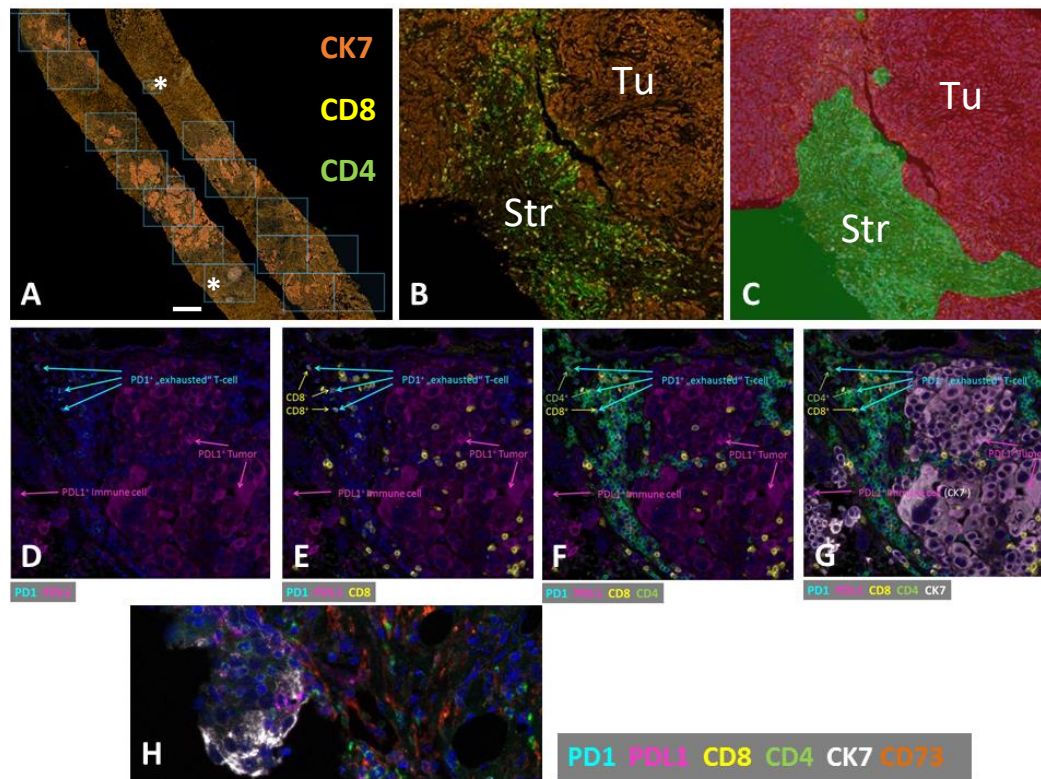
Marker	OR* (95% CI)
<b>Stroma</b>	
CD4	0.60 (0.26-1.38)
CD8	1.14 (0.80-1.61)
CD4/CD8	0.47 (0.20-1.10)
T cell	0.79 (0.37-1.70)
CD73	1.27 (0.67-2.39)
PD1 in CD4	0.93 (0.52-1.68)
PD1 in CD8	0.78 (0.37-1.60)
PDL1	1.24 (0.71-2.15)
<b>Tumor</b>	
CD4	0.73 (0.46-1.16)
CD8	0.95 (0.70-1.27)
CD4/CD8	1.09 (0.80-1.49)
T cell	0.69 (0.34-1.38)
CD73	0.90 (0.47-1.72)
PD1 in CD4	1.01 (0.58-1.74)
PD1 in CD8	0.58 (0.23-1.45)
PDL1	1.02 (0.70-1.49)

\*OR per interquartile range

## Supplementary Figures



Supplemental Figure 1: Design of the WSG ADAPT umbrella trial and the treatment patterns within the ADAPT TN substudy. pCR, pathological complete response; Standard NACT, neoadjuvant therapy according to national guidelines.



**Supplementary Figure 2:** Multiplexed immunohistochemistry (IHC) and multispectral imaging (MSI): **(A)** After sequential IHC staining and labeling with the OPAL IHC detection reagent (Akoya Biosystems), the full biopsy area was scanned (Vectra Polaris, Akoya Biosystems). The resulting overview image that allows selecting the relevant regions of interest (ROIs), based on a rough separation of light emission in the red/orange (CK7), yellow (CD8) and green (CD4) spectrum by band pass filters but not sufficient for full separation of the six markers included in the multiplex panel. Therefore, ROIs of different sizes (small = 466 x 349  $\mu\text{m}$ , medium 931x 698  $\mu\text{m}$ , or large 1396 x 1047  $\mu\text{m}$ ; shown here are small and large fields) were selected for MSI analysis. In the current study, the full available tumor area including adjacent stroma, was included, except ROIs containing brisk stromal perivascular infiltrates (asterisks) or obvious technical artefacts such as folds or scratches. **(B)** Representative ROI selected for further analysis. **(C)** Result of the tumor-stroma classification using the INFORM software (Akoya Biosystems). After MSI, pseudocolors were assigned to the different markers. **(D, E, F, G)** show incremental views of the same ROI adding single markers of the multiplex IHC panel, and **(H)** a full panel depicting stromal CD73 expression.