Detection of the Proximity of PD1 and PD-L1 in Human FFPE Tissues using a Newly Developed Chromogenic in situ Proximity Ligation Assay

Navinci

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Background

Blocking the interaction between the negative immune checkpoint proteins PD-L1/PD-1 in PD-L1-positive tumors by anti-PD-L1 antibodies is a major and challenging therapeutic avenue of cancer immunotherapy. Today, anti-PD1/PD-L1, is the most widely applied and prescribed anticancer therapy in about 50 identifiable solid cancer types. PD1 and PD-L1 are used either as single reagents or as adjuvant therapy in combination with other treatments. Despite the recent success of immune checkpoint inhibitors, many patients do not benefit from these therapies, and predictive biomarkers improving patient stratification are needed[1].

PD-L1 IHC is commonly used as a biomarker, but the correlation between PD-L1 expression levels and PD1/PD-L1 interaction is not always linear[2]. The interaction between PD-L1 and PD-1 has yet to be visualized in situ. Molecular assays compatible with FFPE-tissues to accomplish this would significantly aid in the selection of patient responders to anti-PD-L1 or anti-PD-1 therapy and the deselection of non-responders.

Navinci Diagnostics has established the first and next generation of *in situ* proximity ligation assay as a pioneering technology to image proteins and protein complexes via pairwise antibody binding and localized signal amplification in situ[3]. The technology improved detection specificity over conventional immunohistochemistry, and it afforded a unique ability to reveal interactions and modifications of proteins in cells and tissues (figure 1).

Recently, Navinci has developed the first commercial Proximity Ligation Assay for specific detection of PD1/PD-L1 interactions *in situ.* The kit includes two Navenibodies conjugated to proprietary oligo arms (depicted as orange antibodies in the illustration below). If the Navenibodies are in proximity, they generate a rolling circle amplification reaction, leading to a strong and distinct dot (figure 2).

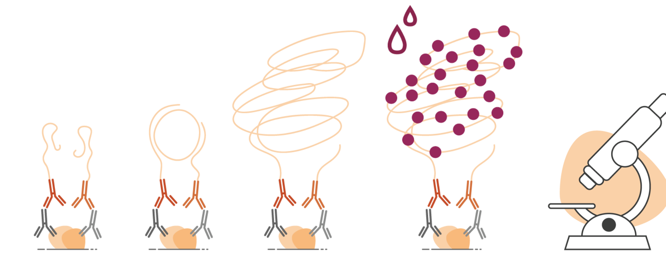

Figure 1. Schematic overview of the NaveniBright™ proximity ligation assay.

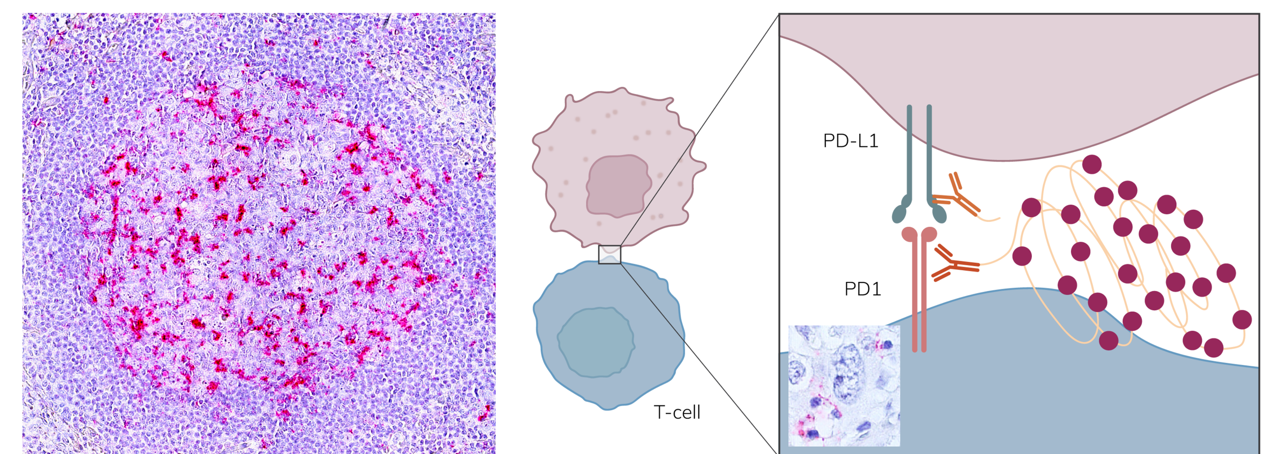

Figure 2. Model of proximity ligation assay for PD-L1 interaction with PD1

Results

The PD-L1 antibody was paired with the PD1 antibody, and a proximity ligation assay was optimized on tonsil tissues and lymph nodes. In both tissues distinct isPLA signals were detected, indicating PD-1 and PD-L1 proximity. Specific membranous staining was seen in germinal centers of the hodgkin's lymphoma (figure 2). All controls were negative. We applied the PD1-PD-L1 assay to detect the interaction in several types of tumour samples. High-intensity contact signal was observed in NCSCL, melanoma metastasis, and colon cancer (figure 3). In NCLC we noted two primary signal clustering, one for tumour clusters (intertumoral lymphocytes) and one for stroma (lymphocytes located in intertumoral stromal regions and, most likely, not interacting directly with cancer cells). On some occasions, the distribution in the stroma was very uneven, with only one spot of highly positive lymphocytes. In this case, we added the third score for 'stroma hot spots' (figure 4).

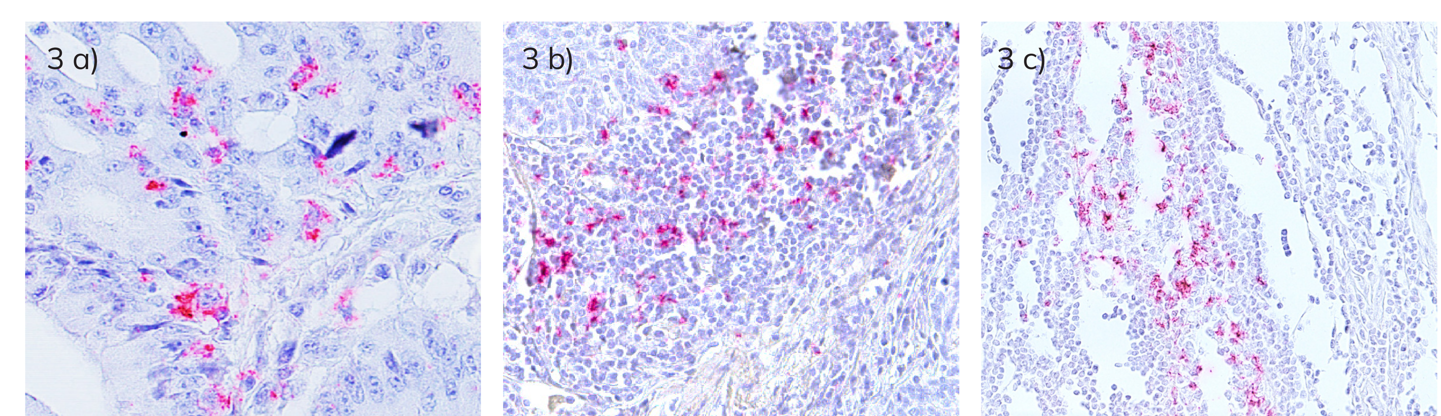

Figure 3. Exemplary images of the interaction between PD1 and PD-L1 in colon cancer and NSCLC.
a) Colon adenocarcinoma, grade III, b) Squamous cell carcinoma, IB, c) Lymph node malignant melanoma.

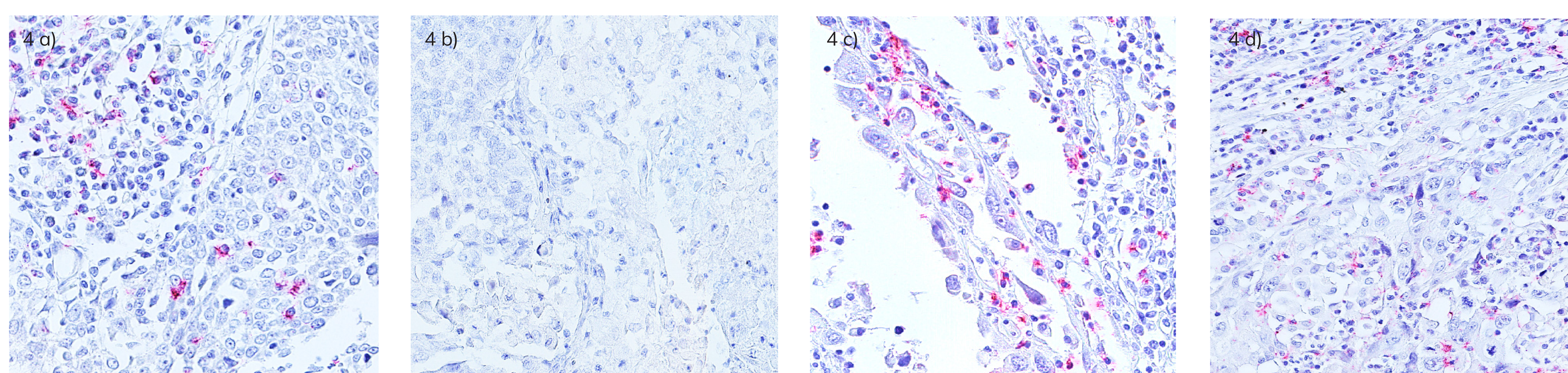

Figure 4. Interaction between PD1 and PD-L1 in various types of lung cancer and metastatic lymph node.
a) Lung, squamous cell carcinoma, IB, malignant,
b) Lung, squamous cell carcinoma IB, malignant,
c) Lung, Papillary carcinoma IB, malignant,
d) Lymph, metastatic carcinoma.

Materials and methods

A specific *in situ* proximity ligation assay against an interaction between PD1 and PD-L1 was developed based on the **NaveniBright™** kit. The interaction between PD1 and PD-L1 was visualized in FFPE tissues acquired from Acovos and Biomax. In short, the tissue was incubated with a pair of monoclonal antibodies (anti PD1 anti PD-L1), followed by ligation of oligonucleotides and subsequent rolling circle amplification. The RCA product was then detected using chromogens and the proximity ligation assay signal assessed by brightfield microscopy. The signal was annotated in collaboration with Histo.one (Sweden).

Conclusions

Navinci Diagnostics designed a new bright field technology for ultra-sensitive detection of interactions and established an immuno-oncology test to analyze PD1-PDL1 in human tissue samples. This assay has a potential to advance the studies of targets for immuno-modulation therapies and drug development.

References

[1] Robert, C. A decade of immune-checkpoint inhibitors in cancer therapy. Nat Commun 11, 3801 (2020).
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[3] Klaesson A, et al., Improved efficiency of in situ protein analysis by proximity ligation using UnFold probes. Sci Rep. 8(1):5400 (2018).

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