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Precision Diagnostics for Immunotherapy: Detecting PD1-PDL1 Interactions in the *In Situ* Microenvironment of Cancer Tissues.

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Background

Immune checkpoint inhibition (ICI) therapies can provide a durable anti-tumor effect in non-small cell lung cancer (NSCLC). But only 20 % of NSCLC patients respond to ICI. Therefore, better predictive biomarkers are highly warranted for optimal patient selection.

Aims

- Validation of an in situ assay detecting the interaction between PD1 and PDL1 in diagnostic patient samples.
- Test the ICI response predictive capacity of PD1-PDL1 interactions.
- Compare the performance of the PD1-PDL1 interaction to the current clinical standard of PDL1 tumor proportion score (TPS).

Methods

1) 2nd generation proximity ligation assay* (PLA) for PD1-PDL1 interaction **Navinci Diagnostics AB, Uppsala*
2) Primary (anti-PD1 & anti-PDL1) and secondary probe-conjugated antibodies are added.
3) Digestion and ligation of unfold probes.
4) Rolling circle amplification and AP-conjugated detection oligo incubation.
5) Chromogenic signal development and counterstain.
6) Quantification of the percentage of positive cells using an automated QuPath pipeline.

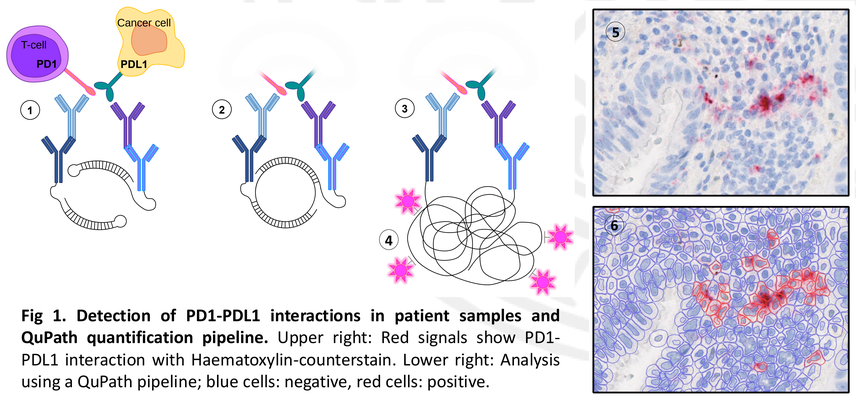


Fig 1. Detection of PD1-PDL1 interactions in patient samples and QuPath quantification pipeline. Upper right: Red signals show PD1-PDL1 interaction with Haematoxylin-counterstain. Lower right: Analysis using a QuPath pipeline; blue cells: negative, red cells: positive.

Results

Only a subset of tissues with PD1 and PDL1 co-expression show PD1-PDL1 interactions.

Analysis of 296 early-stage NSCLC patients in a TMA revealed that although PD1 and PDL1 are required for PD1-PDL1 interactions, only 54.4% of patients positive for both showed these interactions, indicating a clinically relevant patient subgroup.

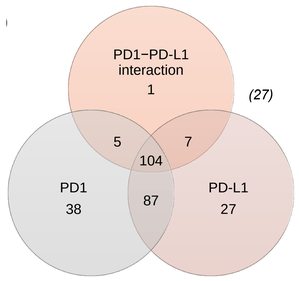
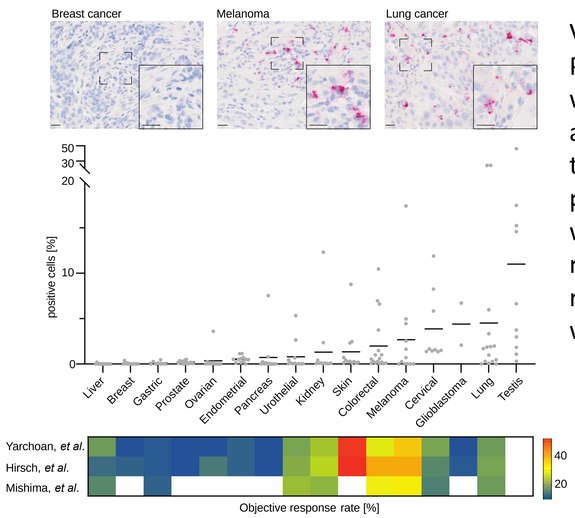


Fig 2. Venn diagram illustrating PD1 and PDL1 single expressions (IHC) and their interaction (PLA).

PD1-PDL1 interaction varies among cancer types and is positively associated with reported response rates.



Varying levels of PD1-PDL1 interaction were observed among solid cancer types. A general positive association with literature–reported objective response rates to ICI was noted.

Fig 3. Percentage of cells positive for PD1-PDL1 interactions across different solid tumor types. Pan-cancer TMAs were analyzed for PD1-PDL1 interaction via PLA. Bars represent mean values. Objective ICI response rates are based on previous reports. White boxes: NA.

PD1-PDL1 interactions predict longer survival post ICI in NSCLC.

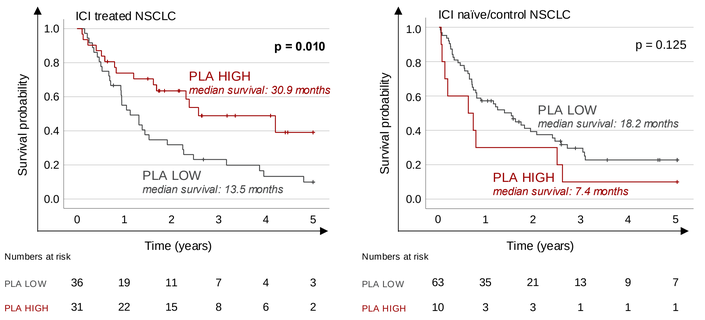


Fig 4. Kaplan Meier analysis of ICI-treated or ICI-naïve advanced NSCLC patients stratified according to low and high PD1-PD-L1 interaction scores (based on best cut-off strategy).

In a multivariable Cox regression model including histology, stage, age, sex, and smoking history, PD1-PDL1 interaction was the only variable associated with survival.

high vs low $HR_{adjusted}$: 0.45 (95%CI 0.23-0.89), p = 0.022

A formal interaction test between PD1-PDL1 interaction and ICI treatment was significant (p=0.006).

PD1-PDL1 interaction surpasses the PDL1 TPS.

The clinical standard of PDL1 TPS assessed by IHC was not associated with improved survival in ICI treated NSCLC patients.

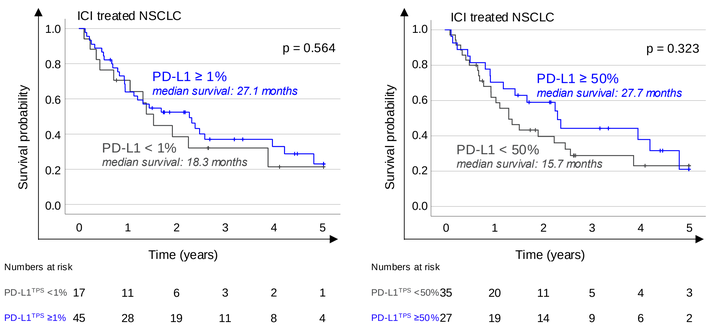
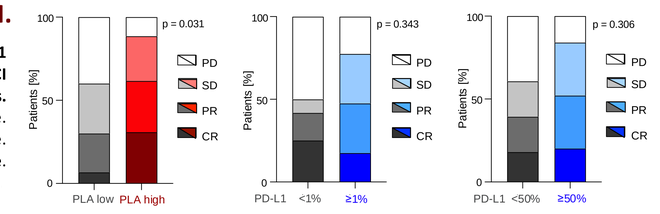


Fig 5. Kaplan Meier analysis of ICI-treated patients stratified by PDL1 TPS (with clinical cut-offs).

High levels of PD1-PDL1 interactions are associated with complete responses to ICI.

Fig 6. PD1-PDL1 interaction and ICI response status. CR=complete response. PR=partial response. SD=stable disease. PD=progressive disease.



Patients with high levels of PD1-PDL1, but resistant to ICI, show expression of other immune regulatory proteins.

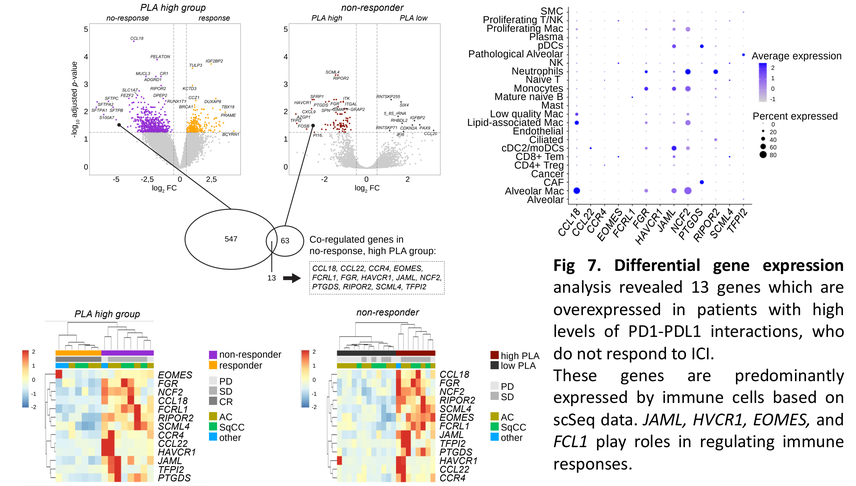


Fig 7. Differential gene expression analysis revealed 13 genes which are overexpressed in patients with high levels of PD1-PDL1 interactions, who do not respond to ICI.
These genes are predominantly expressed by immune cells based on scSeq data. *JAML, HVCR1, EOMES,* and *FCL1* play roles in regulating immune responses.

Conclusions

PD1-PDL1 interaction is a predictive biomarker for favourable outcome and complete response in ICI-treated NSCLC patients.

PD1-PDL1 interaction surpasses the current clinical standard of PDL1 TPS.

NSCLC patients, with high PD1-PDL1 interaction, but resistent to ICI express other immune regulators.

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