#5139

PD1-PDL1 interaction as a superior predictor for response to immune checkpoint therapy in NSCLC patients.

Patrick Micke[1*], Amanda Lindberg[1*], Rebecca Artursson[1], Louise Hellberg[1], Hui Yu[1], Max Backman[1], Johan Botling[1], Hans Brunnström[2], Artur Mezheyeuski[3], Johan Isaksson[1], **Carina Strell**[1,4]

[1]Department of Immunology, Genetics and Pathology, Uppsala University, Sweden. [2]Division of Pathology, Lund University, Sweden. [3]Molecular Oncology Group, Vall d'Hebron Institute of Oncology, Barccelona, Spain. [4]CCBIO, Department of Clinical Medicine, Bergen University, Norway. *Equal contribution

#5139

Background

Immune checkpoint inhibition therapies (ICT) 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

- Development of an in situ assay detecting the interaction between PD1 and PDL1 in diagnostic patient samples.
- Test the ICI response predictive capacity of the PD1-PDL1 interaction.
- 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 hematoxylin counterstain.
6) Quantification of the percentage of positive cells using an automated QuPath pipeline.

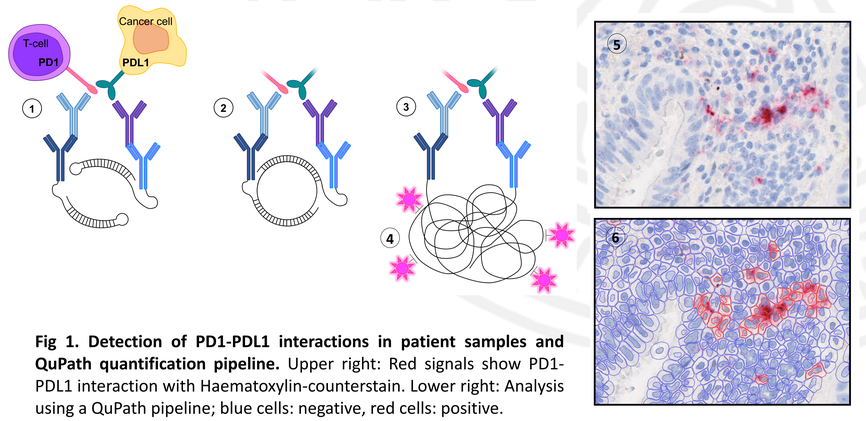


Fig 1. Detection of PD1-PDL1 interactions in patient samples and QuPath quantification pipeline. Upper right: Red signals show PD1-PDL1 interaction with Haematoxylin-counterstain. Lower right: Analysis using a QuPath pipeline; blue cells: negative, red cells: positive.

Results

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

Analysis of 305 early-stage NSCLC patients in a TMA revealed that although PD1 and PDL1 are required for PD1-PDL1 interactions, only 54% of patients positive for both showed these interactions, indicating a clinically relevant patient subgroup.

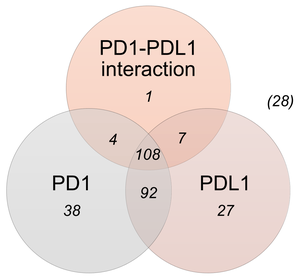
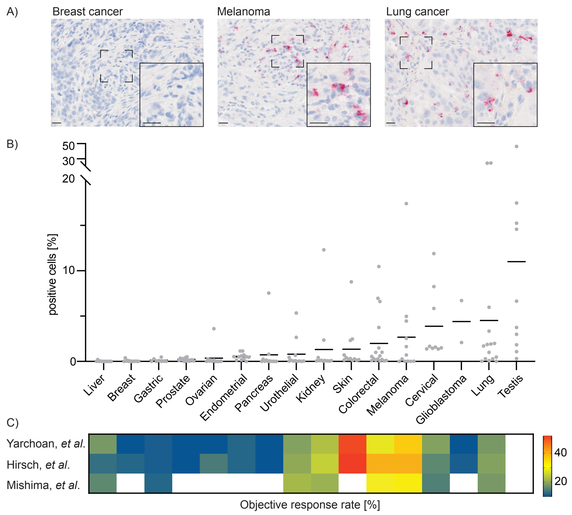


Fig 2. Venn diagram illustrating PD1 and PDL1 single expressions (IHC) and their interaction (PLA).

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 ICT 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 ICT response rates are based on previous reports. White boxes: NA.

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

High PD1-PDL1 interaction is associated with improved survival post-ICT in NSCLC patients, unlike in matched control patients.

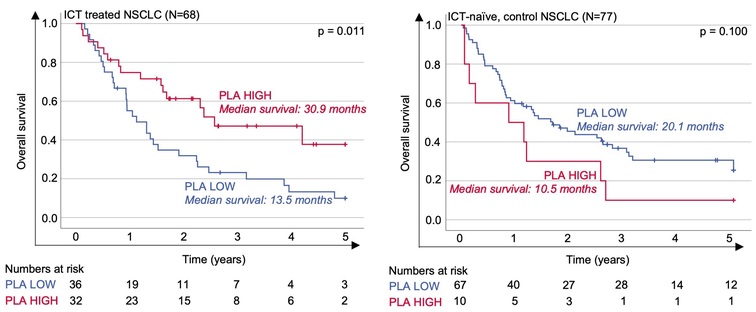
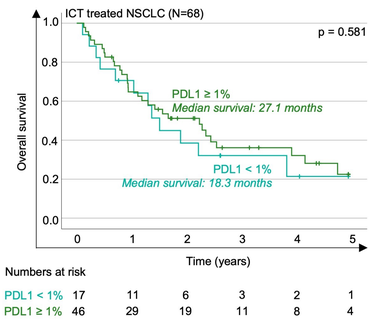


Fig 4. Kaplan Meier analysis of ICI-treated or ICT-naïve advanced NSCLC patients stratified according to low and high PD1-PD-L1 interaction scores (based on best cut-off strategy).

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 $HR_{adjusted}$: 0.46 (95%CI 0.24-0.90), p = 0.023

A formal interaction test between PD1-PDL1 interaction and ICT treatment was significant (p=0.018).



PD1-PDL1 interaction surpasses the PDL1 TPS.

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

Fig 5. Kaplan Meier analysis) of ICT treated patients stratified by PDL1 TPS (with clinical 1% cut-off).

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

χ^2 test showed that a higher PLA scores are associated with complete response to ICT (p = 0.028).

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

Conclusions & Future perspectives

The 2nd generation PLA is suitable tool to study PD1-PDL1 interactions in diagnostic tissue sections.

PD1-PDL1 interactions are associated with a favourable outcome and complete response in ICT-treated NSCLC patients.

PD1-PDL1 interaction is a predictive biomarker for ICT response in NSCLC, and surpasses the current clinical standard of PDL1 immunohistochemistry

Validation studies with regard to cut-off optimization are warranted.

Fig 7. Multiplex staining image including PD1-PD-L1 PLA probe.

Multiplex staining assays are developed to correlate the PD1-PDL1 interaction to other signaling pathways to explain resistance mechanisms, e.g., TGFβ- and STAT3-signaling.

Correspondence:
Carina.strell@igp.uu.se