Quantitative assessment of PD-L1 protein expression on macrophages and tumor cells as predictive markers of response to neoadjuvant durvalumab and chemotherapy in triple negative breast cancer (TNBC)

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INTRODUCTION & OBJECTIVES

In the IMpassion 130 trial, TNBC patients treated with Atezolizumab and nab-Paclitaxel that showed immune cell expression of PD-L1 showed better overall survival. The goal of this analysis was to assess association between quantitative PD-L1 expression, in various tissue compartments, and pathologic complete response (pCR: ypT0/N0) to neoadjuvant anti-PD-L1 therapy concurrent with nab-paclitaxel (100 mg/m2) x 12 followed by ddAC x 4 chemotherapy in stage I-III triple negative breast cancer (TNBC).

METHODS

Pre-treatment core needle biopsies (n=69) were obtained from patients who participated in a Phase I/II clinical trial (NCT02489448). Of these 24 patients samples were excluded due to a range of clinical and technical issues. The final analysis had 45 patients (pCR = 18, non-pCR = 27). The slides were stained using a previously validated Ultivue DNA-based Ultimapper® kit (CD8, CD68, PD-L1, Cytokeratin/Sox10 and Hoechst counterstain for nuclear staining).

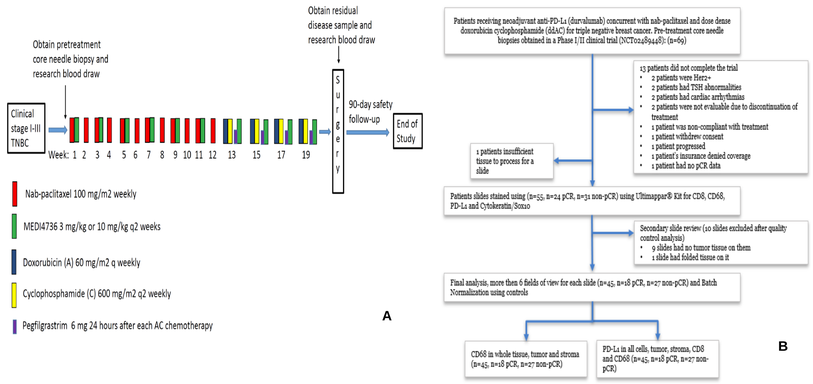
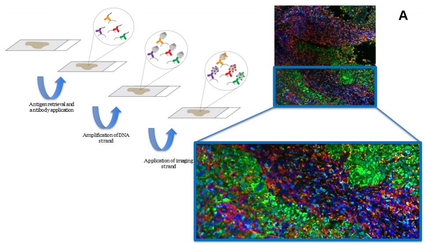


Figure 1: A. Clinical trial design and **B.** Consort diagram for the clinical study



Antibodies	PD-L1	CD8	CD68	Pan CK / SOX10	DAPI
Clone	73-10	C8/144B	KP-1	(AE1/AE2) / (BC34)	Hoechst
Isotypes / Species	Mouse				NA
Dilution	1:100				NA
Antigen retrieval	EDTA pH9				NA
Channel	Cy5	FITC	TRITC/Cy3	Cy7	DAPI

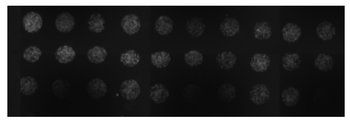


Figure 2: A. Illustration of the mechanism of the Ultimapper® assay. **B.** Anti body details of the Ultimapper® Assay **C.** PD-L1 Index Array Control

METHODS

Briefly, mouse monoclonal antibodies with a short DNA strand attached to the Fc region were amplified and attached with a complimentary DNA strand attached to a fluorophore followed by an amplification procedure. We stained 8 batches of slides with 2 control slides for each batch to assess for variability between batches (CD8, CD68 and PD-L1). The images were analyzed by molecular compartmentalization without segmentation using AQUA software (version 3.2.2.1) for each marker in different compartments (tumor, stroma and CD68 for PD-L1). A column graph and Mann-Whitney U-test was used to analyses the results for statistical significance between pCR and non-pCR. P-values shown are not adjusted for multiple comparisons.

RESULTS

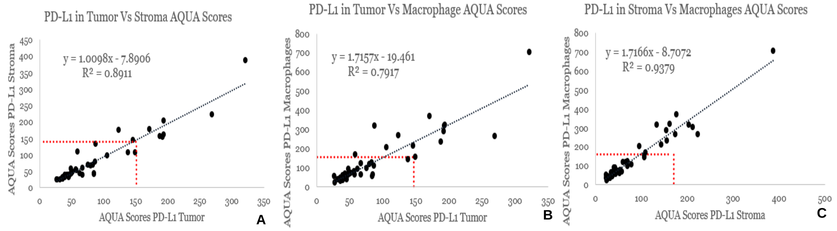


Figure 3. Comparative regression analysis between PD-L1 Positivity between different compartments with the red dotted line (…) showing AQUA scores more then 150 as positive **A.** Tumor verses Stroma, **B.** Tumor verses Macrophages (CD68+) and **C.** Stroma verses Macrophages.

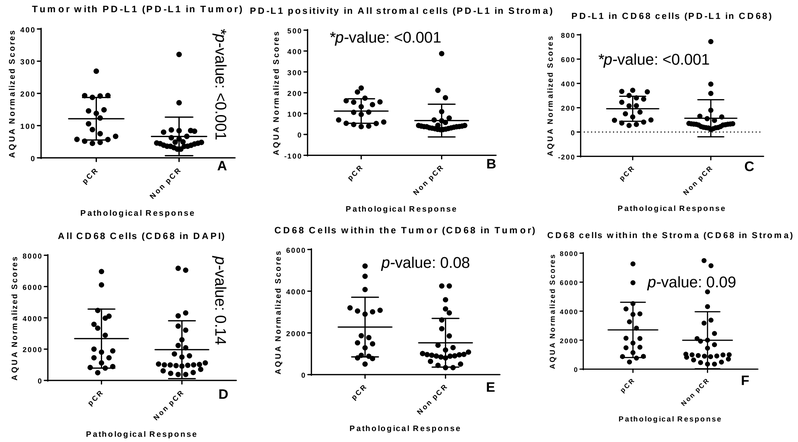


Figure 4. Comparative analysis between the pathological clinical response with non-pathological clinical response in terms of **A.** Expression of PD-L1 in the Tumor cells, **B.** Expression of PD-L1 in all stromal cells, **C.** Expression of PD-L1 in Macrophages (CD68+). **D.** presence of CD68 cells, **E.** presence of CD68 cells tumor, **F.** presence of CD68 cells in Stroma.

RESULTS

Reproducibility between batches was excellent (CD68; R2 = 0.86 to 0.94 and PD-L1; R2 = 0.87 to 0.98). PD-L1 expression was significantly higher in the tumor compartment, the stromal compartment, and in the CD68 compartment in pCR patients compared to non-pCR. However, comparative analysis between the pCR and non-pCR patients showed no difference in the overall presence of CD68 in any of the molecular compartments.

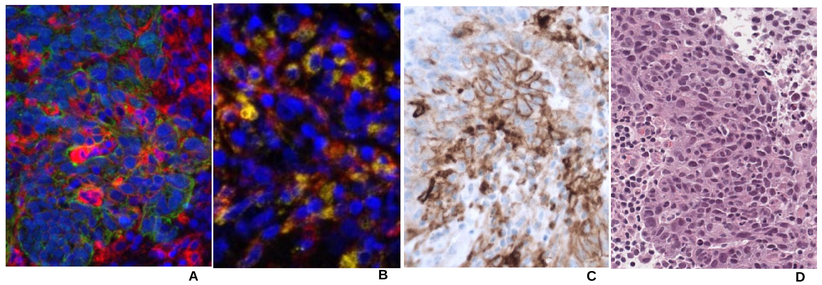


Figure. 5 Tumor PD-L1 expression and Immune cell expression of PD-L1 by immunofluorescence. **A.** Composite image of PD-L1 positive Tumor cells by immunofluorescence, **B.** Composite image of both PD-L1 positive macrophages (orange) and PD-L1 negative macrophages (yellow) by immunofluorescence staining, **C.** PD-L1 positive tumor cells DAB staining on a serial section and **D.** Hematoxylin and Eosin staining of a serial section.

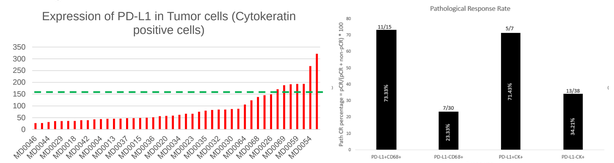


Figure. 6 A. Expression of PD-L1 in tumor cells **B.** pathological response rate in macrophages and tumor cells.

CONCLUSIONS

- Expression of PD-L1 in tumor cells is associated with response to Durvalumab and chemotherapy in this neoadjuvant TNBC trial.
- Similar to NSCLC, PD-L1 expression in TNBC tumor cells is seen using an assay which is more sensitive than FDA approved TNBC SP142 assay
- Expression of PD-L1 immune cells in stroma and the CD68 expression compartment (predominantly macrophages) is associated with response to Durvalumab and chemotherapy in this neoadjuvant TNBC trial.
- This result, the association of PD-L1 expression in macrophages with outcome, has also been seen in melanoma[1] and lung cancer[2]. Further study is required to validate this potential predictive biomarker in TNBC.

REFERENCES

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2. Immune cell PD-L1 co-localizes with macrophages and is associated with outcome in PD-1 pathway blockade therapy Y.Liu, J. Zugazagoitia, F.S. Ahmed, B.S. Henick, S.Gettinger, R.S. Herbst, K.A. Schalper and D.L. Rimm Clin Cancer Res October 15 2019 DOI: 10.1158/1078-0432.CCR-19-1040