VALIDATION OF PD-L1 DYNAMIC EXPRESSION ON EXTRACELLULAR VESICLES AS A PREDICTOR OF RESPONSE TO IMMUNE-CHECKPOINT INHIBITORS AND SURVIVAL IN NON-SMALL CELL LUNG CANCER PATIENTS

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Background Immune-checkpoint inhibitors (ICIs) revolutionized the treatment of advanced non-small cell lung cancer (NSCLC).¹⁻³ To date, tissue PD-L1 immunohistochemistry is one of the leading biomarkers for prediction of ICIs response but has several limitations.⁴⁻⁵ Extracellular vesicles (EVs) are cell-derived structures involved in cell communication and represent a potential minimally invasive alternative to predicting ICI response.⁶⁻⁹ Based on this and our preliminary results presented at SITC 2020,¹⁰ we hypothesize that EV PD-L1 predicts response to ICIs in NSCLC.

Methods This study evaluates an exploratory cohort of advanced/metastatic NSCLC patients receiving ICIs (cohort A) and a validation cohort receiving Pembrolizumab+docetaxel or docetaxel alone (PROLUNG Phase 2 randomized trial) (cohort B).¹¹ Plasma samples were collected pre-treatment (T1) and at 3 treatment cycles (T2) (figure 1A). Response was assessed by computed-tomography scan at 3 (cohort A) and 6–8 treatment cycles (cohort B) according to mono- or chemotherapy combination therapy. Patients were classified as responders (partial, stable, or complete response) or non-responders (progressive disease) by RECISTv1.1.¹² EVs were isolated by serial ultracentrifugation and characterized following ISEV recommendations.^{13,14} Tissue PD-L1 expression was measured by standardized immunohistochemistry (SP263, 22C3, or 28–8 clones)⁵ and EV PD-L1 expression by immunoblot and its ratio was calculated as EV PD-L1 T2/T1. Cut-offs from the exploratory cohort were applied to the validation cohort, being EV PD-L1 ratio <0.85 = Low.

Results Paired samples from 30 ICIs, 23 pembrolizumab+docetaxel, and 15 docetaxel treated patients were analyzed. In cohort A, non-responders showed higher EV PD-L1 ratio than responders (p=0.012) (figure 1B) with an area-under-the-curve (AUC) of 77.3%, 83.3% sensitivity, and 61.1% specificity, while the tissue PD-L1 was not predictive (AUC=50%). As a validation, pembrolizumab+docetaxel treated non-responders showed higher EV PD-L1 ratio (p=0.036) than responders with an AUC=69.3%, sensitivity=75%, and specificity=63.6%, outperforming the tissue PD-L1 (figure 1C). No statistically significant differences were observed in the docetaxel group (p=0.885). Moreover, ICIs patients with higher EV PD-L1 ratio showed shorter progression-free survival (PFS) (HR=0.30, p=0.066) and overall survival (OS) (HR=0.17, p=0.016) (figure 1D) which was also observed in the pembrolizumab+docetaxel cohort with shorter PFS (HR=0.12,

p=0.004) and OS (HR=0.23, p=0.010) (figure 1E). EV PD-L1 ratio did not predict survival in docetaxel-treated patients.

Abstract 23 Figure 1 (A) Study design and methodology. (B) EV PD-L1 ratio predicts response to ICIs in 30 NSCLC patients from the discovery cohort A and outperforms tissue PD-L1. (C) EV PD-L1 ratio is predictive for response to pembrolizumab+docetaxel in 23 NSCLC patients but not in 15 patients receiving docetaxel alone from cohort B. (D) Higher EV PD-L1 ratio predicts shorter PFS and OS in 30 patients from the discovery cohort A treated with ICIs. (E) Higher EV PD-L1 ratio is associated with shorter PFS and OS in 23 patients treated with pembrolizumab+docetaxel but not in patients treated with docetaxel alone. Abbreviations: CT: Computed tomography, EV: Extracellular vesicle; HR: Hazard Ratio; ICIs: Immune-checkpoint Inhibitors; IHC: Immunohistochemistry; NR: Non-Responders; OS: Overall Survival; p: p-value; PFS: Progression-free survival; R: Responders [Created with BioRender].

Conclusions We demonstrated that treatment-associated changes in EV PD-L1 levels are predictive of response and survival in advanced NSCLC patients treated with ICIs. This model, if confirmed in a large prospective cohort, could have important clinical implications, guiding treatment decisions and improving the outcome of patients receiving ICIs.

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Ethics Approval Patients consented to Institutional Review Board–approved protocol, A.O. Pappardo, Messina, Italy for cohort A and Thoracic Oncology Unit, Instituto Nacional de Cancerología (INCan), México City, México in case of the cohort B. Biological material was transferred to the University of Maryland School of Medicine, Baltimore for EV analysis under signed MTA between institutions MTA/2020–13111 & MTA/2020–13113.

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