Background Immune-checkpoint inhibitors (ICIs) have revolutionized the treatment of advanced/metastatic non-small cell lung cancer patients (NSCLC), however, only a small subset of patients derives clinical benefit.1-3 To date, PD-L1 immunohistochemical evaluation is the gold-standard assay and the only approved biomarker, but associated with several limitations due to technical and biological factors such as spatial and temporal tumor heterogeneity.4 5 In this context, liquid biopsies emerge as novel powerful tools that could allow the non-invasive real-time characterization of the tumor PD-L1 status. In particular, extracellular vesicles (EVs), defined as cell-derived double-membrane structures involved in cell communication, hold strong potential as tissue surrogates. Recent studies have suggested that EV PD-L1 cargo can be used as novel biomarkers to predict the response to ICIs in NSCLC patients.6 7 We hypothesize that EV PD-L1 cargo can serve to stratify the response to ICIs in NSCLC patients.

Methods This study enrolled advanced/metastatic NSCLC patients receiving ICI treatment. Plasma samples were obtained at baseline (T1) and at 8 weeks (T2) during the first response evaluation. Patients were classified as responders when showing partial, stable or complete response or as non-responders when manifesting progressive disease following RECIST v1.1.8 Plasma EVs were isolated by standard serial ultracentrifugation methods and characterized according to ISV recommendations.9 10 Tissue PD-L1 expression was measured by immunohistochemistry while EV PD-L1 expression was measured by immunoblot. A predictive model was created by logistic-regression and a bootstrap corrected ROC curve to validate the results.

Results Paired plasma samples from 21 patients were analyzed. PD-L1 tissue expression was not correlated with treatment response (p=0.394) nor matched the baseline EV PD-L1 levels (p=0.37) (figure 1A). However, the dynamics of EV PD-L1 (T1-T2) correlated with the treatment response, observing an increase of PD-L1 expression in non-responders and a decrease or stable levels in responders (p=0.043) (figure 1B). The predictive model reported an AUC=0.85, 90% CI=0.72–0.97, with 74.2% sensitivity and 73.5% specificity (figure 1C). Moreover, the increase of EV PD-L1 was associated with shorter overall survival (HR=4.34, p=0.037) and shorter progression-free survival (HR=5.06, p=0.025) (figure 1D & E).

Conclusions Our preliminary-study showed, for the first time, the predictive and prognostic value of EV PD-L1 dynamic changes in immunotherapy-treated NSCLC patients. Although larger studies are needed to validate these results, this promising biomarker could have important clinical implications, guiding treatment decisions in near real-time and improving the outcome of patients that could benefit from ICIs.

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Ethics Approval All patients consented to an Institutional Review Board–approved protocol (A.O. Papadino, Messina, Italy). Biological material was transfer to the University of Maryland, USA under signed MTA between both institutions (MTA/2020-13111).

REFERENCES
C-REACTIVE PROTEIN (CRP) AS A PROGNOSTIC DYNAMIC MONITORING OF RESPONSE TO IMMUNE CHECKPOINT INHIBITORS. RESULTS FROM A MULTI-CENTER INTERNATIONAL OBSERVATIONAL STUDY

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Background CRP is an acute-phase protein produced primarily in response to interleukin-6 via transcriptional activation of the STAT3. Recent data have provided mechanistic insights into the immune suppressive role of elevated CRP by elucidating its influence on effector T-cell function and antigen presentation. Furthermore, melanoma patients in Checkmate-064 who demonstrate a higher CRP mRNA expression in plasma-derived exosomes are associated with response to anti-PD-1 antibodies in melanoma and NSCLC. Br J Cancer 2018;118:820–824.

Methods Between 2015–2019, 420 adult patients with advanced NSCLC treated with ICIs from a multi-center international cohort were included. CRP level in peripheral blood samples collected up to 2 weeks before starting ICI based therapy was sought to evaluate the role of CRP as a prognostic biomarker in advanced NSCLC treated with ICIs from a multi-center international cohort.

Results Baseline CRP value was available in 75.5% of patients, with 66% having CRP-H. The median CRP was 21.0 mg/l. CRP-H showed a trend for stronger association with squamous histology (73.7% vs. 66% with CRP-N; p=0.062) but did not show an association with the PD-L1 status (0%, 1–49%, or ≥50%). Patients with CRP-H had a lower objective response rate compared with patients with CRP-N (26.9% vs. 47.6% PR; p=0.029). Compared to those with CRP-N (figure 1), patients with CRP-H had a significantly shorter median PFS [3.9 vs. 6.6 months, HR 1.41 95% CI: (1.07–1.86); p=0.0138] and OS (8.6 vs. 14.8 months, HR 1.55 95% CI [1.13–2.14]; p=0.060). In Cox regression analysis, CRP-H was again found to be independently associated with shorter median PFS and OS.

Conclusions This is the largest international real-world dataset demonstrating significantly inferior outcomes associated with CRP >10 mg/l in NSCLC patients treated with ICI based therapies. The potential influence of the immune suppressive effects of elevated CRP and IL-6 on the anti-tumor efficacy of ICIs needs prospective evaluation and could potentially be exploited as a therapeutic avenue in NSCLC.

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Ethics Approval The primary IRB approval for this study was conducted under an ECU (P-MAIF-UMICRB-15-001400). Individual approval was also obtained from the respective IRB of each participating institution.

REFERENCES

BACKGROUND CLEARANCE OF CIRCULATING TUMOR DNA (ctDNA) FOLLOWING CHECKPOINT BLOCKADE (CB) CAN PRECEDE RADIOGRAPHIC RESPONSE, though current state of the art ctDNA detection via targeted panels faces limited sensitivity in low burden disease (figure 1). We previously showed that whole genome sequencing (WGS) of plasma can overcome low input of ctDNA to dynamically track low volume malignancy using matched tumor tissue. We therefore sought to evaluate