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Plasma Cell-free RNA PD-L1 and Survival with Immune Checkpoint Inhibitor Therapy in Metastatic Non-Small Cell Lung Cancer

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ABSTRACT

Background: *Tissue programmed death-ligand 1 (PD-L1) protein expression is predictive of immune checkpoint inhibitor (ICI) benefit. However, tissue testing can be fraught with tissue acquisition and heterogeneity limitations. Plasma testing can overcome these limitations. However, the overall survival predictive benefit of plasma PD-L1 assays have not been well characterized.*

Methods: *Patients with stage IV non-small cell lung cancer (NSCLC) and plasma cell free RNA PD-L1 by polymerase chain reaction (PCR) expression were identified and assessed for overall survival. Sixteen patients treated with front-line ICI-based regimens were assessed and represented a real-world patient population with over half with a performance status of 2 or greater. Ten contemporaneous patients at the same institution treated with chemotherapy alone were also identified and assessed.*

Results: *With a median follow-up of 33 months, median overall survival was 13 months with a 30% 3-year OS for the ICI treated patients compared to a median OS of 3 months and a 10% 3- year OS for those treated with chemotherapy alone. Comparative log-rank test p-value = 0.014 and a hazard ratio 0.376 (95%-CI 0.134-1.057).*

Conclusions: *A plasma cell free RNA PD-L1 by PCR assay was associated with a statistically significant survival benefit from ICI-based treatment compared to chemotherapy in the first line treatment of a real-world patient population of advanced NSCLC.*

Keywords
Plasma PD-L1, Predictive immune biomarker, Liquid biopsy, cfRNA.

Introduction
Tissue programmed death-ligand 1 (PD-L1) protein expression is the recognized predictive immune biomarker of front-line immune checkpoint inhibitor (ICI) based therapy benefit compared to standard chemotherapy in advanced non-small cell lung carcinoma (NSCLC).

However, as with any tissue biomarker testing, tissue PD-L1 protein testing can be fraught with tissue acquisition limitations, tissue site sampling heterogeneity, monoclonal antibody assay

variability, pathologist interpretation variability, and imprecise predictive cut-off levels of immunohistochemical (IHC) staining [1-5].

A liquid biopsy immune biomarker predictive of ICI benefit would not be constrained by these tissue-testing limitations and could also easily allow dynamic assessment of PD-L1 expression with treatment and upon cancer recurrence and/or progression. However, prior plasma PD-L1 assays of soluble PD-L1 by enzyme-linked immunosorbent assays have not been predictive of ICI benefit. Elevated levels of soluble protein PD-L1 were associated with poorer survival with ICI treatment [6,7]. Secreted PD-L1 proteins have also been shown to contain decoy PD-L1 variants as a mediator of ICI treatment resistance (8). Circulating tumor cell PD-L1 expression has also not been a helpful plasma-based immune biomarker. It has an overall poor correlation with tissue PD-L1 expression and has not been associated with predictive ICI treatment benefit [9-10].

A notable exception of an effective plasma-based immune biomarker is extracellular vesicle (EV) PD-L1 expression. An EV PD-L1 protein research assay demonstrated that the dynamic changes in the EV PD-L1 protein were predictive of ICI treatment durability. Increasing EV PD-L1 was associated with non-responders with a decrease seen in patients with an ICI response [11]. PD-L1 mRNA expression by droplet digital PCR in plasma-derived exosomes has also demonstrated a similar dynamic change correlating with ICI response [12]. This emphasizes the potential of a plasma-based PD-L1 assay having longitudinal ICI predictive benefit, however neither PD-L1 EV assay was evaluated as a pre-treatment predictor of ICI benefit, just having a dynamic correlation with response.

mRNA PD-L1 expression is a potential predictive immune biomarker. The use of mRNA for PD-L1 testing carries the potential for a more precise standardization without the confounding IHC interpretation variability or protein expression heterogeneity. Correlation between tissue PD-L1 mRNA and tissue PD-L1 protein expression has yielded conflicting findings. Levels of tissue mRNA expression correlated with PD-L1 protein tumor cell expression with the Dako 28-8 monoclonal antibody IHC staining percentages in NSCLC [13]. There was a similar tissue PD-L1 RNA expression correlation with the Dako 22C3 monoclonal antibody IHC staining in NSCLC and other solid tumors [14]. However, other studies have identified low concordance.

There was only 59% concordance between tissue mRNA PD-L1 in-situ hybridization compared to tissue PD-L1 protein [15]. In a study comparing tissue RNA PD-L1 by PCR with IHC PD-L1 protein 22C3, SP263, and SP 142 assays, 51 of the 167 patient samples tested were discordant with no tumor cell PD-L1 staining yet RNA PD-L1 expression. Of those patients without tumor cell staining, 57% demonstrated immune cell PD-L1 protein expression [16]. In the CLOVER study of 437 NSCLC patients across all stages, there was low agreement between PD-L1 RNA by PCR and PD-L1 protein by the same three IHC assays, concluding PCR PD-L1 RNA is not equivalent to IHC assays, but can identify PD-L1 IHC negative patients [17].

Tissue PD-L1 mRNA expression is suggestive of ICI treatment benefit. Conroy et al. concluded PD-L1 mRNA expression is comparable to PD-L1 protein expression by IHC both analytically and clinically in predicting ICI response in NSCLC [14]. In another study, tissue mRNA qPCR was stated to have only weak correlation with tissue PD-L1 protein. However, high PD-L1 mRNA expression was associated with improved long-term benefit of ICI treatment, whereas low PD-L1 RNA levels had a high negative predictive value of 0.92 for absence of long-term benefit emphasizing the need for further validation of PD-L1 mRNA [18].

Plasma cell free mRNA (cfRNA) testing can be difficult because of RNA fragility and poor extraction efficiency. However, advances in liquid biopsy technology have successfully brought plasma RNA testing into the clinic [19,-20]. Ishiba et al. reported plasma cfRNA PD-L1 by PCR detectable across various cancers with no reported detection in the tested healthy individuals [21]. In the twelve patients in that study with parallel plasma and tissue samples available, there was concordance between the plasma cfRNA PD-L1 expression and the tissue PD-L1 protein expression and stated to be predictive of ICI response, however, OS benefit was not reported [21]. Raez et al. reported cfRNA PD-L1 expression by PCR in a variety of metastatic cancers, including 52 NSCLC patients. That study noted dynamic changes in the cfRNA PD-L1 expression with ICI treatment response, but due to lack of follow up with the COVID pandemic, survival outcomes were not reported [22].

Our aim was to evaluate the association of plasma cfRNA PD-L1 expression and treatment based clinical outcomes in metastatic NSCLC patients. In this retrospective real-world patient experience, we report the median and landmark 3-year OS in metastatic NSCLC patients who demonstrated positive plasma cfRNA PD-L1 expression and were treated with ICI-based therapy compared to chemotherapy alone.

Methods

This is a single-institution, retrospective observational study performed at the Brody School of Medicine at East Carolina University (Greenville, NC, USA) with patients treated at the Vidant Medical Center (now ECU Health Medical Center). In order to assess a landmark 3-year OS, patients with pathologically confirmed NSCLC and positive plasma cfRNA PD-L1 expression by PCR were identified through the institutional thoracic oncology program database from November 2018 through July 2019 (n = 92). Patients with stage I/II/III NSCLC, stage unknown, or with the presence of a targetable oncogenic driver mutation/fusion were excluded. There were no other clinical or laboratory exclusion criteria. Patients were treated based upon the current available standard of care during that time period with the local treating

oncologist making the final treatment decision. Patients with stage IV NSCLC meeting these criteria and who received their treatment at Vidant Medical Center (now ECU Health Medical Center) were identified and utcomes assessed based upon receiving either ICI-based treatment or chemotherapy alone treatment. The Brody School of Medicine at East Carolina University Institutional Review Board approved this study.

The 'IO cohort' consisted of sixteen patients with metastatic NSCLC who demonstrated plasma cfRNA PD-L1 expression and were treated with first-line ICI-based therapies. Thirteen patients received combination anti-PD-1/PD-L1 ICI plus chemotherapy regimens and three patients anti-PD-1/L1 ICI alone. No patients received definitive concurrent chemoradiation therapy or thoracic radiation therapy (RT). Palliative RT with either whole brain RT or Gamma Knife radiosurgery, or palliative stereotactic body RT were undertaken as indicated upon the recommendation of the treating oncologist. The 'ChemoRx cohort' consisted of ten contemporaneously identified metastatic NSCLC patients with plasma cfRNA PD-L1 expression who received first-line platinum-based doublet chemotherapy alone. Median and 3-year landmark OS outcomes in the IO cohort were compared to the ChemoRx cohort.

Plasma for testing was collected before any treatment. Blood was collected in a single 10-ml EDTA tube. The cfRNA PD-L1 expression testing was performed at the Circulogene CLIA/CAP accredited laboratory (Birmingham, AL, USA). Circulogene is a commercial liquid biopsy vendor with a proprietary patented pre-analytical linear-in-situ-amplification technology. The cfRNA PD-L1 Gene Expression assay is a real-time PCR-based assay with PD-L1 specific PCR primers. The demonstrated limit of detection for cfRNA PD-L1 was 1.0 copy/uL. Tissue PD-L1 protein expression testing with the Dako 22C3 monoclonal antibody was requested in all patients.

Ten patients in the IO cohort did have simultaneous plasma and tissue PD-L1 expression results. Tissue PD-L1 was reported as ≥ 50% in six patients and ≥ 1% in four patients. Six of the total IO cohort of sixteen patients (37%) were either tissue PD-L1 negative or unknown due to tissue quantity not sufficient for testing.

OS was assessed from the date of diagnosis and either death or censored follow-up. Median follow-up was 33 months. OS analysis was performed by AnalystSoft StatPlus Kaplan-Meier and log-rank test p-value and hazard ratio (HR) survival analysis. The pre-specified endpoint was median and 3-year OS.

Results

The IO cohort and ChemoRx cohort had similar advanced NSCLC histology and clinical presentations. As typical of a real-world advanced NSCLC patient population, half had an ECOG performance status (PS) of 2 or greater, 20-30% symptomatic brain metastases, and one-third with bone metastases, all predictors of poor ICI and chemotherapy treatment benefit (Table 1).

Table 1: Clinical presentations of the IO cohort and ChemoRx cohort treated patients with plasma cfRNA PD-L1 expression.

IO COHORT (N = 16)		CHEMORX COHORT (N = 10)
GENDER	8 Females/8 males	10 males
AGE	Median age 65 (range 55-85)	Median age 69 (range 42-81)
HISTOLOGY	75% non-squamous 25% squamous	70% non-squamous 30% squamous
ECOG PS	ECOG PS 1 = 8 ECOG PS ≥2 = 8	ECOG PS 1 = 4 ECOG PS ≥2 = 6
BRAIN METASTASES	5 (31%)	2 (20%)
BONE METASTASES	7 (44%)	3 (30%)

The IO treated cohort had a statistically improved OS compared to the ChemoRx treated cohort. The IO cohort patients had a median OS of thirteen months with a 30% 3-year OS. In comparison, the ChemoRx cohort had a median OS of three months and 3-year OS of 10%.

Comparative log-rank test p-value = 0.014 HR of 0.376 (95% CI, 0.14-1.057) (Figure 1). There was no OS difference in the IO cohort whether tissue PD-L1 was positive, negative, or unknown. Given the clinically known poorer OS differences of both ICI and chemotherapy treatment in patients with an ECOG PS of 2 or worse compared to ECOG PS of 0 or 1, OS was compared between the ECOG PS 2 or greater patients and the ECOG PS 1 patients in the IO cohort. There was no difference in OS between the IO cohort patients of ECOG PS 2 or greater and ECOG PS 1 (Figure 2).

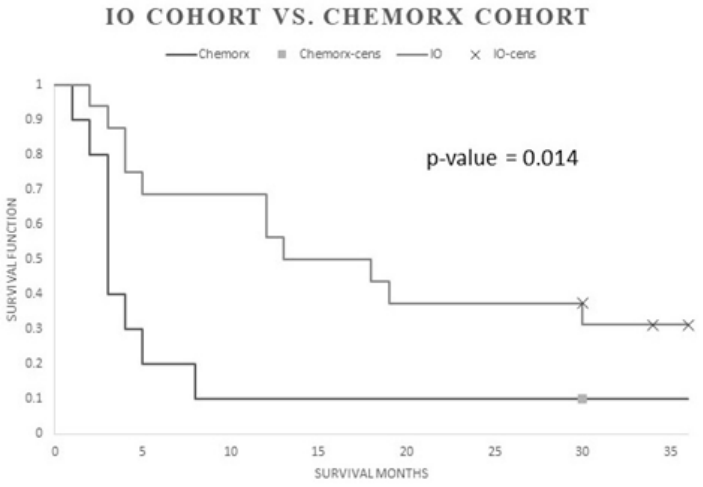

Figure 1: Overall survival of the IO cohort compared to the ChemoRx cohort treated patients with plasma cfRNA PD-L1 expression (p-value = 0.014).

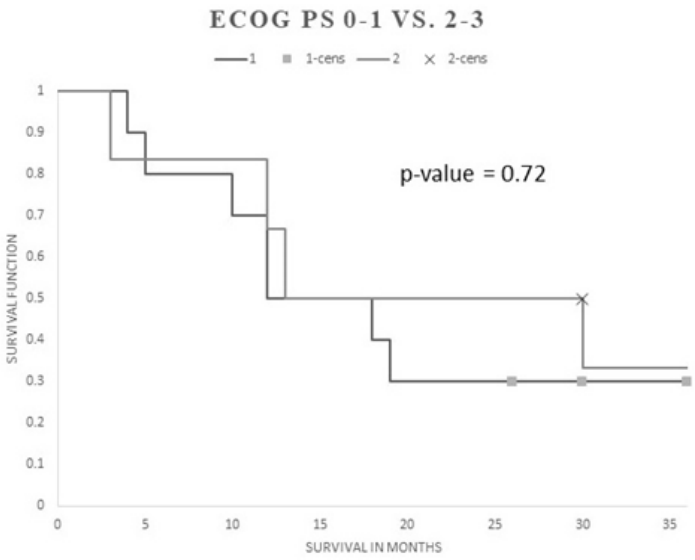

Figure 2: Overall survival of ICI treated patients ECOG PS 1 versus ECOG PS 2 or greater (p-18 value = 0.8289).

Discussion

Just as in the pharma sponsored ICI clinical trials demonstrating tissue PD-L1 protein expression was predictive of an improved OS of ICI compared to chemotherapy in first-line therapy [23,24], plasma cfRNA PD-L1 by PCR expression was associated with an improved OS of ICI-based treatment compared to chemotherapy in our symptomatic metastatic NSCLC population. Within this real-world patient experience and an expected poorer OS, the clinical outcomes of our ICI- based treated patients with plasma cfRNA PD-L1 expression demonstrated a similar median OS and 3-year OS of 30% as the large prospective ICI clinical trials based on tissue PD-L1 protein expression. Even when 37% of patients were either tissue PD-L1 negative or unknown, patients with positive plasma cfRNA PD-L1 expression still achieved an improved ICI treatment benefit.

Real-world data invariably shows poorer clinical outcomes than clinical trial outcomes. That becomes most evident in patients with an ECOG PS of 2. What data is available with chemotherapy studies, ECOG PS 2 patients demonstrated a median OS of 3.9 months and 6% 2- year OS such that this four-chemotherapy regimen phase III trial amended the ongoing protocol excluding ECOG PS 2 patients [25]. This supports an expected OS difference between the chemotherapy treated patients in clinical trials limited to an ECOG of 0 or 1 with our chemotherapy treated population with over half of the patients with an ECOG of 2 or greater.

There remains an open debate whether any NSCLC ECOG PS 2 patients without a targetable mutation or fusion should even be treated with ICI since they were not represented in any of the pharma sponsored ICI clinical trials leaving oncologists without any outcomes data in that symptomatic patient population compared to chemotherapy and what data there is, indicates a much poorer outcome than patients with a PS of 0 or 1 [26-28]. The Flatiron Health database in non-squamous NSCLC patients with an ECOG PS of ≥2 and tissue PD-L1 expression of ≥ 50% treated with ICI alone, reports a median OS of 5.2 months and 3-year KM estimated OS of 16.7%. In ICI-chemotherapy treated non-squamous NSCLC patients with an ECOG PS ≥ 2, median OS was 6.3 months with a KM estimated 3-year OS of just 10.3%. Both median OS and KM estimated 3-year OS approach only half of ECOG PS 0 or 1 patient survivals treated with ICI-based regimens [29]. Bone metastases are associated with a cold tumor immune microenvironment and has become a recognized unfavorable metastatic compartment of ICI treatment outcome benefit irrespective of ECOG PS or liver metastases [30]. Over 40% of patients in our patient population had bone metastases further emphasizing the potential unfavorable ICI treatment outcomes of our real-world experience.

In our population, over half had an ECOG PS of 2 or greater and one-third symptomatic brain metastases, such that only one-third of our patients would have been eligible for a pharma sponsored ICI clinical trial limited to asymptomatic or minimally symptomatic ECOG PS of 0 or 1 patients and excluding those with untreated symptomatic brain metastases. The expected poor outcomes in the ChemoRx cohort would not account for the comparative difference as the IO cohort clinical presentations were similar with poor ICI benefit prognostic factors. Even with these unfavorable ICI benefit patients in our IO cohort, the ICI-based treatment OS outcomes in the ECOG PS 2 patients associated with plasma cfRNA PD-L1 expression was not inferior to our ECOG PS 1 patients or clinical trial outcomes data and was better than reported real-world data in the Flatiron Health database.

We felt that a landmark OS with prolonged follow-up would best reflect the clinical utility of plasma cfRNA PD-L1 expression and ICI treatment outcome. Response rates and progression free survival have been inconsistent early surrogates of ICI treatment OS, and a lack of an early response does not preclude an ICI OS benefit [31-33]. A pooled analysis of first-line ICI randomized trials failed to show a strong correlation between PFS or response rates with OS emphasizing the importance of having mature OS data as the most important endpoint for first- line ICI trials [34,35].

There are limitations of this reported patient experience. It is a retrospective collection of outcomes data treated at a single institution and not a prospective multi-institutional randomized comparison. Our presented outcomes data also only reflects patients with plasma cfRNA PD-L1. Similar outcomes data with ICI-based treated patients who were plasma PD-L1 negative treated at our institution was not captured in this comparative cohort study. That comparison is being assessed in another cohort comparison. Even with these limitations and the modest patient sample size, to our knowledge it does represent the largest patient experience of plasma cfRNA PD-L1 expression and ICI-based treatment compared to chemotherapy alone OS outcomes.

Conclusions

In a real-world patient experience of symptomatic metastatic NSCLC patients, plasma cfRNA PD-L1 expression was associated

with a statistically significant and clinically meaningful median and 3-year landmark OS benefit with ICI-based systemic treatment compared to chemotherapy. Plasma cfRNA PD-L1 expressing patients still received the outcome benefit of ICI treatment whether tissue PD-L1 was negative or unknown. Our data lends support for needed further and expanded study of the potential predictive benefit and clinical utility of plasma cfRNA PD-L1 as a predictive immune biomarker.

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