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*Article*

# Plasma Cell-free RNA PD-L1 Expression and Overall Survival of Immune Checkpoint Inhibitor Therapy in Advanced Non-Small Cell Lung Cancer

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**Simple Summary:** Immune therapy of cancer has made controlling and possibly curing advanced lung cancers possible. Tissue staining for PD-L1 is one of the current predictive biomarkers of immune therapy benefit, however it can be limited by the lack of sufficient tissue and the heterogeneity of the PD-L1 testing. Identifying a predictive immune biomarker in a simple blood test would be a great benefit for patients. In this real-world patient experience, patients with advanced non-small cell lung cancer with positive plasma cell-free RNA PD-L1 expression, achieved a statistically significant and clinically meaningful survival benefit with immune therapy compared to standard chemotherapy. The plasma PD-L1 expression was positive even in patients with negative tissue PD-L1 or did not have sufficient tissue for testing extending the benefit potential of immune therapy for more patients. This study supports further research and testing of plasma cell-free RNA PD-L1 expression and immune therapy benefit across all cancers.

**Abstract:** Tissue programmed death ligand-1 (PD-L1) protein expression is predictive of immune checkpoint inhibitor (ICI) benefit. However, tissue PD-L1 can be fraught with tissue acquisition and heterogeneity limitations. Plasma testing can overcome these limitations. However, the overall survival (OS) predictive benefit of plasma PD-L1 assays have not been well characterized. Patients with stage IV non-small cell lung cancer (NSCLC) and plasma cfRNA PD-L1 by PCR expression were identified and assessed for OS. 16 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. 10 contemporaneous patients at the same institution treated with chemotherapy alone were also identified and assessed. With a median follow-up of 33 months, median OS 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). Plasma cfRNA PD-L1 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; liquid biopsy; cfRNA; immune checkpoint inhibitor; predictive immune biomarker; NSCLC

## 1. 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

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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].

Two points of clinical trial data supporting tissue PD-L1 as a predictive immune biomarker also emphasize its limited predictive impact. First, in KEYNOTE-042 with advanced NSCLC comparing pembrolizumab versus chemotherapy, even with tissue PD-L1 protein IHC staining of ≥50%, only 39% of patients responded to the ICI with a Kaplan-Meier (KM) estimated 3-year overall survival (OS) of 30-40% and 25% in the chemotherapy alone arm. In the subgroup of patients with PD-L1 staining of 1-49%, there was less than a 5% absolute difference between the ICI and chemotherapy treated groups with an insignificant hazard ratio (HR) of 0.92. Even with high ≥50% PD-L1 expression, there is only an absolute 15% OS difference between single agent pembrolizumab and standard chemotherapy [6]. In KEYNOTE-189 comparing pembrolizumab plus chemotherapy to chemotherapy alone, patients with ≥50% tissue PD-L1 expression treated with concurrent chemotherapy plus the ICI had a 2-year OS of 52.2% compared to those treated with chemotherapy alone having a 2-year OS of 37.1%, again with an absolute benefit difference of just 15% in these clinical trials [7]. At best, more than half of patients do not receive an immune therapy benefit predicted by tissue PD-L1 expression and the predictive absolute difference benefit is small.

A plasma-based 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. A plasma-based immune biomarker would have great clinical utility in guiding and evaluating ICI therapies in the clinic. However, prior plasma PD-L1 assays of soluble PD-L1 by enzyme-linked immunosorbent assays have not been predictive of ICI benefit. In fact, elevated levels of soluble protein PD-L1 were associated with poorer survival with ICI treatment indicating it is a predictive biomarker for poor prognosis [8,9]. Secreted PD-L1 proteins have also been shown to contain decoy PD-L1 variants as a mediator of ICI treatment resistance [10]. 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 been associated with a poorer prognosis in patients treated with ICI [11,12]. 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 was predictive of ICI treatment durability. Increasing EV PD-L1 was associated with non-responders with a decrease seen in patients with an ICI response [13]. PD-L1 mRNA expression by droplet digital PCR in plasma-derived exosomes has also demonstrated a similar dynamic change correlating with ICI response [14]. This emphasizes the potential of a plasma-based PD-L1 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. There is a clear need for better predictive immune biomarkers overall and predictive plasma immune biomarkers in particular.

RNA PD-L1 expression is a potential predictive immune biomarker. The use of RNA for PD-L1 testing carries the potential for a more precise standardization without the confounding IHC interpretation variability or protein expression heterogeneity. Tissue PD-L1 RNA by RT-qPCR has been shown to correlate with tissue PD-L1 protein expression. 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 [15]. There was a similar tissue PD-L1 RNA expression correlation with the Dako 22C3 monoclonal antibody IHC staining in NSCLC and other solid tumors [16]. Plasma cell free RNA (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 [17,18]. Plasma cfRNA PD-L1 by PCR has been detectable across various cancers with no reported detection in healthy individuals. In the twelve patients in this study that had parallel plasma and tissue samples available, there was concordance

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between the plasma cfRNA PD-L1 expression and the tissue PD-L1 protein expression [19]. The tissue and plasma RNA PD-L1 expression studies were predictive of ICI response; however, OS benefit was not reported.

Although the available tissue and plasma PD-L1 RNA expression data correlates with tissue PD-L1 protein expression, the predictive clinical outcomes of OS are not well characterized and limited to a general ICI response correlation only. Given the potential prolonged durability of ICI therapies, a true OS assessment requires several years of follow-up. Reports of KM estimated OS with short follow-up will invariably overestimate the true OS. In KEYNOTE-189 comparing chemotherapy with ICI to chemotherapy alone, the first publication with a median follow-up of 10.5 months reported the KM-curve holding up at the 60% range in the intention-to-treat population through 18 months [20]. However, in the protocol specified final analysis with a much longer median follow-up of 31 months, the OS of the same intention-to-treat population fell down to 30% at the 3-year mark [7]. Another blood-based immune biomarker of blood-based tumor mutational burden (TMB) also emphasizes the importance of prolonged follow-up. Response rates increased with higher bTMB but there was no difference in progression free survival. However, with longer median follow-up extended out to 36 months, a significant OS benefit with atezolizumab alone surfaced with bTMB ≥16 that was not discernible with 24 months of median follow-up [21]. Other studies have evaluated surrogate ICI treatment OS markers. However, 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 [22-24]. OS with prolonged follow-up is the gold standard of true ICI benefit to demonstrate the clinical utility of an immune biomarker.

The aim of our study is to report our patient experience and OS outcomes with prolonged follow-up of patients with stage IV NSCLC who had plasma cfRNA PD-L1 expression and were treated with front-line ICI-based regimens to strengthen the foundation to support further research evaluating the clinical utility of plasma cfRNA PD-L1 expression as a predictive immune biomarker. In our real-world patient experience, plasma cfRNA PD-L1 expression was associated with a statistically significant and clinically meaningful correlation with OS benefit with ICI-based treatment compared to chemotherapy.

## 2. Materials and Methods

This is a single-institution, retrospective observational study performed at the Brody School of Medicine at East Carolina University with patients treated at the Vidant Medical Center (now ECU Health Medical Center). 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. A total of 26 patients with stage IV NSCLC meeting these criteria and who received their treatment at Vidant Medical Center (now ECU Health Medical Center) were identified within that total cohort. Patients with advanced NSCLC receiving first-line ICI-based regimen treatment (n =16) or received chemotherapy alone (n = 10), were assessed for OS. This study was approved by the Brody School of Medicine Institutional Review Board.

Plasma for testing was collected before treatment. Blood was collected in a single 10-ml EDTA tube. The cfRNA PD-L1 expression by PCR testing was performed at the Circulogene CLIA/CAP accredited laboratory (Birmingham, Alabama). 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. Circulogene reports plasma PD-L1 results as either (a) not

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detected, (b) ≥ 1%, or (c) ≥ 50%, using the PCR 30th percentile Ct value corresponding to tissue IHC PD-L1 expression of ≥ 50% and the 66th percentile Ct value corresponding to tissue IHC PD-L1 expression of ≥ 1%. Tissue NGS was requested in all patients for tissue PD-L1 protein expression testing with the Dako 22C3 monoclonal antibody.

The 'IO cohort' consisted of the 16 patients with advanced NSCLC who demonstrated plasma cfRNA PD-L1 expression and 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 chemotherapy radiation therapy (CRT) or thoracic radiation therapy (RT). Palliative RT with either whole brain RT or Gamma Knife radiosurgery, palliative bony RT, or palliative stereotactic body radiation therapy were undertaken as indicated upon the recommendation of the treating oncologist. The 'ChemoRx cohort' consisted of the 10 identified contemporaneous advanced NSCLC patients with plasma cfRNA PD-L1 expression who received first-line chemotherapy alone.

OS was assessed from the date of diagnosis and either death or censored follow-up through August 2021. Median follow-up for this for both the IO and ChemoRx cohorts 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 OS and 3-year OS.

## 3. 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 a 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 patients.

| | IO COHORT (N = 16) | CHEMORX COHORT (N = 10) |
|---|---|---|
| **GENDER** | 8 Females/8 males | 10 males |
| **AGE** | Median age 65 (range 54-85) | Median age 69 (range 42-81) |
| **HISTOLOGY** | 75% non-squamous<br>25% squamous | 70% non-squamous<br>30% squamous |
| **ECOG PS** | ECOG PS 1 = 7<br>ECOG PS ≥2 = 9 | ECOG PS 1 = 4<br>ECOG PS ≥2 = 6 |
| **BRAIN METASTASES** | 5 (31%) | 2 (20%) |
| **BONE METASTASES** | 6 (37%) | 3 (30%) |
| **M1A METASTASES** | 3 (19%) | 2 (20%) |

The IO cohort had a median OS of 13 months with a 30% 3-year OS. The ChemoRx cohort had a median OS of 3 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).

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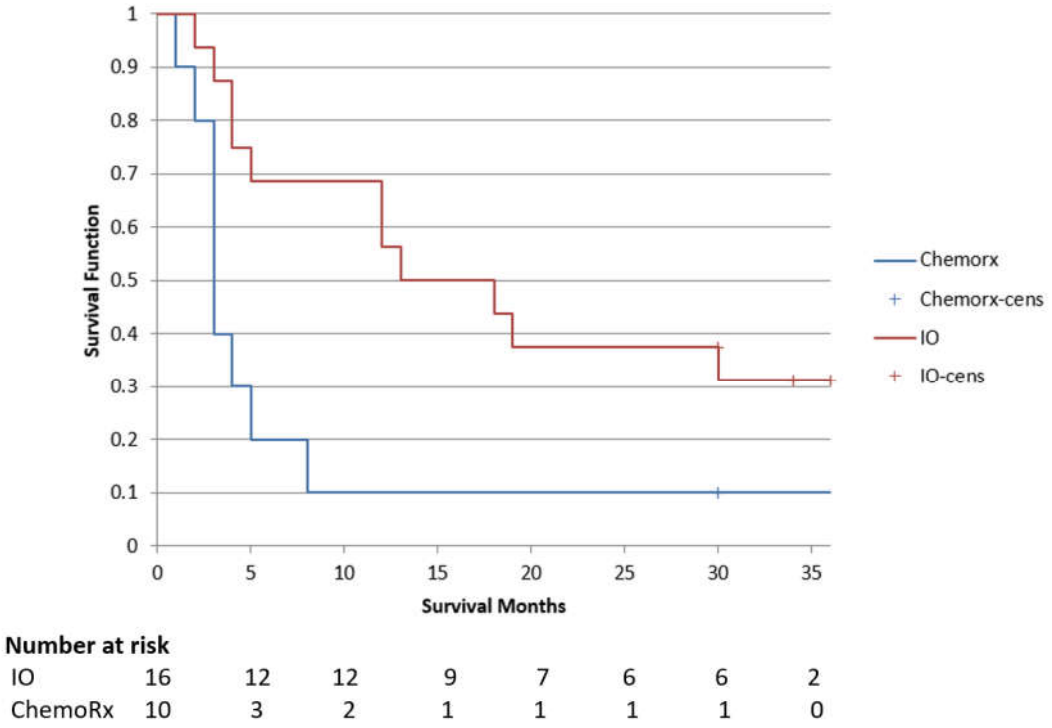


**Number at risk**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| IO | 16 | 12 | 12 | 9 | 7 | 6 | 6 | 2 |
| ChemoRx | 10 | 3 | 2 | 1 | 1 | 1 | 1 | 0 |

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

Individual patients of the IO cohort are detailed in table 2. Within the IO cohort, all but two had cfRNA PD-L1 expression reported as ≥ 50% limiting any assessment of quantitative cfRNA PD-L1 expression and outcomes. However, the two patients with cfRNA PD-L1 expression reported at just ≥ 1% did exceed the median OS of the cohort.

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**Table 2.** IO cohort patients clinical and treatment demographics. pPD-L1 = plasma cfRNA PD-L1 results; tPD-L1 = tissue PD-L1 protein (22C3 results); ChemoIO = combination ICI and chemotherapy; aPD-1 = anti-PD-1 ICI; aPD-L1 = anti-PD-L1 ICI; bev = bevacizumab; SBRT = stereotactic body radiation therapy; WBRT = whole brain radiation therapy' GKRS = Gamma Knife Radiosurgery; * = patient in a complete response for 26 months with a small cell transformation recurrence and death.

| Age-gender | Histology | Metastatic sites | ECOG PS | pPD-L1 | tPD-L1 | Treatment | Survival months |
|---|---|---|---|---|---|---|---|
| 61f | Adeno | Bone | 2 | >1% | 90% | aPD-1; t-spine RT | *30 dead |
| 76f | Squamous | Bilateral lung | 1 | >50% | NA | ChemoIO; SBRT | 36 alive |
| 54m | Adeno | Brain, bone | 2 | >50% | >50% | ChemoIO; WBRT | 12 dead |
| 84m | Adeno | Lymph nodes | 1 | >50% | 1% | aPD-1; SBRT | 12 dead |
| 65f | Adeno | Brain, bone, pleura | 1 | >50% | 0% | ChemoIO | 19 dead |
| 67f | Adeno | Pleura, lymphangitic | 1 | >50% | 100% | ChemoIO | 12 dead |
| 55m | Adeno | Bone, LN | 1 | >1% | 0% | ChemoIO | 30 alive |
| 60f | Adeno | Bone, LN | 2 | >50% | 100% | ChemoIO | 13 dead |
| 54f | Adeno | Brain, liver, pleura | 1 | >50% | 100% | ChemoIO; brain GKRS; salvage aPD-L1 + bev | 18 dead |
| 85f | Adeno | Bilateral lung | 3 | >50% | 1% | ChemoIO; aPD-1 + bev at recurrence | 30 alive |
| 58m | Squamous | Brain, liver | 2 | >50% | 0% | ChemoIO | 4 dead |
| 64m | Adeno | Brain | 1 | >50% | NA | ChemoIO | 4 dead |
| 74m | Squamous | LN | 2 | >50% | 95% | aPD-1; aPD-1 at recurrence | 36 alive |
| 64m | Adeno | LN | 2 | >50% | >1% | ChemoIO | 34 alive |
| 65m | Squamous | Bone | 2 | >50% | NA | ChemoIO | 2 dead |
| 65m | Squamous | Liver, lung | 2 | >50% | >1% | ChemoIO | 3 dead |

Ten patients did have simultaneous plasma and tissue PD-L1 expression results. Plasma and tissue PD-L1 expression were both reported as ≥ 50% in 5 patients and another 4 patients demonstrated plasma PD-L1 expression reported at ≥ 50% with tissue PD-L1 expression reported at ≥ 1%. One patient with plasma PD-L1 expression of ≥ 1% demonstrated tissue PD-L1 expression of 90%. Six of the total IO cohort of 16 patients (37%) were either tissue PD-L1 negative or unknown due to the tissue quantity not sufficient (QNS) for testing.

Given the clinically known poorer OS differences of both ICI and chemotherapy treatment in patients with an ECOG PS of 0 or 1 compared to an ECOG PS of 2, OS was compared in the ECOG PS 1 and ECOG PS 2 or greater patients in the IO cohort. There was no difference in OS between the IO cohort patients of ECOG PS 1 compared to ECOG PS 2 or greater (figure 2; p-value = 0.8289).

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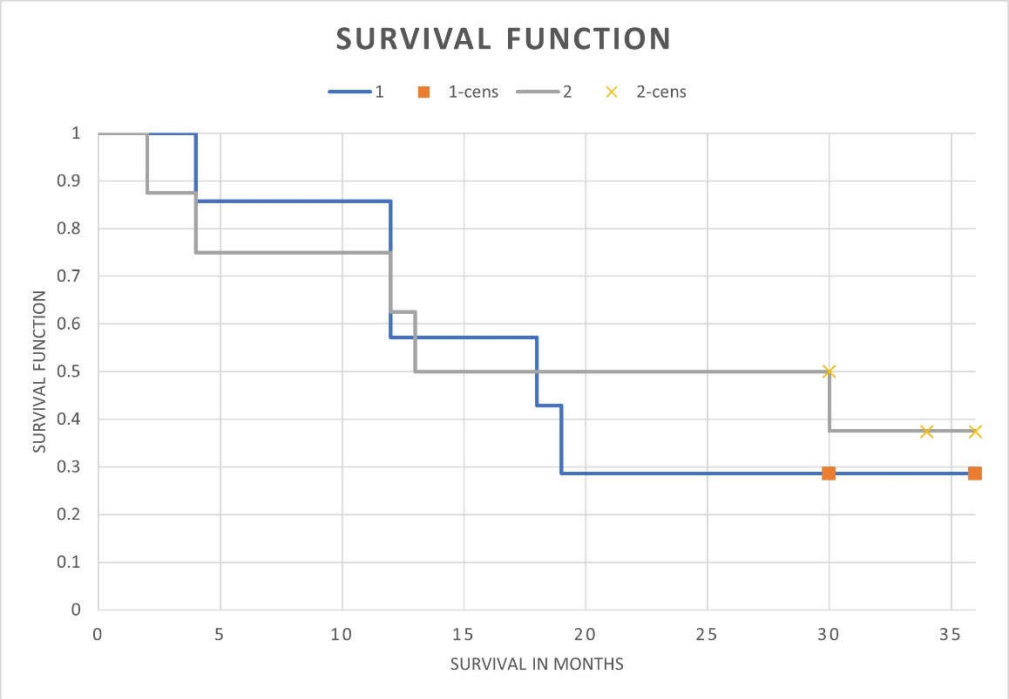


**Figure 2.** OS of ICI treated patients ECOG PS 1 versus ECOG PS 2 or greater.

## 4. Discussion

In a real-world patient population of symptomatic advanced NSCLC patients with cfRNA PD-L1 expression, patients treated with ICI based-regimens demonstrated a significantly improved median and 3-year OS compared to treatment with chemotherapy. Just as in the Pharma ICI clinical trials with tissue PD-L1 protein expression predictive of an improved OS of ICI compared to chemotherapy and similar relative HR but better 3-year OS benefit with ICI plus chemotherapy, plasma cfRNA PD-L1 expression predicted for an improved OS of ICI-based treatment compared to chemotherapy [6,7]. Even within a real-world patient experience and expected poorer OS, the clinical outcomes of our ICI-based treated patients with plasma cfRNA PD-L1 expression demonstrated a similar median OS and most importantly a 3-year OS of 30% as the large prospective ICI clinical trials based on tissue PD-L1 protein expression.

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]. 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 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 [26-29]. In our real-world 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.

Real-world data from 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, identifies a median OS of 5.2 months and 3-year KM estimated OS of 16.7%. In ICI plus 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 what ECOG PS 0 or 1 patient survivals treated

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with ICI-based regimens were [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. Even with the unfavorable ICI benefit patients in the IO cohort, the ICI-based treatment OS outcomes in the ECOG PS 2 patients predicted by plasma cfRNA PD-L1 expression was not inferior to our ECOG PS 1 patients and was superior to published ECOG PS 2 real-world OS data with tissue PD-L1 protein expression and ICI-based therapy.

The ICI treated patients' OS associated with plasma cfRNA PD-L1 expression in this real-world patient experience well likely underestimates the OS that would be predicted in a very different and very select patient population treated in the ICI clinical trials that tissue PD-L1 protein predicted. Similarly, the ChemoRx cohort OS outcomes also reflect a completely different patient population treated with an expected much poorer OS than those treated in the ICI clinical trials. With over half of the patients in the ChemoRx cohort having an ECOG PS of 2, the reported median OS of 3 months and 3-year OS of 10% are very close to the published PS 2 chemotherapy treated data with a median OS of 3.9 months and 2-year OS of 6%. The Pharma ICI clinical trials chemotherapy treated arm only including ECOG PS 0 or 1 patients, are also very close to the expected much better median OS and 2-year OS of 16% of that population [25]. Treatment with cross over ICI also plays a factor improving OS in the ICI clinical trials. None of our patients received salvage ICI therapies. The OS outcomes of the ICI-based and chemotherapy treatment arms in both the ICI clinical trials and our real-world data accurately reflect what would be expected for the different patient populations treated.

There are often-forgotten issues when any liquid versus tissue molecular marker test comparison is discussed, First is the simple fact that tissue may not be accessible, available, or sufficient for the testing. In our ICI treated IO cohort, 37% of the patients were either tissue PD-L1 negative or were tissue QNS for molecular testing. This tissue lack/QNS issue has been reported to be as high as 44% [31]. The second issue is the dynamic changes of PD-L1 expression with treatment and with cancer recurrence and/or progression [32,33]. A prior tissue biopsy assessment of PD-L1 expression has no utility as a current predictive immune biomarker. Third is the significant additional tissue acquisition cost compared to a simple blood test, along with scheduling needs and often delays in obtaining tissue, potential tissue biopsy complications, and at home quality time lost for patients [34,35]. These issues further emphasize the clinical impact and importance to patients of having a plasma-based immune biomarker. The positive plasma cfRNA PD-L1 expression was able to overcome the tissue PD-L1 negative expression and tissue QNS issue in our patient experience. This becomes vitally important outside of NSCLC where positive PD-L1 expression is the selection threshold of ICI-based treatment. With a predictive plasma cfRNA PD-L1 expression assay, patients without sufficient tissue and/or when their cancer recurs or progresses, who would otherwise miss out and not get ICI treatment due to the lack of tissue testing, could be identified, and receive the potential durability benefit of ICI treatment.

There are well acknowledged limitations of this reported patient experience. It is a retrospective collection of outcomes data and not a prospective randomized comparison, there is a modest patient population size, and all treatment was at a single institution. Our presented outcomes data also only reflects patients with plasma cfRNA PD-L1 expression. Similar outcomes data with ICI-based treated patients who were plasma PD-L1 negative treated at our institution was not captured in this study. There was no longitudinal testing to determine dynamic cfRNA PD-L1 changes and outcomes.

As with all data with these limitations, larger prospective clinical outcomes studies are needed. In particular, a more precise correlation of the cfRNA PD-L1 PCR Ct-values with ICI OS and a better understanding of any potential immune tumor biology differences and ICI treatment outcomes in the PD-L1 plasma positive/tissue negative and

conversely PD-L1 plasma negative/tissue positive subsets of patients are vitally important for subsequent studies.

## 5. Conclusion

In our real-world patient experience, plasma cfRNA PD-L1 expression was associated with a statistically significant and clinically meaningful survival benefit with ICI-based systemic treatment compared to chemotherapy. The 30% 3-year OS parallels the tissue PD-L1 protein predictive Pharma ICI clinical trials outcomes. It also reflects one of the largest patient experiences with plasma cfRNA PD-L1 expression and ICI-based treatment OS clinical outcomes supporting the potential benefit and clinical utility of plasma cfRNA PD-L1 as a predictive immune biomarker.

**Author Contributions:** Conceptualization: PRW, MM; Methodology: PRW; Software: PRW, SJ; Validation: PRW, MM; Formal Analysis: PRW; Investigation: PRW, MM, SJ, PN, NS; Resources: PRW, PN, NS; Data Curation: MM, SJ; Writing—Original Draft Preparation: PRW, RBL; Writing—Review and Editing: All authors; Visualization: PRW, MM, SJ, RBL; Supervision: PRW, MM; Project Administration: PRW, PN, NS, TP, CC; Funding Acquisition: MM.

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**Institutional Review Board Statement:** The study was approved by the institutional review board of the Brody School of Medicine at East Carolina University (NO: UMCIRB 20-002795).

**Informed Consent Statement:** Patient consent for this de-identified retrospective analysis was waived by the Brody School of Medicine IRB. However, individual specific cancer treatment consent was obtained in all patients.

**Data Availability Statement:** Data is contained within the article.

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**Conflicts of Interest:** PRW is Emeritus faculty of the Brody School of Medicine at East Carolina University and a current employee of Circulogene. MM has received funding from Circulogene. RBL is on the board of Circulogene. The other authors have no conflicts of interest to declare. The sponsors had no role in the design, execution, interpretation, or writing of the study.

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