Baseline Biomarkers of Immune Checkpoint Inhibitor Clearance and Efficacy in Cancer Cachexia

Research Thesis

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by

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Abstract

Background: Cancer cachexia is a complex metabolic syndrome that is characterized by the loss of body weight, specifically skeletal muscle and adipose tissue. It leads to a lower quality of life and accounts for an estimated ~20% of cancer-related mortalities. Despite cancer cachexia's impact on patient survival, it remains underrecognized and underdiagnosed. Immune checkpoint inhibitors (ICIs) are monoclonal antibodies that target the body's immune checkpoint proteins to stimulate the immune system to fight cancer. ICI therapy has vastly improved the treatment of certain cancers but only a limited number of patients respond. The reasons for this variable response are largely unknown but the rate of ICI clearance has been shown to be a predictor of response to ICI therapy. Also, clearance has been shown to coincide with some markers of cancer cachexia. This study aimed to determine baseline relationships among biomarkers of cachexia, ICI clearance, and ICI efficacy using clinical data from cancer patients. Identifying biomarkers that link cancer cachexia and ICI clearance to outcomes may help to increase our understanding of the relationships between cancer cachexia, elevated ICI clearance, and durable responses to ICIs.

Methods: The study population is part of the ongoing non-interventional clinical trial, OSU20001. Data from 48 patients with either non-small cell lung cancer (n=37) or renal cell carcinoma (n=9), receiving pembrolizumab (n=28) or nivolumab (n=17) were analyzed which included computed tomography images, baseline clinical lab results and cytokine signatures taken at predetermined timepoints.

Results: Baseline biomarkers of interest were analyzed by linear regression against lean mass index (LMI) and ICI baseline clearance (ICI CL). In ICI CL vs. biomarkers, IL-6 ($p=0.024$), albumin ($p=0.038$), ferritin ($p=0.0084$), and absolute monocyte count ($p=0.0016$) all gained significance. In LMI vs. biomarkers, adiponectin ($p=0.022$), absolute neutrophil count ($p=0.022$), and platelet ($p=0.038$) gained significance. In addition to linear regression, a survival with competing risk analysis was completed based on patient's either receiving a nivolumab or pembrolizumab treatment. In patients with nivolumab, CC5a ($p=0.032$), CCL5 ($p<0.001$), SerpinE1 ($p=0.001$), CRP ($p=0.01$), Beta2m ($p=0.002$), pentraxin2 ($p<0.001$), Alpha2m ($p=0.024$), and absolute eosinophil count ($p=0.007$) were all significant with CCL5, SerpinE1, CRP, Alpha2m, and absolute eosinophil count would be thought to increase risk of death. In patients with pembrolizumab, the biomarkers that obtained a significant p-value were ICAM1 ($p=0.019$), Ferritin ($p=0.049$), TIMP1 ($p=0.038$), AST ($p=0.02$) and LDH ($p=0.045$) with ICAM, ferritin, and TIMP1 thought to increase risk of death.

Conclusions: The IL-6 and albumin results agreed with previous literature findings as known markers of cachexia and clearance. There were several interesting results such as the significant relationships between BDNF, adiponectin, and platelet, and ICI CL or cachexia. Ferritin was the one biomarker that was associated with cachexia, ICI CL, and survival, suggesting that baseline ferritin could be a helpful biomarker in determining a patient's response to ICI therapy. This new information could be helpful in determining the relationship between cancer patients, cachexia, and the variable response to ICI therapy.

Background

Cancer cachexia is a multifactorial syndrome characterized by the loss of body weight with specific losses in skeletal muscle mass, that cannot be fully reversed by nutritional support and leads to reduced physical function [1]. Ultimately, cancer cachexia leads to a lower quality of life and is a contributor to cancer-related mortality [1]. Cancer cachexia is a complex metabolic syndrome and is broadly associated with poor outcomes in many therapeutic strategies [1]. Cancer cachexia is estimated to impact ~50% of all cancer patients, and accounts for ~20% of cancer related mortalities depending on disease and staging [2, 3]. Despite cancer cachexia's impact on patient survival, the syndrome continues to be unrecognized and underdiagnosed due to the multifaceted disease pathogenesis and lack of consensus definition of baseline measures required for analysis [4].

Self-reported weight loss is the most common form of diagnosis for cachexia, but it has many limitations due to the difficulty in representing cachexia accurately across a wide range of patients, therapies, cancer type, as well as variability in self-reporting [4]. An alternative approach to assess cancer effects on patient body composition is through the use of computed tomography (CT) images taken as part of routine care for cancer patients. CT scan analysis has been shown to accurately represent whole body composition and has been used to aid in the determination of muscle surface area and density at the level of the 3rd lumbar vertebrae (L3) [4]. Despite this advanced analysis for cancer cachexia induced body composition changes using CT scans, there are few baseline markers available to assess patients with cachexia and the link to outcomes involving immune checkpoint inhibitor therapy.

The body's immune system has the capability to determine which cells are normal to the body and which cells are cancerous and out of cell cycle arrest. Recently, new therapies for the treatment of cancer leverage the immune system to fight off cancerous malignancies. This is done by targeting immune checkpoint receptors that are found on immune cells. Checkpoint receptor binding will inhibit an immune response, and cancer cells can use these checkpoints to evade anti-tumor immunity [5]. These checkpoints are now targeted in therapy as there are drugs that can bind to target checkpoint receptors and inhibit their signaling in order to stimulate the anti-tumor immunity and attack the cancer cells in the body [5]. One type of immune checkpoint inhibitors (ICI) are PD-1 inhibitors, or anti PD-1. These include the monoclonal antibodies (mAb), pembrolizumab and nivolumab. The use of ICI mAb therapies has greatly improved the treatment of certain cancers offering benefit over chemotherapy in non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC), and more [6, 7]. However, current estimates state that only 20-40% of patients respond to this type of treatment, with little knowledge of underlying mechanisms behind this variation in response [6]. Rate of ICI clearance (CL) has been shown to be a strong predictor of response to ICI therapy. Patients with rapid rates of ICI CL display reduced overall survival compared to patients with low rates of ICI CL, and this observation is unaffected by dose and subsequent drug exposure [8]. This indicates that rate of ICI CL is a biomarker, but not a cause, of response to ICI therapy. It has been observed in these studies that elevated rate of ICI CL coincides with markers of cancer cachexia, such as reductions in body weight and hypoalbuminemia [8]. There is a need for studies to identify biomarkers for, and to increase our understanding of the relationship among cancer cachexia, elevated ICI CL, and durable responses to ICI therapy.

There are several published reports of cytokines and clinical lab metrics that have been shown to be predictive for baseline ICI CL and survival outcomes. Cytokines are circulating proteins that regulate the immune system and can contribute to anti-cancer activity. Cytokines that have been associated with ICI CL include: TNFα, IL-6, and ferritin [9]. It has been shown that these cytokines can predict clearance values that correlate with elevated risk of adverse events, poor prognosis, and a shorter overall survival with treatment [10]. However, these biomarkers have primarily been evaluated for clearance and do not directly evaluate associations with either cachexia or outcomes. Therefore, in the on-going OSU20001 clinical trial, patient individual ICI CL rates have been estimated and will be evaluated to determine relationships among circulating biomarkers of ICI CL, cancer cachexia, and response to ICI therapy. This study aims to identify circulating cytokines, clinical lab metrics, and biomarkers that relate ICI CL, cachexia, and therapeutic outcomes.

Materials and Methods

Human Subjects:

The study population used for analysis was part of the ongoing non-interventional clinical trial, OSU20001, overseen by head PI Dwight Owen, MD (Internal Medicine - Medical Oncology, The Ohio State University). Data from a total of 46 patients with either NSCLC (n=37) or RCC (n=9), receiving pembrolizumab (n=28) or nivolumab (n=17) were used in this analysis. Patients had an average age of 65 (range 38-81) and received standard of care (SOC) therapy plus 200mg infusions of either pembrolizumab or nivolumab every 21 days for the duration of therapy. Patient pharmacokinetic timepoints were taken 30 minutes pre-dose and 30 minutes post dose of every infusion. Pre dose of Cycle 1 day 1 (C1D1) serum samples served as baseline when measuring circulating analytes. CT images, as well as baseline and longitudinal clinical lab results (absolute neutrophil, monocyte, and lymphocyte counts, albumin, aspartate transaminase, and alanine aminotransferase) and cytokine signatures were taken for each patient at predetermined timepoints.

Ethics Approval and Consent

All human subject research has been approved by The Ohio State University Institutional Review Board.

L3 Analysis: [4]

Baseline SOC computed tomography (CT) scans were taken from each enrolled patient. The 3rd lumbar vertebra (L3) was identified using Slice-O-Matic v4.31. Then landmarking, the process of identifying anatomical locations of interest was completed. For the lumbar vertebra, these features are short spinous processes, a large body, and small vertebral foramen. The image was then analyzed by tagging predetermined tissue areas. Tissue density was measured in Hounsfield Units (HU) and the sections of the L3 were tagged in different colors based on their varying HU scale. Each L3 image was tagged and measured for surface area of skeletal muscle (-

29 to +150 HU), intramuscular adipose tissue (-190 to -30 HU), visceral adipose tissue (-150 to -50 HU), and subcutaneous adipose tissue (-190 to -30 HU). The next step involved the analysis of the tag results. To measure skeletal muscle index (SMI) the skeletal muscle cross-sectional surface area (total CSA, cm^2) was normalized to patient height (CSA/height^2, cm^2/m^2). Lean mass index (LMI) was attained by taking the established sex specific SMI cutoffs and dividing it by the patient SMI [4]. An LMI value greater than 1 would indicate a skeletal muscle index that is below the threshold of cachexia and a value below 1 indicating no cachexia as patient lean mass is above validated thresholds of cachexia.

Pharmacokinetic Analyses:

Unbound patient pembrolizumab (n=26) and nivolumab (n=15) from clinical plasma samples were measured by ELISA as previously described [11, 12]. Pharmacokinetic parameters were estimated with a nonlinear mixed effects model in NONMEM, Version 7.3. Patient ICI concentrations were fit to a linear two compartment model that has been previously published and a posthoc, first-order conditional estimation method was utilized to estimate individual patient pharmacokinetic parameters [13, 14].

Best Overall Response: [15]

Each enrolled patient that underwent SOC treatment was assessed at baseline and study follow-ups to determine the best overall response (BOR) per Response Evaluation Criteria in Solid Tumors (RECIST) criteria. RECIST is the measure of the response of a tumor size in comparison to baseline and with treatment. The BOR score is determined based on a 1-4 scale. 1 being complete response and 4 being progressive disease. For analysis, patients that were evaluable for RECIST and BOR data were separated into their respective BOR groups. Each BOR group contained patients with treatments of both pembrolizumab and nivolumab. Patient BORs were plotted against SMI, skeletal muscle density (SMD), cachexia score, and cytokine/clinical lab results. Each resulting graph was analyzed through GraphPad Prism 9.0, licensed to the Ohio State University, using a Kruskal-Wallis statistical test to determine the significance between BOR groups in relation to each covariate factor.

Cytokine analysis

Baseline plasma cytokine concentrations were measured using a custom-built Human Luminex Discovery Assay multiplex kit (catalog #LXSAHM-02, Bio-techne, MN, USA) according to the manufacturer's protocol. All analytes measured included Alpha 2-Macroglobulin, Pentraxin 2/SAP, BDNF, VEGFR3, CCL5, CCL18, Ferritin, Myoglobin, TIMP-1, Adiponectin, C-Reactive Protein (CRP), Serpin E1, beta-2-Microglobulim, CC5a, ICAM-1, IL-1 beta, IL-4, IL-6, IL-10, IL-12, IL-13, IL-33, RAGE/AGER, ACF/c-kit, TNF-a, TNF RII, VCAM-1, and vWF. Briefly, patient baseline plasma samples were centrifuged for 4 min at 596

(x g) and the supernatants diluted to recommended amounts with calibrator diluent. Fifty µl of samples and standard were then plated into a 96-well black plate in duplicate. Samples and standards were then incubated with 50 µl of Microparticle Cocktail for 2 h on a plate shaker at 800 rpm. Following 3 washes with 100 µl of wash buffer using a handheld Magnetic 96-well Separator (Catalog #A14179, Fisher Scientific, Waltham, MA, USA), samples were incubated with 50 µl of biotin antibody cocktail for 1 h, washed, and incubated for 30 min with 50 µl of Streptavidin-PE. After a final wash, microparticles were resuspended in 100 µl of wash buffer and measured on a Luminex Magpix instrument (Luminex, Austin, TX, USA) according to manufacturer's guidelines.

Statistics:

Spearman's rank correlation coefficient was used for linear regression analysis and conducted in R Studio v4.3.2 (R Core Team, Vienna, Austria). Survival with competing risk analysis was carried out using RStudio with an alpha level of alpha = 0.05. Comparison of BOR group values was done using a non-parametric one-way ANOVA, or Kruskal-Wallis test.

Results

Given the relationship of ICI CL, cachexia, and efficacy, we sought to understand if there were relationships between measured baseline biomarkers of each variable. In this study 46 patients were enrolled with either NSCLC (n=37) or RCC (n=9), receiving either pembrolizumab (N=28) or nivolumab (N=17). Of this total data set only, n=34 patients were evaluable for baseline ICI CL (Table 1).

BOR

There were no significant results among any covariates compared to BOR groups (Data not shown). This could be due to having a low number of patients (n=21) evaluable for BOR per a RECIST criteria.

Linear Regression

Baseline biomarkers of ICI CL, efficacy, and cachexia were analyzed by linear regression against LMI and baseline ICI CL in order to better understand the relationship between them. When comparing baseline ICI CL versus circulating biomarkers by linear regression (Table 2), there were observed relationships that were expected and also unexpected. IL6 (Figure 1A) and albumin (Figure 1B) levels both significantly correlated with ICI CL with p-values of 0.024 and 0.038, respectively. IL6 had an R value of 0.42 with a positive correlation, while albumin had an R value of –0.38 with a negative correlation. Both IL6 and albumin are well-studied biomarkers. IL6 is an inflammatory marker associated with cachexia, while albumin is a marker of ICI CL in

relation to a shared mechanism of protein degradation and salvage. In addition to IL6 and albumin, significant correlations between ICI baseline clearance with absolute monocyte count (AMC; Figure 1C) and ferritin (Figure 1D) were observed. Ferritin and AMC displayed significantly positive correlations with CL, with p-values of 0.0084 and 0.0016 respectively.

When comparing patient skeletal muscle mass (in the form of LMI) versus cytokines/clinical lab results by linear regression, there were several interesting results (Table 3). Adiponectin (Figure 2A), absolute neutrophil count (ANC; Figure 2B), and platelet count (Figure 2C) all had significant p-values of 0.022, 0.022, and 0.038, respectively, with positive correlations with LMI. This suggests that higher plasma concentrations of these circulating biomarkers are associated with the severity of lean mass depletion, i.e. cachexia.

Survival with Competing Risk Analysis:

Survival with competing risk analysis was done in R Studio. Concentrations of baseline cytokines and clinical lab results for each enrolled patient were used to determine log(Hazard Ratio), a 95% confidence interval, and p-value. Analysis was separated by treatment into patients who received nivolumab (Table 4) and those who received pembrolizumab (Table 5).

In patients receiving nivolumab treatment, the cytokines that obtained a significant value were CC5a ($p=0.032$), CCL5 ($p<0.001$), SerpinE1 ($p=0.001$), CRP ($p=0.01$), Beta2m ($p=0.002$), pentraxin2 ($p<0.001$), and Alpha2m ($p=0.024$). The clinical lab result that obtained a significant p-value was absolute eosinophil count (AEC; $p=0.007$). The log(Hazard Ratio) and 95% confidence interval were plotted on a forest plot, the cytokines and clinical lab results that had a significant p-value and were to the right of the line of null effect, were CCL5, SerpinE1, and AEC (Figure 3A). Positive log(Hazard Ratio) values would indicate a unit increase in value, which would be associated with a higher risk of event occurring, in this case death.

In patients receiving pembrolizumab treatment, the cytokines that obtained a significant p-value were ICAM1 ($p=0.019$), ferritin ($p=0.049$), and TIMP1 ($p=0.038$). The clinical lab results that obtained a significant value were aspartate aminotransferase (AST; $p=0.02$) and lactate dehydrogenase (LDH; $p=0.045$). Log(Hazard Ratio) and 95% confidence interval were then plotted (Figure 3B). Cytokines and clinical lab results with a significant p-value that were to the right of the line of null effect were ICAM1, and LDH, suggesting that these covariates are associated with an increase in risk of death and decrease in the survival rate. Interestingly, ferritin had a significant negative log (Hazard Ratio) to the left of the null effect line.

Discussion

The goal of this study was to explore baseline relationships between biomarkers of cancer cachexia, ICI CL, and treatment efficacy. The data collected suggests relationships between several known biomarkers as well as demonstrating connections between new ones. When investigating patient body composition (using LMI), in order to approximate cancer cachexia,

versus cytokines and clinical lab results, we observed interesting relationships. An LMI value greater than 1 would indicate a skeletal muscle index that is below the threshold of cachexia and a value below 1 indicating no cachexia as patient lean mass is above validated thresholds of cachexia. LMI in this patient dataset displayed a significant positive correlation with adiponectin, ANC, and platelets. This suggests that as the circulating levels of the cytokine and two clinical lab results increase, the magnitude of cachexia and lean mass depletions also increase. This observation would agree with literature reports of high plasma levels of adiponectin in patients with cancer cachexia [16]. Interestingly, adiponectin has also been implicated as a biomarker of nivolumab clearance in patients [9]. Platelets are associated with the promotion of skeletal muscle regeneration through the recruitment of neutrophils to the injured site [17]. However, platelet activity can be reduced into further stages of skeletal muscle injury and could be contributing to the lack of new growth in patients with prolonged cachexia [17]. Platelet counts have even been implicated as a significant negative predictor of survival in a cachectic clinical population [18]. An interesting clinical lab result that did not achieve significance but had a strong correlation was absolute lymphocyte count (ALC). This suggests that at baseline, lower lymphocyte numbers are associated with increased severity of cachexia. When calculating neutrophil to lymphocyte ratio (NLR), we find that NLR is higher in patients with cachexia than in those without. This supports other known findings suggesting that NLR is an inflammatory response that is significantly elevated in patients with cachexia [19].

When looking at the relationship between baseline ICI CL versus cytokines/clinical lab results, there were observed relationships that were expected. Both IL6 and albumin are well-studied biomarkers of cancer. A positive relationship between ICI CL and circulating IL6 concentrations is consistent with the idea that IL6 is a proinflammatory cytokine; thus, it would be associated with higher levels of CL, and both are considered to be linked with a low survival rate [20]. A negative correlation between baseline ICI CL and albumin levels is consistent with their shared mechanism of protein degradation and is interesting because it suggests that high levels of albumin along with a lower baseline clearance, would be associated with a higher response to ICI therapy and improved survival [21, 22]. In addition to IL6 and albumin, relationships between ICI CL with ferritin, BDNF, and AMC were observed. The relationship with BDNF, a neurotrophin that helps to develop the central nervous system, was close to significant ($p=0.07$). The negative correlation between BDNF and ICI CL could suggest that higher levels of BDNF would be associated with lower baseline CL which could have potential utility as a biomarker of survival on ICI therapy [23]. Ferritin and AMC both displayed significant positive correlations with baseline ICI CL. Studies have highlighted that increased circulating levels of ferritin are correlated with an increased response to ICIs [24]. However, the positive correlation between ferritin and clearance in these preliminary clinical trial results suggests that increased levels of ferritin may be associated with a higher level of baseline CL, which would be a potential biomarker of poor response to ICI therapy. AMC is the count of monocytes at baseline in a patient's blood. It has been shown that higher AMC baseline counts are associated with a lower survival rate [25], so the positive correlation that is shown between AMC in these clinical trial patients and baseline clearance would agree with these findings.

When carrying out survival with competing risk analysis, covariates with a positive log(HR) ratio (i.e. values to the right of the line of null effect), are presumed to favor the control, which, in this study, was the effect of disease progression without treatment. Thus, these covariates would contribute to increased risk of death and decreased survival rate. Patients that were treated with nivolumab had circulating biomarkers at baseline with a positive log(HR) and a significant p-value: CCL5, SerpinE1, and AEC. CCL5 is part of the CCL5/CCR5 axis that plays a role in tumor progression. Higher levels of CCL5 are associated with increasing tumor growth and enhancing metastasis [26]. This cytokine is also associated with increased cancer cell resistance to treatment [26]. The significantly positive log(HR) would support these findings, further suggesting that in this clinical study population, an increased amount of CCL5 at baseline would be associated with an increased risk of death and poor response to ICI therapy. SerpinE1 has been studied in relation to skeletal muscle fibrosis and patients with cancer cachexia are often found with high levels of this cytokine [27].

CRP is a plasma protein that is often linked to high inflammation that is associated with poor cancer prognosis and cancer cachexia [28]. CRP is believed to be involved in cachexia indirectly through regulation of IL-6 levels suggesting that higher levels of CRP would be associated with high levels of IL-6, then potentially leading to poor response to therapy and cancer cachexia [28]. However, despite evidence in literature of high CRP levels being associated with cachexia and poor response to therapy, we see the opposite in which CRP had a significantly negative log(HR) value in the survival analysis. This suggests that higher levels of CRP are associated with decreased risk of death in this limited data set, which would not agree with its role in literature. Future work should be focused on further understanding CRP involvement in ICI response. Lastly, AEC is a count of circulating eosinophils, which are a type of white blood cell that functions to protect the body from pathogens. AEC showed a significantly positive log(HR) value, indicating increased association with death in this data set. Elevated AEC levels have been reported in patients that display shorter overall survival to ICI therapy [29]. Future work should focus on understanding eosinophil involvement in cachexia pathogenesis.

In patients treated with pembrolizumab, ICAM1, and LDH were found with a significantly positive log (HR), while AST, ferritin, and TIMP-1 had a significantly negative log (HR). This suggests that increases in ICAM1 and LDH are associated with an increased risk of death. ICAM1 is a regulator of many tissue functions but as a spliced variant it contributes to inflammation and tumor development [30]. Interestingly, increased circulating levels of TIMP-1 has been correlated with increased CD8+ T cell tumor infiltration, as well as leading to enhanced dendritic cell mediated immunogenicity, offering a potential role of TIMP1 in immune cell signaling axis [31]. Baseline levels of TIMP-1 levels have also been shown to be an important predictor of ICI clearance [9, 10]. Interestingly, there are differing reports of ferritin involvement in ICI treatment. It is an indicator of iron levels within patients and has been suggested that increased ferritin levels lead to an increased response to ICIs [24]. This would agree with the survival analysis in which baseline ferritin levels were a significant predictor of survival with higher levels of ferritin at baseline being associated with decreased risk of death. However, plasma ferritin levels have also been shown to be increased in patients with cachexia versus

without [32]. Ferritin's role in cachexia and skeletal muscle wasting is unclear however, as reports in pre-clinical models have shown that muscle atrophy can be alleviated by ferritin supplementation [33]. Interestingly, there was an observed significant positive relationship between ferritin levels at baseline and rate of ICI CL in this patient data set. Given ferritin's significance in this data set with ICI CL and patient survival, as well as its role in cachexia progression as suggested by literature, it could be a potential biomarker of both survival in ICI therapy and ICI CL that is associated with body composition. Ferritin's potential utility as a biomarker of body composition, ICI CL, and ICI response should be investigated further.

Conclusion

Cancer cachexia is a complex multifactorial syndrome that reduces patients' quality of life. It continues to be underrecognized and underdiagnosed despite being a major contributor to cancer related mortality. It is difficult to recognize and diagnose due to a lack of consensus definition and its variety of symptoms[4]. In addition, patients with cancer cachexia who are receiving ICI therapy may not respond to this treatment. So, while cachexia affects ICI CL and efficacy, there is still an unmet need for biomarkers of response to therapy, body composition, and CL. This study aimed to determine baseline relationships between biomarkers of cachexia, CL, and efficacy. In the evaluation of this limited study population, there were several expected results that agreed with previous literature findings and known markers of cachexia and CL. IL-6 and albumin significantly correlated with rate of ICI CL and are also known biomarkers of cancer cachexia. When comparing the results of the linear regression and survival analysis, there are several biomarkers that had interesting relationships with cachexia, CL, or efficacy such as platelet, CRP, and adiponectin. One biomarker that was associated with ICI CL, and survival was ferritin. Ferritin is an intracellular protein that stores and releases iron, but also highly implicated in cancer cachexia. It is also a factor in muscle wasting as ferritin administration remediated muscle wasting in preclinical models [33]. However, elevated levels of ferritin have been observed in cancer patients experiencing muscle loss [34]. Given the involvement of ferritin in ICI CL, ICI efficacy, and skeletal muscle wasting, our findings might suggest that ferritin could be a useful biomarker in determining a patient's response to ICI therapy. Future work should be focused on further understanding plasma ferritin levels' role in ICI therapy. These novel observations of several biomarkers that relate to all three areas of interest could be helpful in determining the relationship between cancer patients, cachexia, and the variable response to ICI therapy.

Tables and Figures

Table 1. Demographics of patients from OSU20001

Patient Demographics		n=	% of total
Cancer type			
	NSCLC	37	80%
	RCC	9	20%
Immunotherapy			
	Pembrolizumab	15	33%
	Pembro + Chemo	13	28%
	Nivolumab (+ipilimumab)	12	26%
	Nivo + Chemo	4	9%
	Other	1	2%
Sex			
	Male	28	61%
	Female	18	39%
Baseline ECOG			
	0	13	28%
	1	22	48%
	2	11	24%
Age			
	Mean	65 (±9.1)	
	Range	40-81	

Table 2. Linear regression analysis of all analytes (cytokines, clinical lab metrics) versus clearance

CL and Cytokines **Table 2**

Cytokine	Rationale	R Value	P Value
TNF Alpha	Cachexia	0.14	0.45
IL-10	Cachexia	-0.12	0.56
Von Willebrand Factor	Clearance	-0.07	0.73
SCF	Clearance	-0.05	0.79
CC5a	Cachexia	0.00	0.97
CCL18	Cachexia	0.35	0.06
Ferritin	Clearance	0.48	0.008
CCL5/Rantes	Clearance	-0.27	0.14
IL-4	Cachexia	0.18	0.34
RAGE	Clearance	-0.12	0.53
ICAM 1	Clearance	-0.18	0.33
Myoglobin	Clearance	0.12	0.54
C-Reactive Protein	Cachexia/Clearance	0.18	0.34
Serpin E1	Clearance	-0.05	0.78
IL-6	Cachexia	0.42	0.02
Adiponectin	Clearance	-0.11	0.57
TNFRII	Clearance	0.18	0.34
IL-1b	Cachexia	0.22	0.24
BDNF	Cachexia	-0.34	0.07
ANC (neutrophil count)	Clinical Monitoring	-0.01	0.97
AMC (monocyte count)	Clinical Monitoring	0.55	0.002
albumin	Clinical Monitoring	-0.38	0.038
Platelet	Clinical Monitoring	0.04	0.85
ALC (leukocyte)	Clinical Monitoring	-0.01	0.95
Total Bilirubin	Clinical Monitoring	0.09	0.66
ALT	Clinical Monitoring	-0.18	0.35
AST	Clinical Monitoring	-0.04	0.85
LDH	Clinical Monitoring	-0.17	0.42
VEGF-3	Clearance	-0.17	0.36
Beta-2 microglobulin	Clearance	0.09	0.64
Pentraxin-2	Cachexia	0.00	0.97
Alpha-2 macroglobulin	Clearance	0.018	0.93

Figure 1

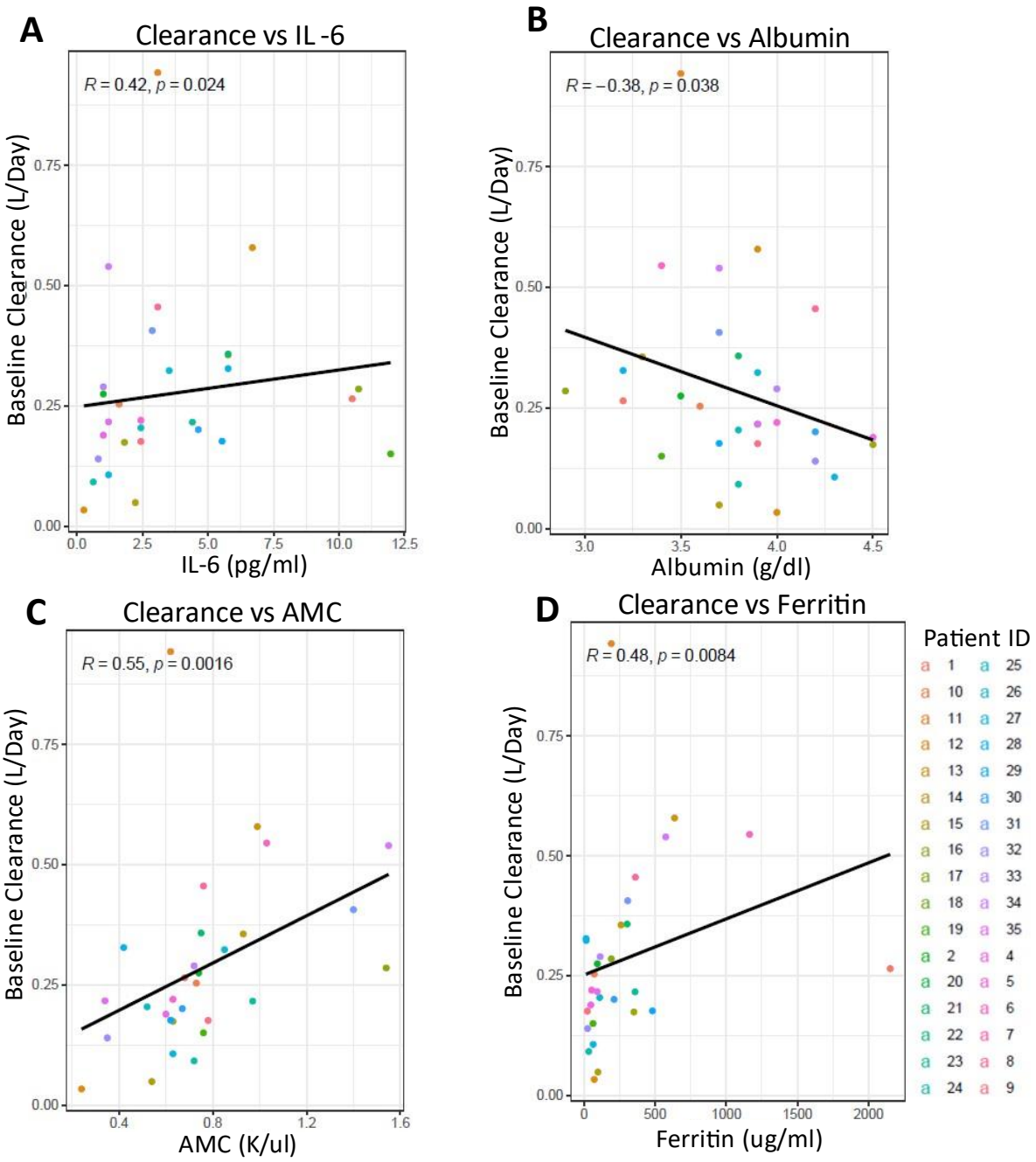

Figure 1. Significant correlations between baseline ICI CL and covariates. Relationships between patient sample analytes (cytokines, clinical lab metrics) and baseline ICI (nivolumab, pembrolizumab) CL were analyzed by linear regression as described in Materials and Methods. Only those analytes that showed a significant ($p<0.05$) relationship are shown.

Table 3. Linear regression analysis of all analytes (cytokines, clinical lab metrics) versus lean mass index

LMI and Cytokines **Table 3**

Cytokine	Rationale	R Value	P Value
TNF Alpha	Cachexia	0.01	0.95
IL-10	Cachexia	-0.01	0.68
Von Willebrand Factor	Clearance	0.28	0.12
SCF	Clearance	-0.04	0.84
CC5a	Cachexia	-0.001	0.97
CCL18	Cachexia	0.02	0.91
Ferritin	Clearance	0.28	0.12
CCL5/Rantes	Clearance	0.09	0.61
IL-4	Cachexia	0.05	0.8
RAGE	Clearance	-0.02	0.92
ICAM 1	Clearance	0.06	0.74
Myoglobin	Clearance	-0.09	0.61
C-Reactive Protein	Cachexia/Clearance	0.12	0.49
Serpin E1	Clearance	0.08	0.69
IL-6	Cachexia	0.10	0.6
Adiponectin	Clearance	0.40	0.02
TNFRII	Clearance	0.12	0.52
IL-1b	Cachexia	-0.04	0.84
BDNF	Cachexia	-0.18	0.32
ANC (neutrophil count)	Clinical Monitoring	0.4	0.02
AMC (monocyte count)	Clinical Monitoring	0.16	0.38
albumin	Clinical Monitoring	-0.15	0.42
Platelet	Clinical Monitoring	0.37	0.04
ALC (lymphocyte count)	Clinical Monitoring	-0.31	0.09
Total Bilirubin	Clinical Monitoring	0.29	0.11
ALT	Clinical Monitoring	-0.23	0.2
AST	Clinical Monitoring	-0.19	0.29
LDH	Clinical Monitoring	0.18	0.36
VEGF-3	Clearance	0.05	0.78
Beta-2 microglobulin	Clearance	0.15	0.39
Pentraxin-2	Cachexia	0.21	0.24
Alpha-2 macroglobulin	Clearance	0.31	0.08

Figure 2

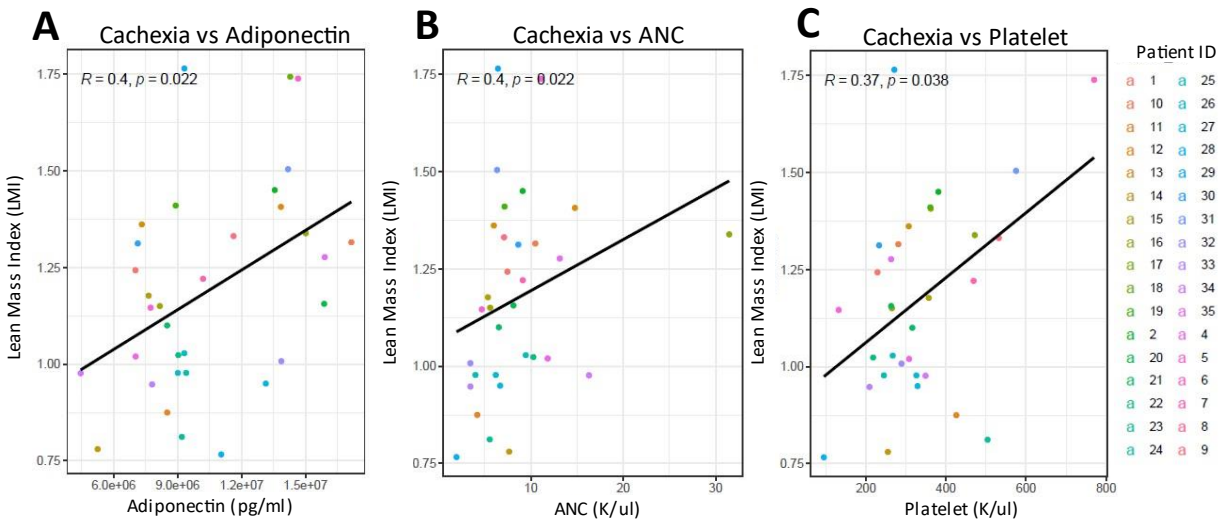

Figure 2. Significant correlations between LMI and covariates.

Relationships between patient sample analytes (cytokines, clinical lab metrics) and patient lean mass index (LMI) were analyzed by linear regression as described in Materials and Methods. Only those analytes that showed a significant ($p<0.05$) relationship are shown.

Table 4. Survival with competing risk analysis of analytes (cytokines, clinical lab metrics) of patients receiving nivolumab treatment

Nivolumab **Table 4**

Cytokine	Log(HR)	95% C.I.	P Value
TNFa	-0.110	-0.24, 0.02	0.100
IL6	0.110	0.21, 0.43	0.500
CC5a	0.150	0.010	0.032
IL10	0.250	-1.1, 1.6	0.700
SCF	0.000	-0.1, 0.2	0.800
IL1b	0.910	-1.1, 2.9	0.400
IL4	-0.01	-0.05, 0.04	0.7
RAGE	0.02	-0.04, 0.08	0.5
VCAM1	0	0, 0	0.3
ICAM1	0	-0.01, 0	0.3
TNFRII	0.08	-0.21, 0.37	0.6
Ferritin	0.02	-0.04, 0.07	0.6
BDNF	-0.09	-0.19, 0.01	0.093
CCL18	-0.03	-0.45, 0.38	0.9
CCL5	0.06	0.03, 0.09	<0.001
Myoglobin	0.04	-0.17, 0.26	0.7
TIMP1	0.06	-0.08, 0.2	4
vWFA2	0.17	-0.18, 0.52	0.3
SerpinE1	0.08	0.03, 0.13	0.001
Adiponectin	-0.02	-0.05, 0.01	0.3
CRP	-0.26	-0.46, -0.06	0.01
VEGFR3	0.000	-0.1, 0.02	0.700
Beta2m	-0.580	-0.94, -0.22	0.002
pentraxin2	-0.010	-0.02, -0.01	<0.001
Alpha2m	-0.08	-0.15, -0.01	0.024
ANC	0.060	-0.18, 0.31	0.600
AMC	-1.000	-3.6, 1.5	0.400
ALC	-0.390	-1.9, 1.1	0.600
AEC	1.700	0.46, 2.9	0.007
Platelet	0.000	0, 0.01	0.700
Albumin	-0.050	-2.2, 2.1	>0.9
Total Bilirubin	-1.600	-4.0, 0.84	0.200
ALT	0.030	0, 0.07	0.063
AST	0.050	-0.02, 0.12	0.200
LDH	0.000	0, 0.01	0.400
Lean Mass Index	0.820	-2.7, 4.4	0.600
SMD	0.08	0.02, 0.13	0.004

Table 5. Survival with competing risk analysis of analytes (cytokines, clinical lab metrics) of patients receiving pembrolizumab treatment

Pembrolizumab **Table 5**

Cytokine	Log(HR)	95% C.I.	P Value
TNFa	-0.02	-0.15, 0.12	0.8
IL6	-0.04	-0.19, 0.1	0.6
CC5a	-0.21	-0.68, 0.26	0.4
IL10	-0.53	-1.1, 0.09	0.094
SCF	0.01	-0.01, 0.03	0.4
IL1b	0.31	-1.5, 2.1	0.7
IL4	0	-0.82, 0.83	>0.9
RAGE	0.02	-0.08, 0.11	0.7
VCAM1	-0.07	-0.33, 0.2	0.6
ICAM1	0.02	0, 0.03	0.019
TNFRII	0.05	-0.2, 0.3	0.7
Ferritin	-0.01	0, -0.02	0.049
BDNF	-0.46	-1.5, 0.54	0.4
CCL18	-0.06	-0.14, 0.02	0.2
CCL5	-0.32	-0.75, 0.12	0.2
Myoglobin	-0.04	-0.27, 0.2	0.8
TIMP1	-0.11	-0.22, -0.01	0.038
vWFA2	-0.05	-0.23, 0.13	0.6
SerpinE1	0.05	-0.02, 0.12	0.13
Adiponectin	0.01	-0.02, 0.03	0.6
CRP	0.04	-0.02, 0.1	0.2
VEGFR3	0.03	-0.06, 0.12	0.5
Beta2m	-0.01	-0.04, 0.02	0.6
pentraxin2	0	-0.05, 0.05	>0.9
Alpha2m	0	-0.04, 0.04	>0.9
AMC	1.6	0.05, 3.2	0.043
AEC	-1.4	-4.5, 1.8	0.4
Platelet	0.01	0, 0.01	0.08
Albumin	0.83	-1.5, 3.1	0.5
Total Bilirubin	-1.5	-5.2, 2.2	0.4
ALT	-0.23	-0.47, 0.01	0.065
AST	-0.1	-0.19, -0.02	0.02
LDH	0.01	0, 0.01	0.045
Lean Mass Index	-0.35	-2.7, 2	0.8
SMD	-0.02	-0.08, 0.04	0.5

Figure 3

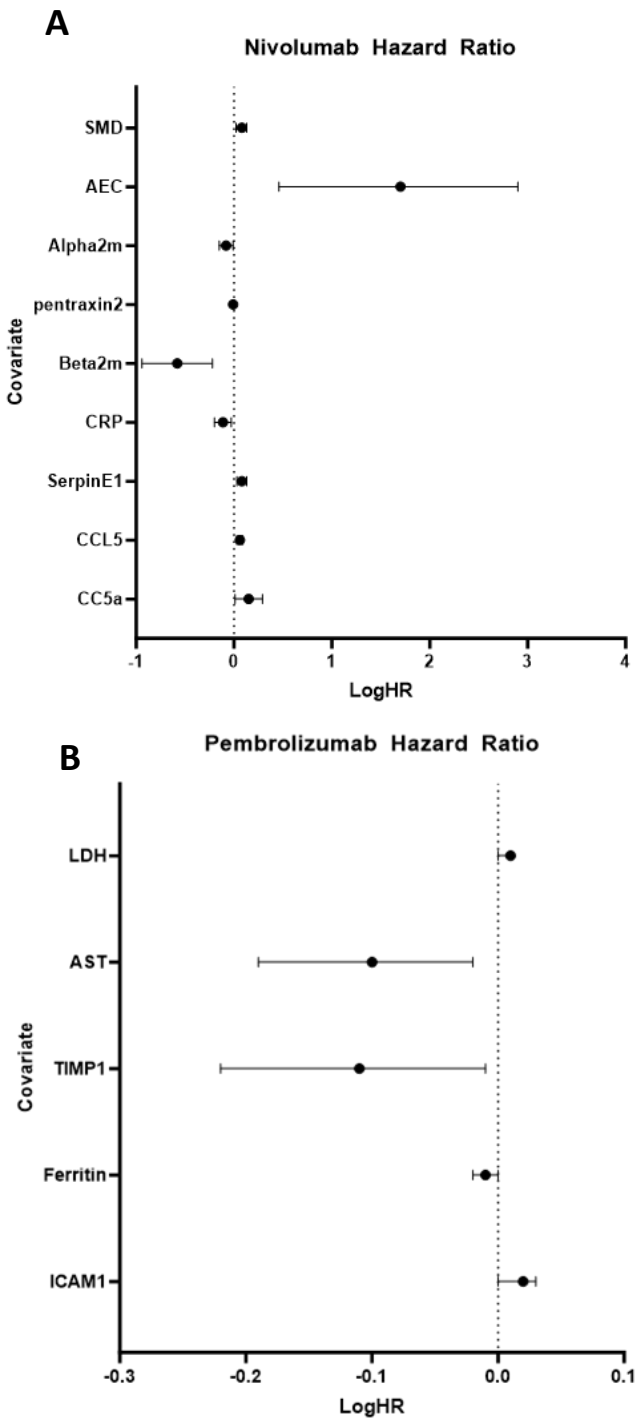

Figure 3. Survival analysis on significant covariates.

A forest plot comparison between analytes' (cytokines, clinical lab metrics) log(HR) (Table 4-5) on the y-axis and the line of null effect on the x-axis with the null difference value at 0. **(A)** based on the patients receiving nivolumab (Table 4). **(B)** based on the patients receiving pembrolizumab (Table 5). Only those analytes that showed a significant ($p<0.05$)

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