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Novel and non-invasive markers of immune checkpoint inhibitor-associated acute kidney injury

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BOSTON UNIVERSITY

ARAM V. CHOBANIAN & EDWARD AVEDISIAN SCHOOL OF MEDICINE

Thesis

**NOVEL AND NON-INVASIVE MARKERS OF
IMMUNE CHECKPOINT INHIBITOR-ASSOCIATED ACUTE KIDNEY INJURY**

by

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DEDICATION

I would like to dedicate this work to my younger self, who dreamed of being where we are today but struggled to believe her dream would come true.

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Words cannot express my gratitude to my principal investigator, Dr. Shruti Gupta, for her invaluable mentorship and support. This endeavor also would not have been possible without Dr. Beth Bragdon, who generously provided her knowledge and feedback. Additionally, I would like to thank Dr. David Leaf, whose expertise was essential for the progression of this research.

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Lastly, I would be remiss in not mentioning my family, especially my parents. Their belief in me has kept my spirits and motivation high during this process. I would also like to thank my cats for all the entertainment and emotional support.

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ABSTRACT

Introduction The introduction of immune checkpoint inhibitors (ICIs), a class of immunotherapy drugs, into the field of hematology/oncology has revolutionized the treatment of many malignancies. The benefits of these medications are enormous, but their use comes with some risks. Through systemic activation of the immune system, ICIs may cause immune-related adverse events (irAEs). One such irAE is acute kidney injury (AKI) directly attributed to the ICI, referred to as ICI-AKI. The consequences of such a diagnosis include temporary or permanent discontinuation of ICI therapy and irreversible loss of kidney function. ICI-AKI is notoriously challenging to diagnose, and the gold standard diagnostic test is a kidney biopsy, which is often contraindicated or difficult to obtain. The incidence of any AKI while on ICIs is 15-20%, though true ICI-AKI is closer to 2-5%. As the breadth of immunotherapy becomes more comprehensive, the need for markers to differentiate true ICI-AKI from other AKI causes becomes more urgent.

Objectives There were two goals for this thesis. Objective 1 was to examine the efficacy of positron emission tomography-computed tomography scan (PET-CT) in diagnosing ICI-AKI, specifically acute tubulointerstitial nephritis (ATIN). Objective 2 was to explore using cell-free RNA (cfRNA) as a biomarker for AKI.

Methods For Objective 1, clinical and radiological data were collected from eight patients with AKI directly attributed to their ICI therapy. These patients had a baseline PET-CT scan performed within 90 days before initiation of ICI therapy and a follow-up PET-CT scan within 14 days before or after ICI-AKI diagnosis. A nuclear radiologist interpreted the scans and provided quantitative data on radiotracer uptake in the renal cortex, referred to as the mean standardized uptake value (SUV_{mean}). We compared baseline and follow-up SUV_{mean} using a two-tailed Wilcoxon signed-rank test with a significance level of 0.05. For Objective 2, a cfRNA analysis of the plasma samples from patients on immunotherapy who develop AKI was conducted. Forty-six samples were taken at the time of AKI (timepoint 1 (TP1)), and 32 had a matched sample 60 days (+/- 30) following AKI (timepoint 2 (TP2)).

Results For Objective 1, an increase in SUV_{mean} was observed in all eight patients during ICI-AKI. The rise in raw SUV_{mean} values was statistically significant (p-value <0.00001). After calculating the average percent change in SUV_{mean} for each patient, the median change was 38.6 (IQR 8.0-58.6). For Objective 2, sixty-one differentially expressed genes (DEGs) were identified at TP1 versus TP2. Two mitochondrial transcripts (MT-ND4 and MT-ATP8) were found whose expression depended on the etiology of AKI (ATIN versus obstruction). Three genes (BNIP3L, HBA1, and FAM210B) were noted to have overlapping differential expression patterns in both AKI severity and timepoint comparisons.

Conclusions While the results for both objectives are preliminary, they are promising for future research on novel and noninvasive indicators of ICI-AKI and the eventual clinical correlations of that research. This analysis indicates that PET-CT imaging can aid in diagnosing ICI-AKI, as radiotracer uptake rises at the time of injury. The DEGs identified in the cfRNA analysis can be further investigated to create a machine model to predict AKI, which later could be expanded into a clinically relevant “liquid biopsy” to diagnose AKI in a non-invasive manner.

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LIST OF ABBREVIATIONS

AKI	Acute kidney injury
ATIN	Acute tubulointerstitial nephritis
ATN/ATI.....	Acute tubular necrosis/injury
BCC.....	Basal cell carcinoma
BMI.....	Body mass index (kg/m ²)
BWH.....	Brigham and Women's Hospital
cfRNA.....	Cell-free ribonucleic acid
CKD.....	Chronic kidney disease
COPD.....	Chronic obstructive pulmonary dysfunction
CPM	Count per million
CTLA-4.....	Cytotoxic T-lymphocyte antigen 4
DEG	Differentially expressed gene
DFCI.....	Dana-Farber Cancer Institute
DNA.....	Deoxyribonucleic acid
DVT	Deep vein thrombosis
eGFR	Estimated glomerular filtration rate (ml/min)
ESRD	End-stage renal disease
F18-FDG	F18-fluorodeoxyglucose
GU.....	Genitourinary
HCST	Hematopoietic stem cell transplant
HIF	Hypoxia-inducible factor

ICI.....Immune checkpoint inhibitor
ICI-AKI Immune checkpoint inhibitor-associated acute kidney injury
ICUIntensive care unit
IQR.....Interquartile range
irAEImmune-related adverse event
KDIGO Kidney Disease: Improving Global Outcomes
mCi..... Millicurie
NSAID Non-steroidal anti-inflammatory
NSCLC..... Non-small-cell lung cancer
OSA.....Obstructive sleep apnea
PD-1 Programmed cell death 1
PD-L1Programmed cell death ligand 1
PET-CT Positron emission tomography-computed tomography scan
PI..... Principal Investigator
PPIProton pump inhibitor
RBC..... Red blood cell
RCC Renal cell carcinoma
RNA.....Ribonucleic acid
RNA-seq Ribonucleic acid sequencing
ROI.....Region of interest
RPDR..... Research Patient Data Registry
RPMRevolutions per minute

RRT.....	Renal replacement therapy
SCC.....	Squamous cell carcinoma
SCr.....	Serum creatinine
SUV.....	Standardized uptake value
TB.....	Tuberculosis
UPCR.....	Urine protein-creatinine ratio
VST.....	Variance stabilization transformation
WBC.....	White blood cell
wbRNA.....	Whole-blood ribonucleic acid
WHO.....	World Health Organization

INTRODUCTION

Cancer and Acute Kidney Injury

Acute kidney injury (AKI) is a sudden episode of kidney failure or kidney damage that happens within a few hours or a few days. By causing a build-up of waste products in the blood due to decreased filtration, AKI increases the risk of chronic kidney disease (CKD) or end-stage renal disease (ESRD). The pathophysiology of AKI has traditionally been divided into three categories: prerenal (e.g., reduced blood flow to the kidney), intrarenal (e.g., direct kidney damage, sepsis, tubular necrosis), and postrenal (e.g., obstruction)¹. Despite advances in cancer treatment, AKI remains a common complication. In a population-based study of patients initiating systemic therapy for a new cancer diagnosis, AKI occurred with an overall incidence of 9.3%^{2,3}.

AKI occurs in cancer patients for a variety of reasons; its manifestation is driven by cancer-related, patient-related, and treatment-related factors³. Multiple myeloma (26%), bladder cancer (19%), and leukemia (15%) were associated with the highest 5-year risk of AKI². Critically ill cancer patients admitted to the intensive care unit (ICU), as well as those who have undergone hematopoietic stem cell transplant (HSCT), are among other high-risk populations. Other risk factors for AKI beyond a cancer diagnosis include advanced age, CKD, diabetes, and concomitant administration of diuretics and renin-angiotensin receptor blockers, antibiotics, and intravenous contrast³.

Conventional chemotherapies (e.g., cisplatin and methotrexate) continue to be the gold standard of treatment for many malignancies; however, these agents are well-known to increase the patient's risk of AKI. While newer therapies (e.g., immune checkpoint inhibitors) have advanced the treatment of a wide range of malignancies, nephrotoxicity remains an essential consideration before and during treatment.

ICI-AKI is Common and Has Important Implications

Many significant advances have been made by innovators in the field of hematology/oncology toward more effective cancer treatment, such as the introduction of immunotherapy. One such class of drugs is immune checkpoint inhibitors (ICIs). Immune checkpoints are inhibitory immunoreceptor proteins on T cells that protect against unfavorable immune responses and maintain self-tolerance⁴; they are to the immune system what breaks are to a moving vehicle. By releasing the natural breaks in immune activation, ICIs enhance the immune system's innate ability to destroy tumor cells⁵. Approved agents target checkpoint pathways mediated by cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), programmed cell death protein 1 (PD-1), and programmed cell death ligand 1 (PDL-1) (Table 1). While ICIs have produced durable responses in patients with cancer by increasing T cell activation, the benefit is not without associated risks.

Table 1. Immune Checkpoint Inhibitors Approved by the Food and Drug Administration. This shows the three primary checkpoint targets of immune checkpoint inhibitors, common medications in each category, and examples of malignancy types treated by these drugs. Abbreviations: PD-1, programmed cell death 1; PD-L1, programmed cell death ligand 1; CTLA-4, cytotoxic T-lymphocyte antigen 4; NSCLC, non-small-cell lung cancer; RCC, renal-cell carcinoma; SCC, squamous cell carcinoma; BCC, basal cell carcinoma.		
Checkpoint	Drug	Indication
PD-1	Nivolumab	Melanoma, NSCLC, RCC, hepatocellular carcinoma, classic Hodgkin's lymphoma, SCC of the head and neck, urothelial carcinoma
	Pembrolizumab	Melanoma, NSCLC, classic Hodgkin's lymphoma, SCC of head and neck, urothelial carcinoma, gastric cancer
	Cemiplimab	Cutaneous SCC, BCC, NSCLC
	Dosarlimab	Endometrial cancer
PD-L1	Atezolizumab	NSCLC, urothelial carcinoma
	Avelumab	Merkel-cell carcinoma, urothelial carcinoma
	Durvalumab	Urothelial carcinoma
CTLA-4	Ipilimumab	Melanoma

As a result of increased and unrestrained activation of the immune system, ICIs may cause multisystem, immune-related adverse events (irAEs), which can be fatal. Although any organ system can be affected, the more frequently diagnosed irAEs affect the gastrointestinal tract, liver, skin, and endocrine systems⁶. An estimated 60-80% of patients receiving ICIs are found to have at least one irAE⁷. A review of 48 monotherapy trials of ICIs (6,938 patients) from 2003 to 2015 identified the frequency of high-grade irAEs among patients treated with a CTLA-4 inhibitor as 31% versus 10% among patients treated with PD-1 inhibitors⁸. Drugs targeting CTLA-4 are generally less cancer cell-specific than those targeting PD-1 and PD-L1, accounting for this disparity in the rate of irAEs⁹.

AKI directly attributed to the ICI (ICI-AKI) is one specific type of irAE with

an estimated incidence of 2-5%, though definitions of ICI-AKI vary across studies^{3,10}—alternatively, the incidence of any AKI while on ICIs is 15-20%¹¹. Thus, we need markers to differentiate true ICI-AKI, which most commonly presents as acute tubulointerstitial nephritis (ATIN), from other AKI causes. There are many ongoing efforts to describe the mechanism of causation of renal irAEs. The pathogenesis has been partially explained by a “multi-hit” hypothesis derived from animal models and extrarenal irAEs. The “first hit” is exposure to ICI, when T cells could potentially lose their tolerance against normal renal tubular epithelium and cause kidney injury. Drug-induced hypersensitivity to ICI by contemporary use of non-steroidal anti-inflammatory drugs (NSAIDs) and proton pump inhibitors (PPIs) is thought to cause the “second hit”⁴. Nonetheless, to this day, we have a limited understanding of the underlying pathophysiology of both renal and extrarenal irAEs.

Until recently, case reports and small single-center case series were the extent of existing data on ICI-AKI. Critical questions regarding risk factors, clinical features, histopathological findings, renal outcomes, and overall survival still need to be answered. Gupta et al. (2021) conducted a multicenter cohort study of adults diagnosed with ICI-AKI between 2012 and 2020 to address these and other critical knowledge gaps¹⁰. Data from 429 patients with ICI-AKI and 429 control patients who received ICIs but did not develop ICI-AKI was collected and analyzed. In multivariable models, lower baseline eGFR, PPI use, and prior or concurrent extrarenal irAEs were associated with an increased risk of ICI-AKI⁹.

ICI-AKI can have severe consequences for patients, including held doses or permanent discontinuation of ICI therapy, irreversible loss of kidney function (which can impact eligibility to receive other anticancer treatments), and prolonged courses of immunosuppression⁹. In published cohorts of patients with ICI-AKI, renal recovery occurred in 40% to 88% of patients, demonstrating that a significant portion of study participants remained with reduced kidney function (presumably CKD) after the AKI episode^{9,11}. One study reported that 18% of their cohort had sustained eGFR decline at 1-year follow-up. The association between AKI and increased mortality among cancer patients treated with an ICI has been demonstrated by several studies^{4,9,11}. In a retrospective analysis of 759 patients with solid organ malignancies treated with ICI, patients who developed AKI had a 1.6-fold (95% CI 1.3-2.1) higher odds of mortality than patients without AKI¹².

Management of ICI-AKI includes temporary cessation of ICI, treatment with glucocorticoids, and halting medications linked with an increased risk of ATIN (a common pathological finding of ICI-AKI), like PPI and NSAIDs. Gupta et al. (2021) reported that when patients were initiated on a course of glucocorticoids within three days of the ICI-AKI diagnosis, they were more likely to achieve renal recovery than patients with later initiation^{4,9}. ICI-AKI is generally highly sensitive to glucocorticoid treatment, with around 90% of cases experiencing some degree of remission^{4,9}. For these reasons, treatment should be initiated immediately following the exclusion of all other causes of AKI and a confident diagnosis of ICI-AKI. As ICIs become more common cancer

therapeutics, the ability to diagnose ICI-AKI swiftly and accurately is essential.

Diagnosing ICI-AKI Is Challenging

As discussed previously, AKI can be caused by a multitude of reasons and is a common affliction of cancer patients. As a result, when a patient undergoing immunotherapy develops AKI, there is often uncertainty concerning its etiology. The Gupta et al. (2021) multicenter cohort study referenced above sought to identify clinical features that may help clinicians identify ICI-AKI versus other causes of AKI. The time course of ICI-AKI described was highly variable but often prolonged; ICI-AKI developed at a median of 16 weeks (IQR 8-32) after ICI initiation, with 11.4% of patients developing ICI-AKI more than a year after ICI initiation⁹. Of the 429 patients with ICI-AKI, 77 (17.9%) had stage 1 AKI, 144 (33.6%) had stage 2, and 208 (48.5%) had stage 3. Thirty-three patients received renal replacement therapy (RRT) (15.8% of those with stage 3 AKI; 7.7% overall)⁹. Extrarenal irAEs preceded or were concurrent with ICI-AKI in 243 patients (56.6%), with rash and hepatitis occurring most frequently⁹. At the time of ICI-AKI, 62% of patients were receiving other medications associated with ATIN, with PPIs being the most common (approximately 50%)⁹.

No clinical features have been identified to be reliably present or absent in patients with ICI-AKI. Therefore, when it comes to the diagnosis of ICI-AKI, renal biopsy remains the gold standard. ATIN is the most common pathologic lesion observed on the biopsies of patients with ICI-AKI, as reported by several studies. For example, Cortazar et al. (2016) found that the primary lesion was ATIN in 12

of 13 patients with ICI-AKI who underwent kidney biopsy¹³. Shirali et al. (2016) determined ATIN to be the primary lesion on kidney biopsy of all six non-small cell lung cancer (NSCLC) patients with ICI-AKI in their study¹⁴. Finally, Gupta et al. (2021) also reported ATIN as the most common lesion seen in kidney biopsy (125/151 biopsied patients (83%))⁹. Clinicians and researchers face two problems when considering biopsy as the primary diagnostic tool for ICI-AKI: (1) despite ATIN occurring most commonly, alternative and co-occurring lesions are present on many biopsies, and (2) kidney biopsy is not always indicated nor appropriate.

Non-Invasive Markers of ICI-AKI are Necessary

The decision of which patients to biopsy is described by Gupta et al. (2020) as “one of the most complex and subjective decisions in nephrology”¹⁵. Clinicians are often tempted to treat patients with suspected ICI-AKI empirically, meaning without performing a biopsy. There is a dire need for non-invasive biomarkers with anatomic specificity to confirm the diagnosis of ICI-AKI and prevent inappropriate discontinuation of immunotherapy. This thesis explores two possible modes for identifying non-invasive predictors of ICI-AKI: positron emission tomography-computed tomography scan (PET-CT) and cell-free RNA. This research hopes to provide a foundation for changing clinical practice to improve patient outcomes.

Utility of PET-CT in Diagnosing ICI-AKI

PET-CT in Extrarenal irAEs

¹⁸F-FDG PET-CT scanning is routinely performed for staging and re-staging tumor growth in patients with cancer. Though predominantly used to detect malignancies, ¹⁸F-FDG PET-CT scanning can also show evidence of tissue inflammation, a hallmark of irAEs¹⁶. However, immune-related inflammatory changes on PET-CT scans may result in interpretive error. Thus, clinicians must be aware of the broad spectrum of non-malignancy inflammatory changes occasionally present on these scans¹⁶. Various articles have reported irAEs, such as thyroiditis, arthritis/synovitis, pneumonitis, colitis, hepatitis, and pancreatitis, to be ¹⁸F-FDG PET-positive^{16,17}. The primary cause of the increased ¹⁸F-FDG uptake noted at inflammatory sites is that cells involved in inflammation actively use glucose. Though more non-specific, increased blood flow and capillary permeability contribute to increased ¹⁸F-FDG uptake at inflammation sites. Many patients with PET-detectable irAEs are asymptomatic, which speaks to the unique capability of PET-CT to enable early identification and early management¹⁷.

Several studies have demonstrated that ¹⁸F-FDG PET-CT effectively and accurately diagnoses irAEs, with some manifestations better represented than others. The utility of ¹⁸F-FDG PET-CT in detecting irAEs in 46 patients treated with an ICI was investigated by Tatar et al. (2022). According to studies cited by Tatar et al. (2022), the frequency of irAEs in patients treated for melanoma with

nivolumab was 68.2%¹⁸. Another study has estimated the incidence of at least one irAE to be 60-80%⁶. The objective of the Tater et al. (2022) study was to compare the incidence of irAEs in their cohort as observed on PET-CT with these previously published percentages. PET-CT analysis diagnosed irAEs at a similar rate to the published approximate incidence, identifying at least one irAE in 61% (28 patients) of the cohort. This is compelling evidence favoring PET-CT as an effective and reliable method for diagnosing a variety of irAEs.

Colitis is generally accepted to be one of the more common manifestations of irAEs, occurring in 35-50% of patients receiving ICIs. It has been reported that ¹⁸F-FDG PET-CT imaging is more accurate in the early detection of colitis due to ICI treatment than plain CT. Schierz et al. (2021) examined ¹⁸F-FDG PET-CT of a 71-year-old patient with melanoma. PET-CT images before treatment did not show abnormal findings in the sigmoidal colon. However, PET-CT images four months after initiating nivolumab treatment showed thickening of the sigmoidal wall, with inflamed diverticula and increased radiotracer uptake without clinical symptoms. Six months after discontinuing nivolumab treatment, an additional scan showed only mild thickening and uptake¹⁷. Returning to the Tatar et al. (2022) study, enteritis and colitis were the most frequently visualized irAEs on PET-CT imaging, apparent in 28.2% of their patient cohort. These patients presented with symptoms that supported the PET-CT findings of colitis, including abdominal pain, bloating, diarrhea, and constipation. Lastly, in a retrospective cohort study of 100 melanoma patients

receiving ipilimumab, Lang et al. (2019) reported increased radiotracer uptake in the colon of 49% of patients with diarrhea¹⁹.

The success exhibited by the above studies is promising evidence for the role of PET-CT imaging in the confirmation of clinically suspected irAEs and in predicting irAEs before the manifestation of clinical symptoms. Two things are essential for clinicians to consider when utilizing PET-CT imaging. As mentioned above, one limitation of using ¹⁸F-FDG uptake to identify irAEs is an interpretive error when inflammation caused by the ICI is interpreted as malignancy (or vice versa). In addition, using ¹⁸F-FDG PET-CT in patients who recently received high doses of glucocorticoids leads to another limitation, as such treatment may cause a false negative or underestimate the metabolic activity of inflammation¹⁷.

PET-CT as a Diagnostic Tool for ICI-AKI

As far as we know, the radiologic characteristics of patients with biopsy-proven ATIN or presumed ICI-AKI have yet to be adequately described beyond select case reports. This is not because ICI-AKI does not cause increased FDG activity on PET-CT imaging but rather due to the rarity of definitive ICI-AKI. Qualls et al. (2019) published one of the first case reports to describe the potential for PET-CT as a diagnostic measure for ICI-AKI²⁰. The case report is of a 56-year-old woman with metastatic vulvar melanoma who developed one week of fatigue, nausea, and vomiting following seven cycles of nivolumab (in addition to palliative radiation). Laboratory studies performed were notable for a serum

creatinine of 4.5 mg/dL, up from a baseline of 0.5 mg/dL, indicating AKI. In need of additional evaluation and management, she was admitted to the hospital.

Despite rehydration, her creatinine continued to rise during the first day of her admission. Her home medications were notable for daily omeprazole (40mg) and aspirin (325mg). Given the high probability of ICI-AKI, a kidney biopsy was indicated but could not be safely performed due to her anticoagulation with aspirin. It was decided to empirically treat her with a pulse dose of intravenous methylprednisolone, followed by a prednisone taper. Given the known association of PPI with ATIN, her omeprazole was discontinued. Her creatinine quickly improved and stabilized after one month of steroids.

It was noted that the patient had a routine staging ¹⁸F-FDG PET-CT scan ten days before her AKI diagnosis, though uptake in the renal cortex was not explicitly commented upon at that time. Given the clinical suspicion for ICI-AKI, a nuclear radiologist retrospectively re-evaluated the PET-CT scan. Sure enough, increased FDG uptake was identified in the renal cortices. The researchers were able to compare these findings to three other PET-CT scans: before initiation of ICI therapy, after ICI initiation but before the onset of AKI, and after recovery from AKI. Using the maximum standardized uptake value (SUV_{max}) to make comparisons, the FDG uptake in the renal cortices was highest on the PET-CT scan taken just before AKI diagnosis.

It is important to note that ICI-AKI (recall that ATIN is the most common lesion) is inflammatory, characterized by a lymphocyte-predominant infiltrate with

plasma cells and eosinophils. This type of infiltrate is highly metabolically active and, thus, will quickly take up FDG, as can be appreciated on ^{18}F -FDG PET-CT imaging. To test the hypothesis that the inflammatory nature of ATIN will drive FDG uptake, Qualls et al. (2019) described the imaging of three patients with non-ATIN AKI. Indeed, the PET-CT imaging of these three patients had either a decrease or no change in FDG uptake at the time of AKI.

Awiwi et al. (2023) published a retrospective case series describing the imaging features of biopsy-proven ICI-AKI²¹. This study looked at and described both CT and PET-CT imaging when available. Their cohort consisted of 34 patients with biopsy-proven ICI-AKI, though only 14 of those patients had PET-CT scans at baseline and ICI-AKI. Their analysis revealed that the renal parenchyma SUV_{max} (quantitative measure of radiotracer uptake) was higher at ICI-AKI than baseline PET-CT, though this difference did not reach statistical significance (4.4. vs. 3.4; $p = 0.051$)²¹. Nonetheless, this finding supports our hypothesis that radiotracer uptake increases during ATIN.

PET-CT as a Diagnostic Tool for ICI-AKI: Next Steps

Despite having limitations, namely that ATIN was not confirmed with a kidney biopsy and being a single case report, Qualls et al. (2019) presented a unique opportunity for diagnostic insight into ICI-AKI using ^{18}F -FDG PET-CT imaging. As immunotherapy becomes more widely utilized and awareness of ICI-AKI increases, more rigorous investigation into this topic is vital.

The study by Awiwi et al. (2023) attempted to build upon the report published by Qualls et al. (2019). While it was successful, the work had significant limitations. One limitation was the small sample size, with only 14 patients having PET-CT scans at baseline and ICI-AKI. Two additional issues were with their methodology for quantifying radiotracer uptake in the renal parenchyma on PET-CT. First, according to the opinion of our nuclear radiologist, SUV_{mean} more accurately represents radiotracer uptake than SUV_{max} in a study of this nature. Awiwi et al. (2023) reported only SUV_{max} , not SUV_{mean} . Second, only one region of interest (ROI) was drawn on each kidney to obtain the SUV_{max} , and only the maximum SUV_{max} value derived from either kidney was included in their data analysis. The study above does not consider how radiotracer uptake may vary throughout the renal parenchyma by only taking one measurement per kidney. Finally, Awiwi et al. (2023) had no control groups in their study. This opens an avenue for future research, where the PET-CT scans of ICI-AKI patients can be compared to those with other etiologies of AKI. By doing so, more robust conclusions could be drawn about whether ATIN is genuinely causing the increase in radiotracer uptake or if any injury to the kidney would cause this effect.

The current study hopes to address some, if not all, of these limitations. This research aims to confirm the results of both Qualls et al. (2019) and Awiwi et al. (2023) and expand upon current knowledge of the ^{18}F -FDG PET-CT imaging features of ICI-AKI.

Possibility for cfRNA as a Biomarker for ICI-AKI

What is cfRNA?

Changes in gene expression, referred to as transcriptomic signatures, have been shown to provide valuable insight into host response to some disease processes. Analysis of whole blood RNA (wbRNA) is one way to identify transcriptomic signatures. It is continually being explored, though there is increasing interest in the possibility of cell-free RNA (cfRNA) as biomarkers. The interest in cfRNA stems from two key differences between this analyte and the more conventional wbRNA. First, cfRNA is released by cells in both the blood and vascularized solid tissues. Thus, cfRNA analysis can reveal information about both systemic immune dynamics and immune-tissue interactions. wbRNA, alternatively, can only provide insight into immune dynamics. Second, wbRNA is primarily extracted from live cells, while cfRNA is released by dying cells. For this reason, cell death pathways and mechanisms of cellular injury that would not be available in wbRNA profiling may be elucidated by cfRNA²².

cfRNA in Tuberculosis

One disease of interest in cfRNA analysis is tuberculosis (TB). Several wbRNA signatures of TB have been identified but have yet to meet recommendations established by the World Health Organization (WHO) for a non-sputum-based triage or diagnostic test²². Chang et al. (2023) conducted the first study to investigate the efficacy of circulating plasma cfRNA, an alternative option to wbRNA, as a biomarker for TB. This study profiled plasma cfRNA from

182 individuals with a cough persisting longer than two weeks, identified from enrollment logs of two independent clinical studies in Uganda, Vietnam, and the Philippines. TB was microbiologically confirmed in 100 of these individuals.

Using differential abundance analysis, 541 genes were identified as expressed differently between TB-positive and TB-negative groups. For example, when compared to the TB-negative group, the TB-positive group was associated with increased macrophage and neutrophil markers and antimicrobial genes. In addition, lung-specific markers were elevated in individuals with TB²². Pathway analysis was performed to confirm the significance of these findings in microbiologically confirmed TB diagnoses. The top pathways associated with TB included pyroptosis signaling, macrophage classical activation signaling, and pathogen-induced cytokine storm signaling²². Indeed, these results suggested that plasma cfRNA could serve as a biomarker in diagnostic tests or assays for TB.

To examine this, the researchers sought to develop a machine-learning model to characterize samples as TB-positive or TB-negative. Only the top 150 genes identified by the differential abundance analysis were normalized (count per million [CPM]) and selected for machine classification models²². The model with the best results included a nine-gene signature, discriminating between TB-positive and TB-negative groups with 89.1% accuracy, 96.2% sensitivity, and 89.7% specificity²². In this cohort, the panel barely failed the specificity requirements for a diagnostic test established by the WHO. However, it did

exceed the optimal criteria for a triage test, which is extremely promising. In addition, the cfRNA signature compared favorably to previously described whole blood signatures, suggesting that cfRNA may be a more robust and effective biomarker for TB²².

cfRNA in Preeclampsia

A similar study examined the role of cfRNA in the early prediction of preeclampsia among pregnant people. The measurement of circulating cfRNA in this study is denoted as a “liquid biopsy,” which can provide an opportunity to study non-invasively, in this case, pregnancy-related complications. The goal of such liquid biopsies would be to alleviate gaps in clinical care by indirectly observing pathogenesis in real-time²³. Using 404 blood samples from 199 pregnant patients, Moufarrej et al. (2022) identified a total of 544 differentially expressed genes (DEGs) between patients who later developed preeclampsia with or without severe features and normotensive patients, with most of these changes occurring within the first 20 weeks of gestation²³. The 544 DEGs were well categorized into two trends throughout gestation. Two hundred sixteen genes (40%) were reduced in preeclampsia samples, reaching a minimum between 13 and 20 weeks. The other 328 genes (60%) were significantly increased in preeclampsia, peaking before 20 weeks. Approximately 13% of DEGs were tissue- or cell-type-specific, with a significant contribution from endothelial cells, consistent with the established pathogenesis of preeclampsia.

Interestingly, the gene expression changes in preeclampsia were irrespective of symptom severity. Taking into consideration that these changes most frequently occurred much before symptom onset, researchers wanted to develop a classifier that could identify patients at risk for preeclampsia at or before 16 weeks of gestation. Their final model included 18 genes and performed well in the discovery cohort, with 85% specificity and 100% sensitivity²³. The model was tested on three other cohorts and again performed well. Results even suggested that differentiation between preeclampsia and other risks, like chronic hypertension and gestational diabetes, could be made by this model.

Lastly, researchers were interested in determining if cfRNA could monitor maternal organ health. The presentation of preeclampsia impacts a variety of organ systems, resulting in consequences such as impaired liver function, renal insufficiency, proteinuria, and epilepsy²³. Indeed, considerable tissue- and cell-type-specific changes were identified in the preeclampsia cohort compared to normotensive patients. Astrocyte signal was heightened before 20 weeks of gestation, while oligodendrocyte and excitatory signals were reduced after 23 weeks. In preeclampsia pregnancies, the signals from placental tissue and syncytiotrophoblasts declined before 20 weeks of gestation. Finally, in the preeclampsia cohort, hepatocyte, kidney, endothelial cell, and smooth muscle contributions were decreased throughout gestation²³. These signatures are consistent with the pathogenesis of preeclampsia and the primary diagnoses of patients in this cohort.

cfRNA Can Predict Cellular Pathophysiology

In hopes of improving the resolution of the liquid biopsy to match invasive procedures more closely, Vorperian et al. (2022) sought to examine cfRNA cell types of origin further. They hypothesized that changes in the cfRNA contributions of different cell types could measure cellular pathophysiology. While they confirmed this in several cases, their analysis of the kidneys is the most relevant to this investigation. Using the proximal tubule epithelial cells, a predominant kidney cell type and locale of concern in injury and disease progression, cfRNA obtained from patients with CKD was compared with healthy controls. A marked decrease in contribution to metabolic activity from proximal tubule cells was identified in the patients with CKD. Proximal tubule deterioration is observed in CKD histology, demonstrating that non-invasive liquid biopsy has a similar resolution to an invasive kidney biopsy²⁴.

cfRNA and ICI-AKI

The clinical utility of cfRNA is broad, with existing applications in various fields, including oncology and bone marrow transplantation, obstetrics, neurodegeneration, and liver disease²⁴. To the current knowledge, there have not been any published reports of cfRNA in the context of ICI-AKI, though the potential is salient. The results of the three studies discussed previously, among others, form the basis of clinical tests to identify, predict, or describe disease processes in a non-invasive manner. Chang et al. (2023) demonstrated the possibility of a cfRNA assay being utilized to detect tuberculosis and suggested

the opportunity for its use in other diseases. By looking at organ health during preeclampsia pregnancies, Moufarrej et al. (2022) showed that renal insufficiency could be predicted by cell-specific cfRNA signatures, aligning with what was published by Vorperian et al. (2022) a few months before.

In conjunction with Dr. Iwijn de Vlaminck, Ph.D., an expert in cfRNA and DNA sequencing at Cornell University, this research aims to explore the utility of liquid biopsies in diagnosing ICI-AKI. We will analyze biological samples collected from patients at Brigham and Women's Hospital and Dana-Farber Cancer Institute (BWH/DFCI) who are receiving an ICI for cancer treatment and later develop AKI.

OBJECTIVES

1. To examine the role of positron emission tomography-computed tomography scan (PET-CT) in diagnosing immune checkpoint inhibitor-associated acute kidney injury (ICI-AKI), specifically acute tubulointerstitial nephritis (ATIN).
2. To explore using cell-free RNA (cfRNA) as a biomarker for acute kidney injury.

METHODS

ICI-AKI PET-CT

ICI-AKI International Consortium

Data from the ICI-AKI consortium developed by the Principal Investigator, Dr. Shruti Gupta, was utilized to conduct this study. To create this consortium, Dr. Gupta conducted an ambitious cohort study of adults diagnosed with ICI-AKI between 2012 and 2020. The team contacted nephrologists and oncologists at 40 major academic cancer centers across North America, Europe, and Asia to identify cases of ICI-AKI. The final cohort consisted of 429 patients with ICI-AKI from 30 different cancer centers.

Data was collected at each site by detailed chart review and entered into REDCap, a secure, web-based platform. Compiled data included the following: demographics and comorbidities; concomitant treatment with nephrotoxic chemotherapies and medications associated with ATIN; prior or concurrent extrarenal irAEs; laboratory data at baseline and at the time of ICI-AKI; kidney biopsy data; treatments received for ICI-AKI; and data on renal recovery, ICI rechallenge, and overall survival.

Definition of ICI-AKI

Patients were eligible for inclusion in the ICI-AKI consortium if they had AKI that was directly attributed to the ICI as adjudicated by the treating provider and if they met either of the following criteria:

- 1) An increase in serum creatinine (SCr) $\geq 100\%$ from baseline or treatment with renal replacement therapy (RRT).
- 2) An increase in SCr $\geq 50\%$ from baseline and at least one of the following:
 - a. ATIN on kidney biopsy.
 - b. ICI therapy is held for at least one cycle due to concern for ICI-AKI.
 - c. Treatment with glucocorticoids due to concern for ICI-AKI.

The closest recorded SCr before ICI initiation was considered as the baseline value. Patients with allograft kidneys or who had ESRD were excluded from the consortium.

AKI stage was determined according to KDIGO (Kidney Disease: Improving Global Outcomes) criteria²⁵, as outlined below:

- Stage 1: increase in SCr ≥ 0.3 mg/dL within 48 h or $\geq 50\%$ within seven days.
- Stage 2: increase in SCr $\geq 2x$ from baseline.
- Stage 3: increase in SCr $\geq 3x$ from baseline, SCr > 4 mg/dL, or RRT.

Inclusion and Exclusion Criteria

In the original standardized case report form used in data collection for the ICI-AKI consortium, collaborators were asked to indicate if each patient had a PET-CT scan at the time of ICI-AKI, defined as within 14 days before or after ICI-

AKI diagnosis. From the cohort of 429 patients with ICI-AKI, it was indicated that 25 patients from 13 different centers had a PET-CT scan at the time of ICI-AKI.

Collaborators at each center were contacted to inquire about their interest in participating in this ancillary study. Only one center declined to participate. The collaborators were provided with the following inclusion and exclusion criteria, with instructions on proceeding with data collection if they deemed the patient eligible for the study.

Inclusion:

- 1) AKI is directly attributed to the ICI, as defined above.
- 2) A follow-up PET-CT scan was performed within 14 days of ICI-AKI.
- 3) A baseline PET-CT scan was performed within 90 days before the initiation of ICI therapy.

Exclusion:

- 1) Previous OR active renal cell carcinoma (RCC) or other genitourinary (GU) malignancy.
- 2) Acute tubular necrosis/injury (ATN/ATI) on kidney biopsy, if obtained.
- 3) Seven or more days of glucocorticoids before the follow-up PET-CT scan.

After exclusions, eight patients from the ICI-AKI consortium were included in the analysis (Figure 1).

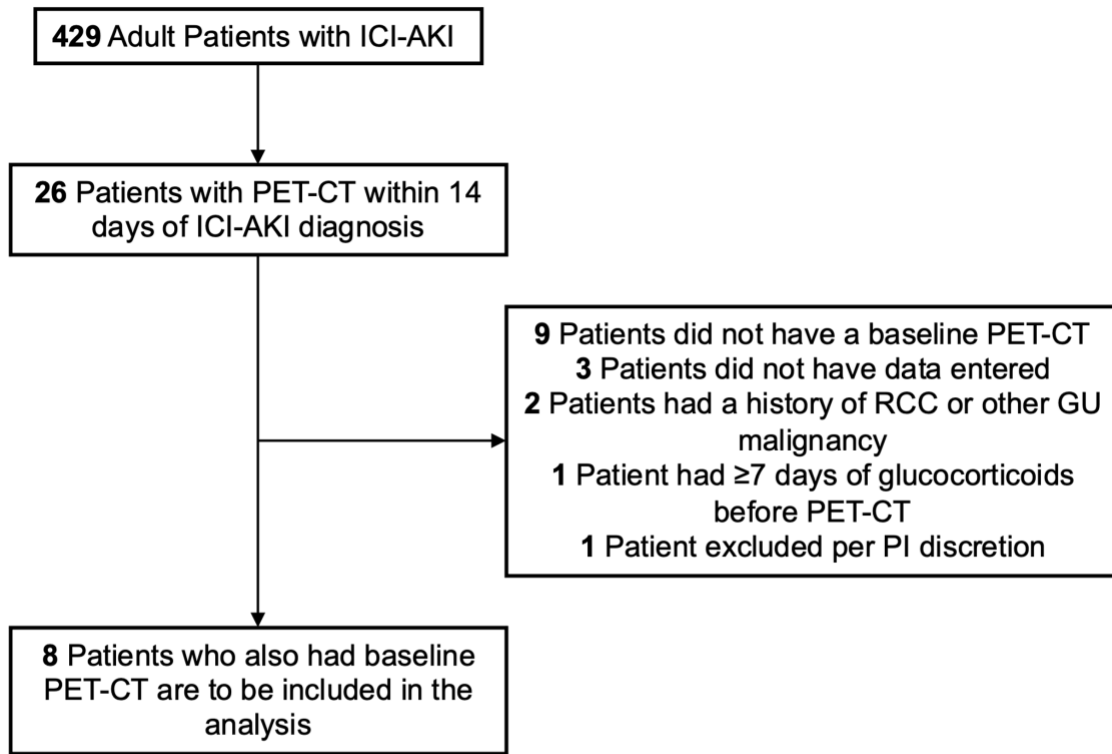


Figure 1. Flow Diagram for Inclusion of ICI-AKI Patients. Flowchart depicting the study population for the ICI-AKI cohort, demonstrating our inclusion and exclusion criteria. Abbreviations: ICI-AKI, immune checkpoint inhibitor-associated acute kidney injury; PET-CT, positron emission tomography-computed tomography scan; GU, genitourinary; PI, principal investigator.

Additional ICI-AKI Cases

Additional ICI-AKI cases were sought out to make the analysis more robust. Unable to identify other ICI-AKI cases from BWH/DFCI that met the inclusion criteria and had PET-CT scans available, it was ultimately decided to contact a collaborator from the ICI-AKI consortium. Drs. Ala Abudayyeh and Muhammad Awiwi at MD Anderson Cancer Center were senior authors on the previously discussed Awiwi et al. (2023) paper, which described imaging

signatures of ICI-AKI, including PET-CT scans²¹. Drs. Abudayyeh and Awiwi graciously agreed to enter clinical and radiological data from their ICI-AKI patients into the REDCap. The process of collecting five additional patients from MD Anderson was ongoing when this thesis was written.

Data Collection

Again, each site collected additional data by chart review using REDCap. This time, the data included details of the PET-CT scans, and study personnel were asked to contact a nuclear radiologist at their home institution for interpretation. The following data were collected:

- 1) Weight (in kg) at baseline and at the time of ICI-AKI.
- 2) Blood glucose (in mg/dl) level in the 24 hours preceding baseline and ICI-AKI PET-CT scans.
- 3) Radiotracer used for baseline and ICI-AKI PET-CT scans.
- 4) Dose (in mCi) of radiotracer used for baseline and ICI-AKI PET-CT scans.
- 5) Uptake time (in minutes) of radiotracer for baseline and ICI-AKI PET-CT scans.

The following instructions were provided for image analysis of each PET-CT scan:

- 1) Draw five 0.5 cm regions of interest (ROIs) in the cortex of each kidney, avoiding the collecting system and any space-occupying lesions such as cysts. The ROIs should represent each kidney's upper, mid, and lower poles.

- 2) Record the SUV_{max} and SUV_{mean} within the ROIs in the table provided.
- 3) All ROIs should be placed in the same location within each kidney for the baseline and follow-up PET-CT scans.

A similar protocol for data collection was followed for the control patients, only changing the type of follow-up PET-CT scan we were looking at.

Statistical Analysis

A paired t-test will demonstrate that radiotracer dose, uptake time, blood glucose, and weight are the same for baseline and AKI scans. A two-tailed Wilcoxon signed-rank test was used to compare SUV_{mean} at baseline vs. AKI, with a significance level of 0.05.

Biomarkers for ICI-AKI: cfRNA

The following methods were adapted from published literature (Chang et al., 2023 and Moufarrej et al., 2022)^{22,23} and other materials provided by personnel at de Vlaminck Lab.

Sample Collection

An alert system was established in the electronic medical record utilized by BWH/DFCI, which notifies study personnel in real-time if any patient with a solid organ malignancy treated with an ICI has a $\geq 50\%$ rise in SCr from baseline. Blood (~12 mL) was collected from eligible patients who consented to the study at two time points. Timepoint 1 (TP1) was at the time of AKI, and timepoint 2 (TP2) was 60 days (+/- 30 days) after the AKI event. Blood was collected through

venipuncture and centrifuged for 15 minutes at 3200 RPM at 4°C. Plasma was carefully pipetted out, aliquoted, and frozen at -80°C.

A total of 78 plasma samples were sent to Dr. Iwijn de Vlaminck, who included 46 TP1 plasma samples and 32 matched TP2 samples in their analysis. Each sample was deidentified, and a study identification number was assigned. Some clinical data was provided along with the samples, including the designation of AKI etiology into one of the following groupings: (1) proven/likely ATIN, (2) possible ATIN, (3) obstruction, (4) prerenal, or (5) other.

cfRNA Isolation, Library Preparation, and Sequencing

Collected plasma was transported on dry ice and stored at -80°C until processed. Samples were thawed and centrifuged at 1300 RPM for 10 minutes at 4°C, and the supernatant was extracted. Using the Norgen Plasma/Serum Circulating and Exosomal RNA Purification Mini Kit (Norgen, 51000), cfRNA was isolated and extracted from the plasma. cfRNA was treated with DNase and subsequently concentrated to 12 µl using the Zymo RNA Clean and Concentrated Kit (Zymo, R1015).

The Takara SMARTer® RNA Unique Dual Index Kit (Takara, 634451) was used to prepare sequencing libraries and barcoded using the SMARTer® RNA Unique Dual Index Kit (Takara, 634451). Libraries were quality-controlled and pooled at equal concentrations. Finally, Illumina NextSeq 2000 Platform (paired-end, 2x50 bp) or Illumina Novaseq 6000 (2x150 bp) was used for sample sequencing.

Road Mapping and Deconvolution

After being filtered for quality and trimmed using BBDUK (v38.90), sequenced samples were aligned to the Gencode GRCh39 human reference genome (v38, primary assembly) using STAR (v2.7.0f) default parameters. The “Tabula Sapiens” human single-cell transcriptomic atlas was leveraged as reference data to determine changes in the cell types of origin of cfRNA associated with ICI-AKI. Cell types contributing cfRNA to the blood were identified using BayesPrism, a Bayesian approach to reduce bulk tissue transcriptomes into fractional cell type components. This method assumes that the transcriptome of RNA in plasma is a linear combination of cell-type-specific RNA contributions. Any cell grouping with more than 100,000 unique molecular identifiers was included in the analysis.

Differential Abundance Analysis

RNA-sequencing (RNA-Seq) is inherently a relative measurement, so it is essential to normalize the data to compare between samples. Many standard statistical methods for exploratory analysis of multidimensional data, like RNA-Seq, work best when the variance of an observable quantity (i.e., gene expression strength) does not depend on the mean. In RNA-Seq, however, variance grows with the mean. Variance stabilization transformation (VST) is the normalization method the de Vlaminck Lab prefers. VST is akin to the more commonly used “counts per million” (CPM) normalization method in RNA-Seq.

However, VST is more sophisticated because it reduces the correlation between variance and average abundance.

After normalizing the data using VST, a negative binomial model was used to perform a comparative analysis of DEGs. Heatmaps were created using the pheatmap package in R (v1.0.12). Correlated samples and genes were clustered hierarchically. Using the differential abundance analysis information, machine learning and model training were used to identify genes that may be important in detecting AKI using cfRNA.

Statistical Analysis

All statistical methods were performed in R version 4.0.2. Groups were compared using two-sided Wilcoxon Rank-Sum tests. The box plots indicate the 25th and 75th percentiles, the band in the box indicates the median, and the whiskers extend to 1.5-fold the interquartile range (IQR).

RESULTS

Results of PET-CT scans for ICI-AKI Diagnosis Study

Baseline Characteristics of ICI-AKI Patients

The initial study population included 429 adults who were receiving an ICI and developed ICI-AKI. After implementing the exclusion criteria (Figure 1), eight patients were included in the PET-CT scan analysis. The median age of the study cohort was 67 years old (range 48-85), and six (75%) were male. Five patients had malignant melanoma, and three had lung cancer. Four patients were receiving pembrolizumab (PD-1), while the other four patients were receiving a combination of ipilimumab (CTLA-4) and nivolumab (PD-1). Only one patient in the cohort was receiving a conventional chemotherapy agent concurrently with the ICI (Patient 7: cisplatin). Extrarenal irAEs were present in 6 (75%) patients (Table 2).

Table 2. Baseline Characteristics of ICI-AKI Patients. Description of the demographics and baseline characteristics, including laboratory values and type of ICI, of the eight ICI-AKI patients included in the analysis. Abbreviations: CKD, chronic kidney disease; DVT, deep vein thrombosis; COPD, chronic obstructive pulmonary dysfunction; OSA, obstructive sleep apnea; ICI, immune checkpoint inhibitor; BMI, body mass index (kg/m²); SCr, serum creatinine (mg/dl); eGFR, estimated glomerular filtration rate (ml/min). eGFR was calculated using the 2021 CKD-EPI equation.

^ARefers to any other irAEs occurring before or concomitant with ICI-AKI.

Pt	Age/ Sex	Malignancy	SCr/ eGFR	Weight (kg)/BMI	Comorbidities	ICI	Extrarenal irAE ^A
1	58/F	Lung adenocarcinoma	0.56/106	55.9/21.8	None	Pembro	None
2	56/F	Melanoma	0.71/100	48.0/17.7	None	Ipi and Nivo	None
3	73/M	Melanoma	0.88/91	93.6/32.5	Atrial fibrillation requiring pacemaker	Ipi and Nivo	Pneumonitis
4	61/M	Melanoma	1.11/76	89.4/26.0	COPD	Ipi and Nivo	Thyroid disease and pneumonitis
5	71/M	Lung squamous cell	1.24/62	65.0/21.7	None	Pembro	Colitis
6	85/M	Melanoma	1.25/56	76.4/24.9	Hypertension, CKD, DVT, ischemic heart disease, and Ramsey Hunt Syndrome	Pembro	Arthritis
7	48/M	Lung squamous cell	0.87/106	119.0/39.8	OSA	Pembro	Fever
8	80/M	Melanoma	1.64/42	85.7/28.7	Hypertension, prostate cancer, and basal cell carcinoma	Ipi and Nivo	Myocarditis

Clinical Description of ICI-AKI

Using the KDIGO criteria, three (38%) patients developed Stage 1 AKI, two (25%) had Stage 2 AKI, and three (38%) had Stage 3 AKI. Seven patients had a urinalysis performed at the time of ICI-AKI. Four patients had leukocyte esterase present, and two had hematuria on urinalysis. Only one patient had proteinuria (1+) on urinalysis. Urine protein-creatinine ratio (UPCR) was reported for only four patients (Table 3).

Two of the eight patients had a renal ultrasound at the time of ICI-AKI diagnosis. The kidneys of these two patients were measured as follows: R 14.0cm/L 13.7cm (Patient 1) and R 11.8cm/L 12.0cm (Patient 2). In addition, two of the eight patients had a renal biopsy at the time of ICI-AKI. The primary lesions identified on the biopsies of these two patients were ATIN (Patient 1) and mesangial proliferative immune complex-mediated glomerulonephritis (Patient 7). None of the patients required RRT for their AKI (Table 3). Glucocorticoids were initiated for treatment of the ICI-AKI in four of the patients, though only one patient had received any dose of glucocorticoid at the time of their ICI-AKI PET-CT scan (Patient 4: initiated glucocorticoid on the same day as scan) (Table 4).

Table 3. Clinical Data for ICI-AKI Patients. If available, clinical characteristics of the ICI-AKI include laboratory values, AKI stage, and kidney ultrasound or biopsy results. Kidney size is determined by ultrasound. Abbreviations: ICI, immune checkpoint inhibitor; AKI, acute kidney injury; UPCR, urine protein-creatinine ratio (g/g); SCr, serum creatinine; RRT, renal replacement therapy; irAE, immune-related adverse event; WBC, white blood cell; RBC, red blood cell; ATIN, acute tubulointerstitial nephritis; NA, not available.

^AAKI staged according to the Kidney Disease: Improving Global Outcomes criteria.

^BRefers to any chemotherapies administered before or concomitant with ICPI.

Pt	Urine sediment	Proteinuria (dipstick/UPCR)	Leukocyte Esterase (dipstick)	Blood (dipstick)	Peak SCr (mg/dl)	AKI stage ^A	Kidney size (cm)	Primary lesion on biopsy	RRT Required?	Other Chemotherapy ^B
1	182 WBCs, 2 RBCs	neg/0.35	3+	neg	2.85	Stage 3	R 14.0 L 13.7	ATIN	No	NA
2	<10 WBCs, 0-2 RBCs, + squamous cells	neg/0.33	1+	1+	4.84	Stage 3	R 11.8 L 12.0	NA	No	NA
3	3-5 WBCs, 3-5 RBCs, 1+ bacteria, 0-2 hyaline casts, + mucus	neg/NA	neg	1+	1.96	Stage 2	NA	NA	No	NA
4	NA	neg/0.1	neg	neg	1.88	Stage 1	NA	NA	No	NA
5	15 leukocytes	1+/0.64	3+	neg	9.13	Stage 3	NA	NA	No	NA
6	NA	NA	NA	NA	2.33	Stage 1	NA	NA	No	NA
7	9 WBCs, 3 RBCs	neg/NA	neg	neg	1.97	Stage 2	NA	Mesangial proliferative immune complex mediated glomerulonephritis	No	Cisplatin
8	17 WBCs, 1 RBC, 1+ bacteria, occ hyaline casts	neg/NA	3+	neg	2.54	Stage 1	NA	NA	No	NA

PET-CT Analysis

All sixteen PET-CT scans included in this analysis (eight baseline and eight AKI) utilized F¹⁸-fluorodeoxyglucose (F¹⁸-FDG) as the radiotracer. There does not appear to be any difference in weight, blood glucose, radiotracer dose, or uptake time between baseline and AKI PET-CT scans in any of these patients (Table 4). However, this will be confirmed with paired t-tests when all data has been collected.

Table 4. PET-CT Scan Details. Description of clinical features relevant to the interpretation of PET-CT scans, as well as details on the dose of radiotracer used, uptake time, the percent increase in SUVmean, and if the patient was on steroids at the time of the AKI scan. Abbreviations: BL, baseline; AKI, acute kidney injury; mCi, millicurie; NA, not available; 18F-FDG, 18-fluorodeoxyglucose.

^AThe radiotracer used for all scans was [¹⁸F]-FDG (F¹⁸-fluorodeoxyglucose).

^BRefers to the timing of PET-CT scan in relation to AKI diagnosis. Negative values indicate that the scan occurred prior to AKI.

Pt	Weight (kg)		Blood glucose (mg/dl)		Dose of radiotracer ^A (mCi)		Uptake time (min)		Days between AKI and scan ^B	Average % Increase in SUVmean	On steroids at time of AKI scan?	If yes, for how long?
	BL	AKI	BL	AKI	BL	AKI	BL	AKI				
1	56	56	92	86	6.7	6.8	109	65	13	132.6	No	-
2	44	48	100	94	15.1	15.0	60	72	-9	59.0	No	-
3	78	94	95	113	19.1	16.0	60	62	-3	57.4	No	-
4	82	89	106	103	16.7	10.0	57	57	5	41.4	Yes	<1 day
5	68	65	96	70	9.8	9.8	53	70	1	35.7	No	-
6	74	76	95	90	8.4	8.5	84	80	-3	22.1	No	-
7	81	119	94	93	14.7	14.2	118	88	14	3.3	No	-
8	NA	86	97	NA	11.0	10.7	67	55	0	1.9	No	-

While data on SUV_{mean} and SUV_{max} were being collected, the collaborating nuclear radiologist at BWH/DFCI advised that SUV_{mean} would be more appropriate for this analysis. As the collected data are paired, with each patient serving as their own control, we decided that average percent change in SUV_{mean} would be more meaningful than reporting raw SUV_{mean} values. The percent change of SUV_{mean} in each ROI for each patient was calculated, yielding five values per patient. Then, these five values were averaged to report one average percent change in SUV_{mean} per patient. A percent change increase was observed

in SUV_{mean} in all patients at the time of ICI-AKI versus baseline, as demonstrated in Figure 2.

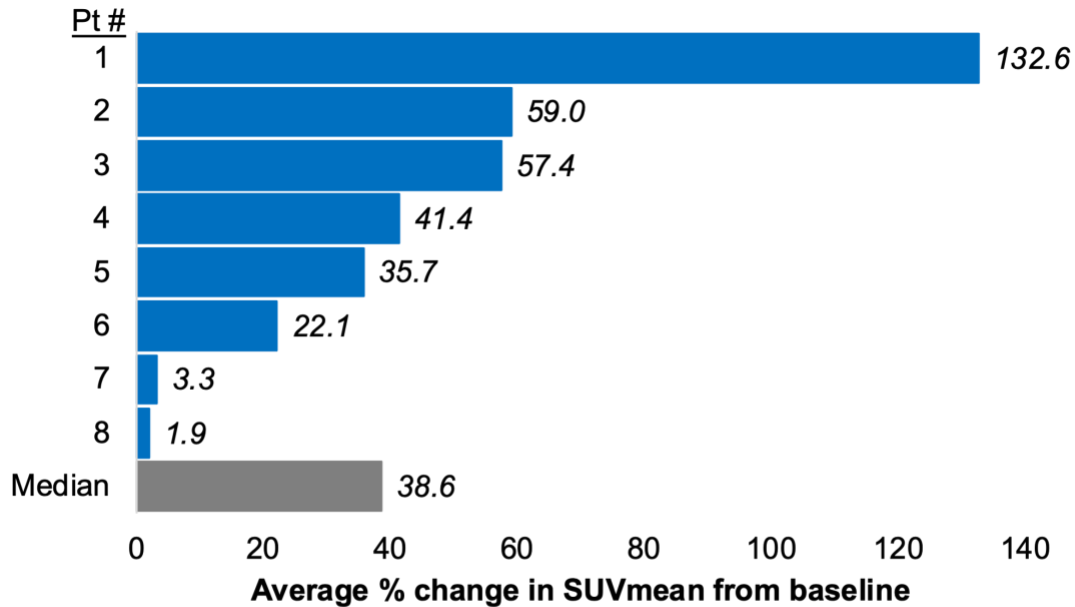


Figure 2. Percent Change in SUV_{mean} . Waterfall plot depicting the average percent change in SUV_{mean} from baseline to ICI-AKI for each patient. The grey bar represents the median percent change in SUV_{mean} among all patients.

The median SUV_{mean} increase was 38.6 (IQR 8.0-58.6). Using raw values, the rise in SUV_{mean} observed in PET-CT scans during AKI compared to baseline was statistically significant (p-value <0.00001, Wilcoxon signed rank).

Preliminary Findings from cfRNA Study

Comparing cfRNA Patterns at AKI and Follow-Up

The first step in this research was to determine whether cfRNA patterns were significantly different at the time of AKI (TP1) versus at follow-up (TP2) to determine if specific cfRNA patterns could indicate the presence of ICI-AKI. Two comparisons were made to do so. The first comparison was between AKI and recovery samples without accounting for patient differences. Fifty-six DEGs were

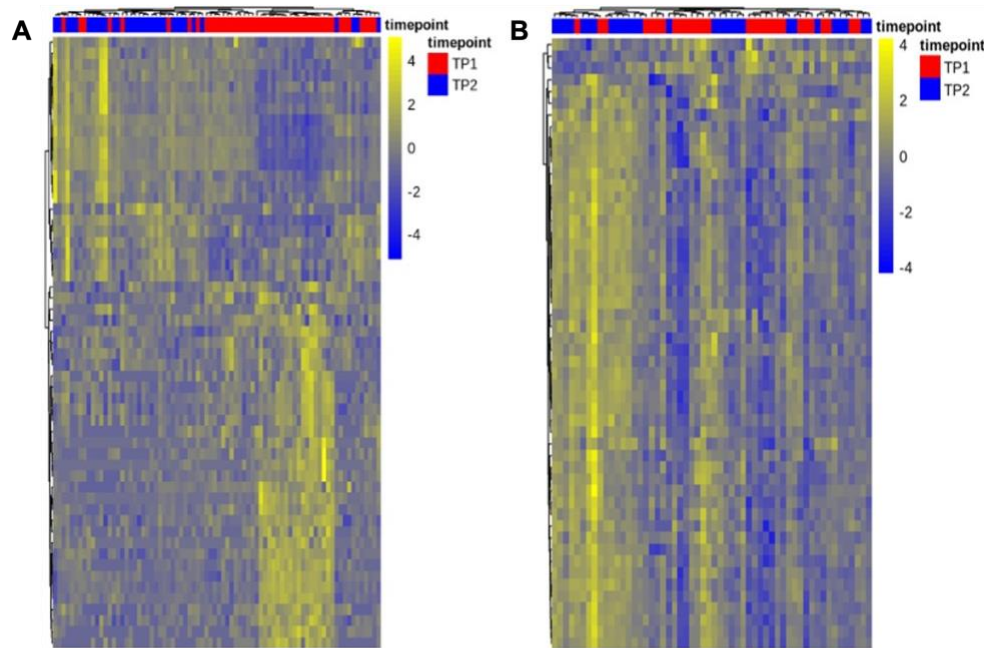


Figure 3. Differential Abundance Analysis Comparing AKI and Recovery. **Panel A** Shows the differential abundance analysis using unmatched samples. 56 DEGs were identified between AKI and recovery samples. **Panel B** shows the differential abundance analysis using patient-matched samples. Now, 61 DEGs were identified between AKI and recovery samples. Abbreviations: DEGs, differentially expressed genes; AKI, acute kidney injury.

identified using this comparison, as represented visually on the heatmap (Figure 3A). The second comparison was also between samples from AKI and follow-up, using only patient-matched samples. There is considerable heterogeneity among

patients in this cohort due to factors including the etiology of AKI. Only matched samples were used to consider these possible differences among patients. In the second comparison, 61 DEGs were identified (Figure 3B). Between these two methods, there were only six common DEGs. This suggests that the differences among the patient cohort confound the results if sample matching is not utilized.

Using only the 61 DEGs from the second comparison (of matched samples), gene ontology was conducted to identify possible biological pathways to explain why these specific genes can be differentially expressed during AKI or at follow-up. It was determined that the two most relevant pathways were (1) oxygen transport and (2) erythrocyte development. Recent research has suggested that hypoxia-inducible factors (HIFs) play an essential role in kidney injury and repair by regulating their target genes. As such, those genes are expected to be upregulated during repair and early recovery. Four differentially expressed genes between TP1 and TP2 were identified: HBA, HBB, ALAS2, and FAM210B. HBA and HBB are related to oxygen transport, while ALAS2 and FAM210B relate to erythrocyte development. As expected, all four of these genes were differentially expressed at follow-up (Figure 4).

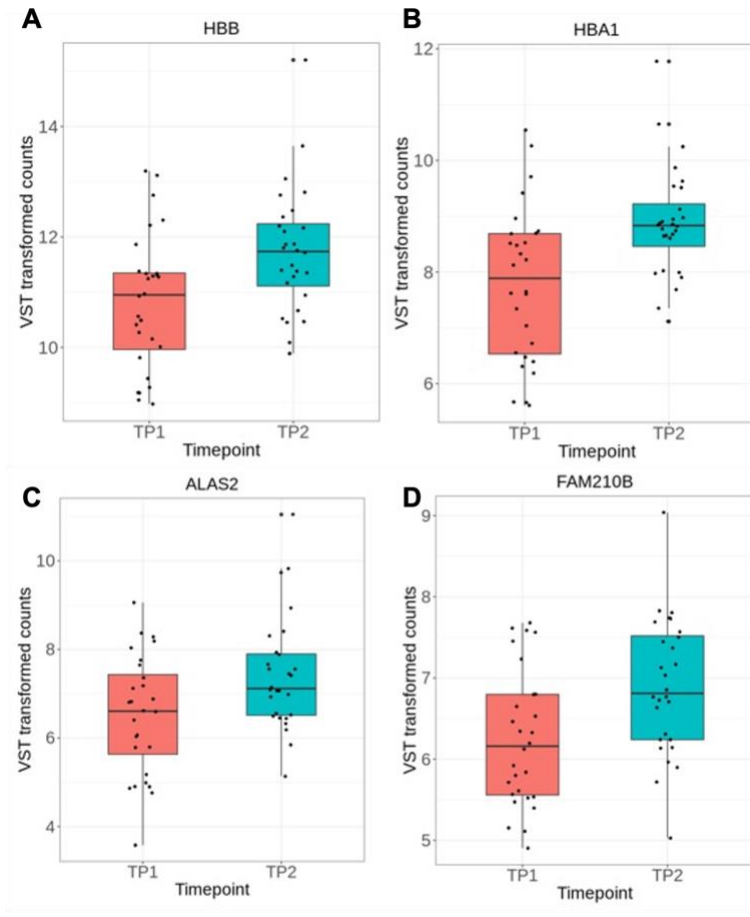


Figure 4. Gene Ontology Analysis. Gene ontology analysis to identify biological processes involved in AKI. The differential abundance analysis of HBB (A), HBA1 (B), ALAS2 (C), and FAM210B (D) at TP1 (during AKI) versus TP2 (60 days +/- 30 after AKI). Y-axis is number of genes present per sample after VST normalization. Abbreviations: AKI, acute kidney injury; VST, variance stabilization transformation.

The cell type of origin of the cfRNA was investigated, but this analysis did not identify any differences in cell type between AKI and follow-up. There was a slight increase in the fraction of erythrocyte erythroid progenitor cells at follow-up, though the difference was insignificant (Figure 5).

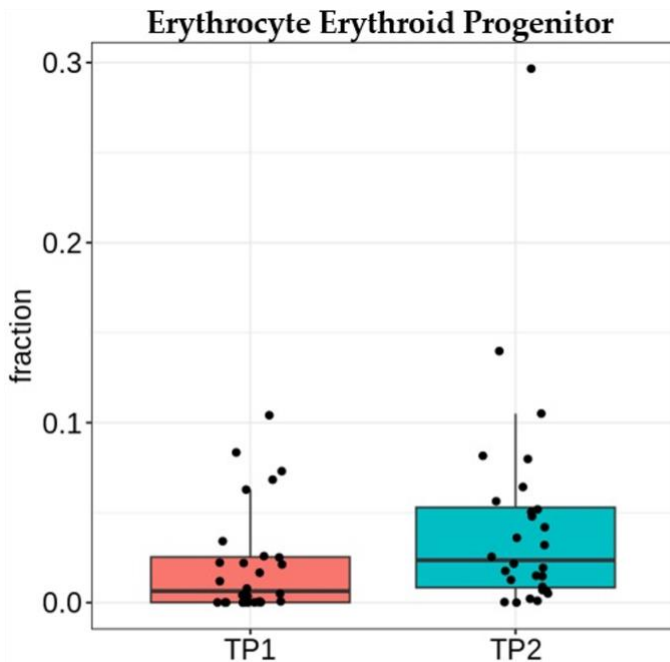


Figure 5. cfRNA Cell Type of Origin Comparison. We wanted to identify differences in cfRNA cell type of origin between TP1 and TP2. The fraction of erythrocyte erythroid progenitor cells was slightly increased at TP2, but this was not statistically significant.

Comparing cfRNA Patterns Across Causes of AKI

While it is crucial to determine biomarkers for AKI of all etiologies, we are particularly interested in whether cfRNA can predict ICI-AKI (or ATIN) versus other AKI etiologies. Using the clinical data provided, we made two comparison groups: (1) ATIN (using proven/likely ATIN and possible ATIN samples) and (2) obstruction (only obstruction samples). The ATIN group had 28 matched samples from 14 patients, while the obstruction group had ten matched samples from five patients. Again, we compared genes differentially expressed at AKI versus at recovery. Indeed, several genes were differentially expressed at AKI versus at recovery within the AKI etiology groups. Figure 6A shows which genes were

upregulated or downregulated during recovery from ATIN versus acute injury.

Figure 6B shows the same but for recovery from obstruction.

Some notable mitochondrial RNA transcripts, such as MT-ND4 and MT-ATP8, were differentially expressed in ATIN and obstruction groups. Many appear to be upregulated at follow-up for ATIN but downregulated when AKI is caused by

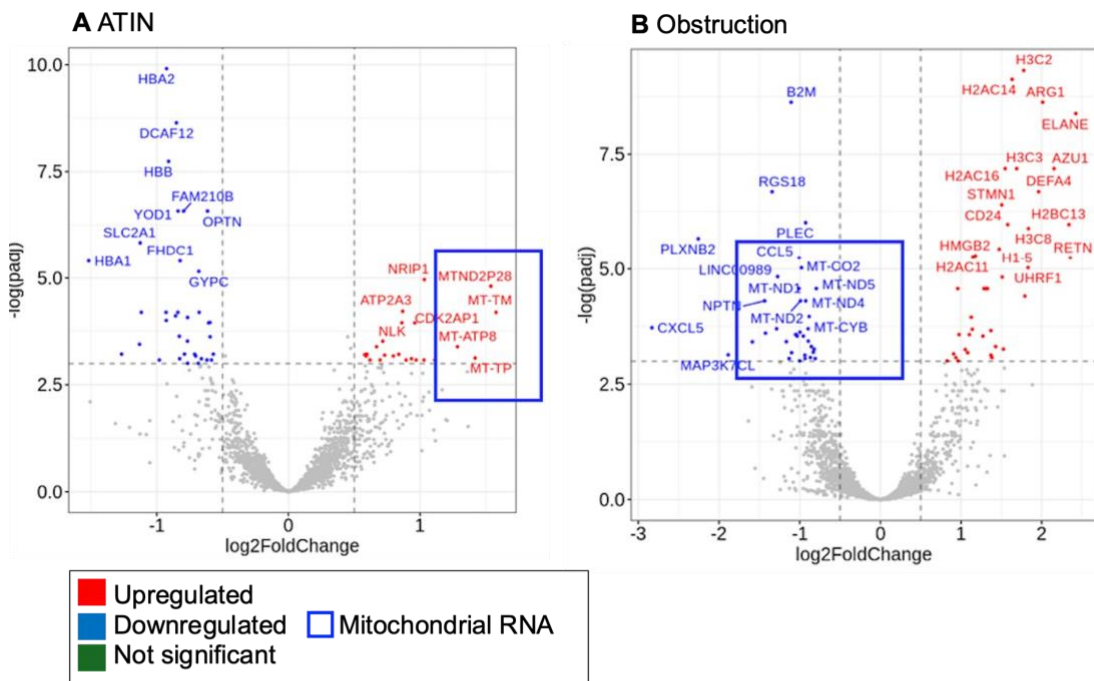


Figure 6. cfRNA Signatures of ATIN versus Obstruction. We wanted to compare cfRNA signatures of injury caused by ATIN versus other etiologies. **Panel A** compares DEGs associated with ATIN at TP1 versus TP2. **Panel B** compares the same but in patients with kidney injury caused by obstruction. The blue box in both panels denotes which DEGs are mitochondrial RNA transcripts. Abbreviations: cfRNA, cell-free ribonucleic acid; ATIN, acute tubulointerstitial nephritis; DEGs, differentially expressed genes; TP1, timepoint 1; TP2, timepoint 2; RNA, ribonucleic acid.

obstruction. The blue boxes in Figure 6 highlight these instances.

cfRNA Predicting Severity of Kidney Injury

The final question in this preliminary analysis was whether cfRNA profiles can predict the severity of AKI. Serum creatinine values determine AKI staging, and the KDIGO criteria were utilized again²⁵. Using only the plasma samples

taken at ICI-AKI (TP1) diagnosis, we compared differentially expressed genes in patients with Stage 1 AKI with those with Stage 2 AKI (Figure 7). By clustering

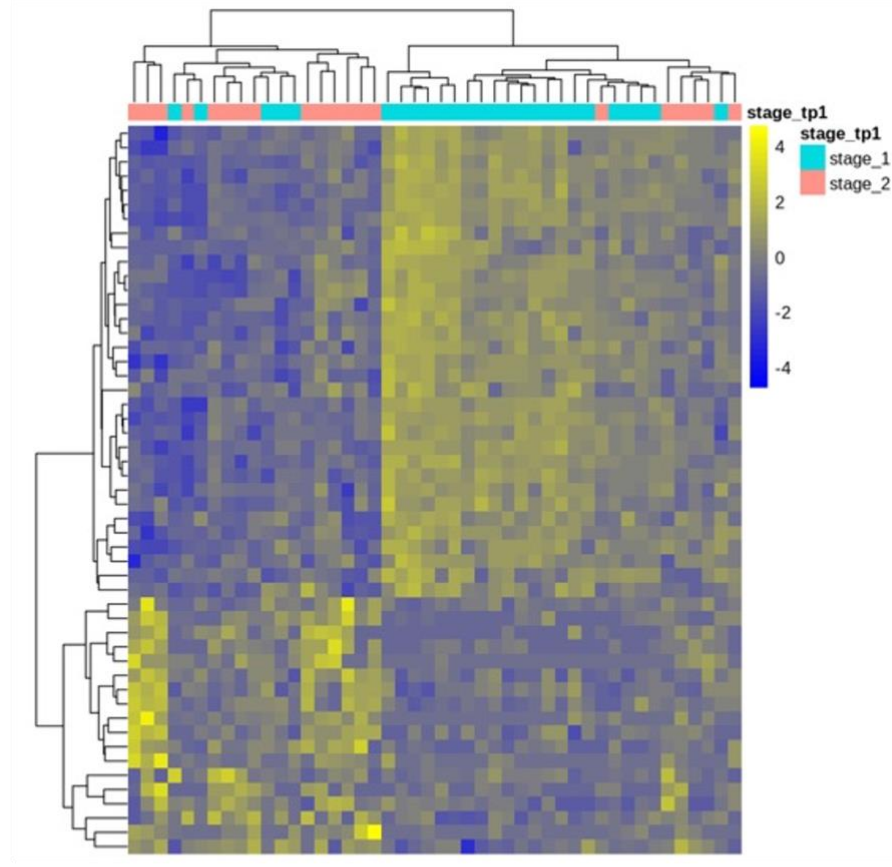


Figure 7. Differential Gene Analysis for AKI Severity. This heatmap shows the differential expression of genes of samples from patients with Stage 1 versus Stage 2 AKI. Color represents the logarithmic fold change for each gene; yellow corresponds to upregulation, and blue to downregulation. By clustering the heatmap, we can appreciate some trends in abundance depending on AKI severity. Abbreviations: AKI, acute kidney injury.

this heatmap, we can appreciate some similarity in gene expression counts within Stage 1 and Stage 2 samples. Color represents the logarithmic fold change for each gene; yellow represents a positive fold change (upregulation), while blue represents a negative fold change (downregulation) of that gene.

Given that this differential expression analysis suggested the potential for cfRNA to predict AKI severity, we decided to compare it with the analysis of acute versus follow-up samples (Figure 3B). Interestingly, we did identify some overlapping genes between these two analyses. In other words, we found three genes that were differentially expressed in both AKI severity and time point comparisons. These three genes are BNIP3L, HBA1, and FAM210B (Figure 8). Further analysis into the expression of these genes and their role in kidney injury may be interesting for future research and model building.

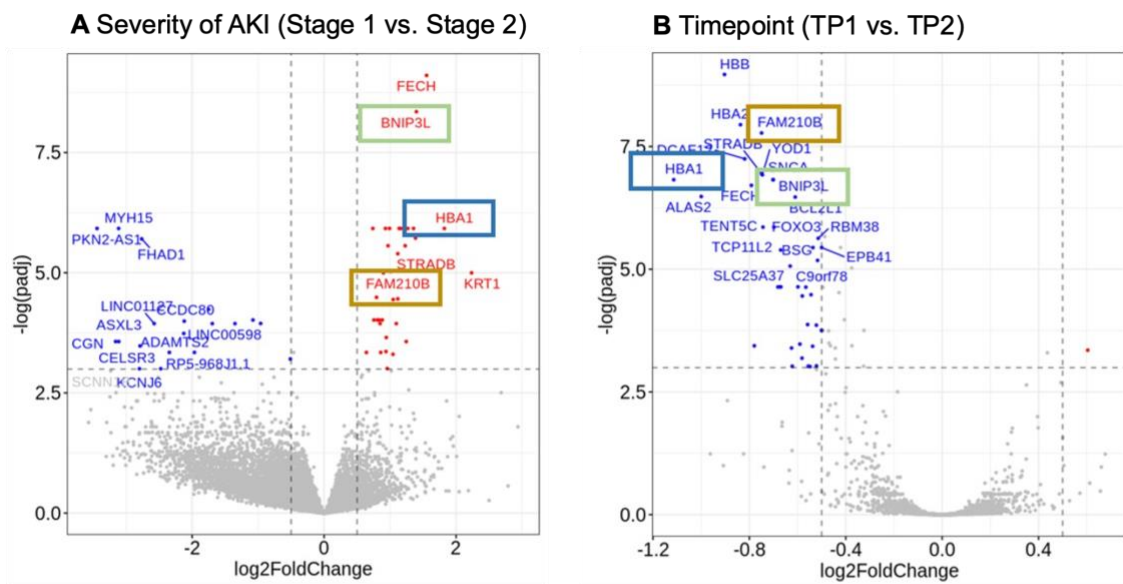


Figure 8. Common DEGs for AKI Severity and Timepoint. Panel A shows the differential expression of selected genes when comparing Stage 1 versus Stage 2 AKI. Panel B is the differential expression of selected genes when comparing TP1 versus TP2 sample collections. The rectangular boxes highlight three genes that were differentially expressed in both models. Abbreviations: AKI, acute kidney injury; TP1, timepoint 1; TP2, timepoint 2.

DISCUSSION

PET-CT Project

Utilizing an international multicenter consortium of 429 patients with ICI-AKI, the quantitative differences identified in the ^{18}F -FDG PET-CT scans from eight patients at two different time points were described. Baseline PET-CT scans were performed in the 90 days preceding the initiation of ICI therapy. The second time point was at ICI-AKI, which included PET-CT scans within 14 days of diagnosis. We first discussed the clinical information relevant to these patients and their ICI-AKI. Then, we described a perceived increase in SUV_{mean} raw values across the patient cohort. Finally, we identified a statistically significant trend in SUV_{mean} percent change between baseline and ICI-AKI using these data.

While the findings presented in this thesis are compelling, definitive statements regarding the efficacy of ^{18}F -FDG PET-CT scans in diagnosing ICI-AKI (more specifically, ATIN) versus other etiologies of AKI can only be made with having control groups. As such, two control groups were established for this study (Table 5). Patients have been identified as controls, and a collaborating

Table 5. Control Groups for PET-CT Scan Project. Classification of patients for each control group. Abbreviations: ICI, immune checkpoint inhibitor; AKI, acute kidney injury.	
Control Group 1	Control Group 2
Patients who: <ul style="list-style-type: none">• Receive a nephrotoxic chemotherapy agent other than an ICI• DO develop AKI	Patients who: <ul style="list-style-type: none">• Receive an ICI• Do NOT develop AKI

nuclear radiologist is interpreting the PET-CT scans. When data collection is complete for the control patients, a similar analysis will be conducted to compare SUV_{mean} at the different time points. As the hypothesis is that ATIN specifically causes increased radiotracer uptake, SUV_{mean} is anticipated to be unchanged in both control groups.

Control Group 1 consisted of patients who received a nephrotoxic chemotherapy agent other than an ICI and developed AKI after initiation of that treatment. In searching for patients to include in this group, it became clear that it was rare for patients to have a follow-up PET-CT scan that coincides with their AKI. Several methods were utilized to identify enough control patients. First, the Research Patient Data Registry (RPDR), a clinical database for over 6.5 million individuals who receive care from Mass General Brigham and DFCI in Massachusetts, was used. An RPDR query was submitted to search for patients who received cisplatin (a nephrotoxic chemotherapy agent) and obtained their laboratory and radiology data. Using other patient cohorts curated by the study PI, Dr. Gupta, over 100 additional patients were reviewed for eligibility.

Inclusion Criteria for Control Group 1:

- 1) AKI of any etiology, defined as a $\geq 2x$ increase in SCr from baseline, occurring in the 90-365 days following treatment initiation.
- 2) A baseline PET-CT scan within 90 days before treatment initiation.
- 3) A follow-up PET-CT scan within 14 days before or after AKI diagnosis.

Control Group 2 consisted of patients who received an ICI but did not develop AKI. We used another RPDR query to search for appropriate patients.

Inclusion Criteria for Control Group 2:

- 1) No SCr values were $\geq 25\%$ higher than baseline.
- 2) Baseline PET-CT scan within 90 days before ICI initiation.
- 3) Follow-up PET-CT scan 90-365 days following treatment initiation.

The exclusion criteria for Control Group 1 and Control Group 2 were the same as those discussed for the ICI-AKI group (see Methods section).

As the patients in Control Group 1 do not have ATIN but rather a different etiology of AKI, an increase in SUV_{mean} at the time of AKI versus baseline is not expected to be seen. If this is true, a more well-informed statement regarding the role of ATIN in increasing radiotracer uptake in the renal cortex at the time of injury can be made. Patients in Control Group 2 have received an ICI but did not develop AKI, so we do not expect an increase in SUV_{mean} here either. If this is true, the ICI itself and its general systemic effects can be confidently ruled out as the cause of increased radiotracer uptake in the renal cortex.

Comparing SUV_{mean} values among all three patient groups will make this study more impactful. Including two control groups will fortify the value of the findings and thus distinguish the results from the existing literature on this topic. With positive results that confirm the hypothesis, this project may contribute to changing the clinical practice of diagnosing ICI-AKI. For patients for whom ICI-AKI is clinically suspected, the PET-CT scan could replace kidney biopsy for a

definitive diagnosis. Deciding to forego an invasive procedure would be especially easy for patients who already receive serial re-staging PET-CT imaging. A PET-CT scan can be ordered at most prominent cancer centers or inpatient hospitals for patients who do not have them regularly. This prospect is both exciting and extremely promising for improving patient outcomes.

Of course, this project has limitations, the most significant being the small patient cohort. With how difficult identifying eligible patients for Control Group 1 has proven to be, it is unlikely that we will achieve a one-to-one ratio with the total number of ICI-AKI patients, though that ratio remains the goal. Ideally, we would also like more ICI-AKI patients in the cohort. The positive results of this study could prompt more clinicians to order PET-CT scans when their patient has ICI-AKI, thereby increasing the patient pool for future research on this topic.

cfRNA Project

The preliminary analysis of cfRNA utilized plasma samples from 46 patients with AKI while receiving an ICI. All patients had a sample from the time of AKI (TP1), while 32 patients had a matched follow-up sample (TP2) collected 60 days (+/- 30 days) after the AKI event. We had three primary questions when looking at these samples: (1) can cfRNA signatures distinguish between TP1 (AKI) and TP2 (follow-up), (2) does the etiology of AKI impact the cfRNA signatures, and (3) can cfRNA signatures predict the severity of AKI. While these limited results cannot definitively answer these questions, the findings provide an excellent foundation for future research.

The identification of 61 DEGs when comparing AKI versus follow-up samples suggests that the expression patterns of specific genes, as quantified in a liquid biopsy, could predict AKI. Unfortunately, we could not identify genes that were predictive of AKI. We did, however, delve deeper into four genes that, when upregulated, differentiated follow-up samples from matched AKI samples. These genes (HBA, HBB, ALAS2, and FAM210B) are involved in erythrocyte development and oxygen transport. HBA and HBB are genes that code for hemoglobin's alpha and beta globin chains, respectively. When we compared cfRNA signatures between Stage 1 and Stage 2 AKI, we found the same HBA gene to be upregulated in more severe (Stage 2) cases of AKI. Two other genes (BNIP3L and FAM210B) were also differentially expressed in both comparisons. This overlap may be important when selecting biomarkers for model building. We also identified that different causes of AKI (ATIN versus obstruction) show different mitochondrial encoded cfRNA patterns with differentially abundant genes.

Future research on this subject is necessary and worthwhile, given the potential cfRNA holds as a biomarker for AKI. We are interested in comparing the plasma cfRNA results with matched urine samples, which may be more sensitive to kidney injury. In addition, we hope to expand the sample size to increase statistical power and reduce heterogeneity among patients included in the analysis.

BIBLIOGRAPHY

1. Goyal A, Daneshpajouhnejad P, Hashmi MF, Bashir K. Acute Kidney Injury. In: *StatPearls*. StatPearls Publishing; 2023. Accessed December 14, 2023. <http://www.ncbi.nlm.nih.gov/books/NBK441896/>
2. Kitchlu A, McArthur E, Amir E, et al. Acute Kidney Injury in Patients Receiving Systemic Treatment for Cancer: A Population-Based Cohort Study. *JNCI: Journal of the National Cancer Institute*. 2019;111(7):727-736. doi:10.1093/jnci/djy167
3. Gupta S, Gudsoorkar P, Jhaveri KD. Acute Kidney Injury in Critically Ill Patients with Cancer. *Clinical Journal of the American Society of Nephrology*. 2022;17(9):1385. doi:10.2215/CJN.15681221
4. Rao Ullur A, Côté G, Pelletier K, Kitchlu A. Immunotherapy in oncology and the kidneys: a clinical review of the evaluation and management of kidney immune-related adverse events. *Clinical Kidney Journal*. 2023;16(6):939-951. doi:10.1093/ckj/sfad014
5. Seethapathy H, Zhao S, Chute DF, et al. The Incidence, Causes, and Risk Factors of Acute Kidney Injury in Patients Receiving Immune Checkpoint Inhibitors. *Clinical Journal of the American Society of Nephrology*. 2019;14(12):1692. doi:10.2215/CJN.00990119
6. Meraz-Muñoz A, Amir E, Ng P, et al. Acute kidney injury associated with immune checkpoint inhibitor therapy: incidence, risk factors and outcomes. *Journal for ImmunoTherapy of Cancer*. 2020;8(1):e000467. doi:10.1136/jitc-2019-000467
7. Postow MA, Sidlow R, Hellmann MD. Immune-Related Adverse Events Associated with Immune Checkpoint Blockade. *New England Journal of Medicine*. 2018;378(2):158-168. doi:10.1056/NEJMra1703481
8. Khoja L, Day D, Wei-Wu Chen T, Siu LL, Hansen AR. Tumour- and class-specific patterns of immune-related adverse events of immune checkpoint inhibitors: a systematic review. *Annals of Oncology*. 2017;28(10):2377-2385. doi:10.1093/annonc/mdx286
9. O'Reilly M, Mellotte G, Ryan B, O'Connor A. Gastrointestinal side effects of cancer treatments. *Therapeutic Advances in Chronic Disease*. 2020;11:2040622320970354. doi:10.1177/2040622320970354

10. Gupta S, Short SAP, Sise ME, et al. Acute kidney injury in patients treated with immune checkpoint inhibitors. *Journal for Immunotherapy of Cancer*. 2021;9(10):e003467. doi:10.1136/jitc-2021-003467
11. Seethapathy H, Zhao S, Strohbehm IA, et al. Incidence and Clinical Features of Immune-Related Acute Kidney Injury in Patients Receiving Programmed Cell Death Ligand-1 Inhibitors. *Kidney International Reports*. 2020;5(10):1700-1705. doi:10.1016/j.ekir.2020.07.011
12. García-Carro C, Bolufer M, Bury R, et al. Acute kidney injury as a risk factor for mortality in oncological patients receiving checkpoint inhibitors. *Nephrology Dialysis Transplantation*. 2022;37(5):887-894. doi:10.1093/ndt/gfab034
13. Cortazar FB, Marrone KA, Troxell ML, et al. Clinicopathological features of acute kidney injury associated with immune checkpoint inhibitors. *Kidney International*. 2016;90(3):638-647. doi:10.1016/j.kint.2016.04.008
14. Shirali AC, Perazella MA, Gettinger S. Association of Acute Interstitial Nephritis With Programmed Cell Death 1 Inhibitor Therapy in Lung Cancer Patients. *American Journal of Kidney Diseases*. 2016;68(2):287-291. doi:10.1053/j.ajkd.2016.02.057
15. Gupta S, Cortazar FB, Riella LV, Leaf DE. Immune Checkpoint Inhibitor Nephrotoxicity: Update 2020. *Kidney360*. 2020;1(2):130-140. doi:10.34067/KID.0000852019
16. Cherk MH, Nadebaum DP, Barber TW, Beech P, Haydon A, Yap KS. 18F-FDG PET/CT features of immune-related adverse events and pitfalls following immunotherapy. *Journal of Medical Imaging and Radiation Oncology*. 2022;66(4):483-494. doi:10.1111/1754-9485.13390
17. Schierz JH, Sarikaya I, Wollina U, Unger L, Sarikaya A. Immune Checkpoint Inhibitor–Related Adverse Effects and 18F-FDG PET/CT Findings. *Journal of Nuclear Medicine Technology*. 2021;49(4):324-329. doi:10.2967/jnmt.121.262151
18. L'Orphelin JM, Varey E, Khammari A, Dreno B, Domp Martin A. Severe Late-Onset Grade III-IV Adverse Events under Immunotherapy: A Retrospective Study of 79 Cases. *Cancers*. 2021;13(19):4928. doi:10.3390/cancers13194928
19. Lang N, Dick J, Slynko A, et al. Clinical significance of signs of autoimmune colitis in 18F-fluorodeoxyglucose positron emission tomography-

- computed tomography of 100 stage-IV melanoma patients. *Immunotherapy*. 2019;11(8):667-676. doi:10.2217/imt-2018-0146
20. Qualls D, Seethapathy H, Bates H, et al. Positron emission tomography as an adjuvant diagnostic test in the evaluation of checkpoint inhibitor-associated acute interstitial nephritis. *Journal for ImmunoTherapy of Cancer*. 2019;7(1):356. doi:10.1186/s40425-019-0820-9
 21. Awiwi MO, Abudayyeh A, Abdel-Wahab N, et al. Imaging features of immune checkpoint inhibitor-related nephritis with clinical correlation: a retrospective series of biopsy-proven cases. *European Radiology*. 2023;33(3):2227-2238. doi:10.1007/s00330-022-09158-8
 22. Chang A, Loy CJ, Lenz JS, et al. Circulating Cell-Free RNA in Blood as a Host Response Biomarker for the Detection of Tuberculosis. Published online January 11, 2023:2023.01.11.23284433. doi:10.1101/2023.01.11.23284433
 23. Moufarrej MN, Vorperian SK, Wong RJ, et al. Early prediction of preeclampsia in pregnancy with cell-free RNA. *Nature*. 2022;602(7898):689-694. doi:10.1038/s41586-022-04410-z
 24. Vorperian SK, Moufarrej MN, Quake SR. Cell types of origin of the cell-free transcriptome. *Nature Biotechnology*. 2022;40(6):855-861. doi:10.1038/s41587-021-01188-9
 25. Leaf DE, Waikar SS. End Points for Clinical Trials in Acute Kidney Injury. *American Journal of Kidney Diseases*. 2017;69(1):108-116. doi:10.1053/j.ajkd.2016.05.033

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