TITLE:
EARLY THERAPEUTIC MONITORING OF RESPONSE TO THERAPY WITH SERIAL ULTRASOUND IN METASTATIC RENAL CELL CARCINOMA

Document History	Notes
Version 1.0 Date: 19 Aug, 2020	Initial Approval
Version 2.0 Date: 16 Jan, 2021	Revisions include: • updated list of co-investigators • relaxation of allowable time window for 6-week US exam (+/- 15 days instead of +/- 8 days) • option added to obtain consent from Legally Authorized Representatives (LARs) • option added to obtain written consent from English-speaking participants/LARs online via RedCAP
Version 3.0 Date: 28 Apr, 2021	Revisions include: • updated list of co-investigators • clarification of inclusion criteria (section 3.1) • inclusion of other ultrasound transducers
Version 4.0 Date: 28 Jun, 2021	Revisions include: • updated list of co-investigators • correction of protocol title
Version 5.0 Date: 12 Jul, 2022	Revisions include: • updated list of co-investigators • additional exploratory study arm 2 defined: patients receiving any non-immune checkpoint inhibitor treatment (e.g., TKI monotherapy, combination of TKI with other kinase inhibitor, localized tumor therapy such as radiation, chemo- or radioembolization) • participant population expanded to patients referred to Stanford from outside institutions for participation in this study • clarification added to study calendar on definition of start of treatment • minimum time period of 10 days between 'week-3' and 'week-6' ultrasound exams defined in study calendar • use of RedCAP for electronic signatures on consent forms replaced by Adobe Sign • correction of typos and unclear wording
Version 5.0 Date: 27 Jul, 2022 (revisions after start of SRC review)	Revisions include: • triple eligibility check replaced by single eligibility check by treating provider (incl. APPs)
Version 6.0 Date: 4 Oct, 2022	Revisions include: • updated list of co-investigators • option added for non-licensed, qualified staff to perform research ultrasound exams
Version 7.0 Date: 21 Nov, 2022	Revisions include: • AE evaluation schedule revised, now tied to study procedures instead of SOC visits
Version 8.0 Date: 30 Jan, 2023	Revisions include: • management of ultrasound imaging data and associated PHI clarified (section 8.2) • Appendix A: lines for provider initials and date for subjective exclusion criterion added

Version 9.0 Date: 1 May, 2023	Revisions include: • updated list of co-investigators • criteria for removal from study clarified (section 5.1) • data management procedures upon study closure clarified (section 8.2)

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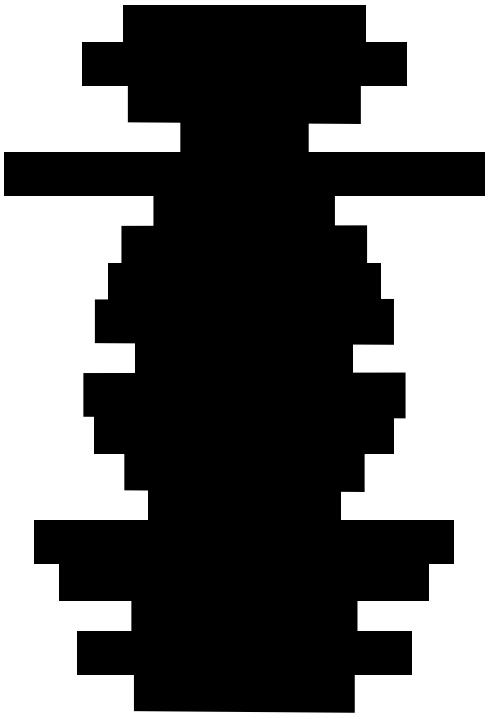

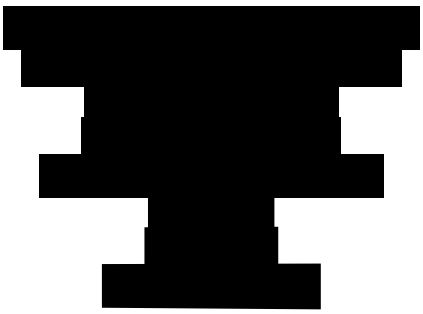

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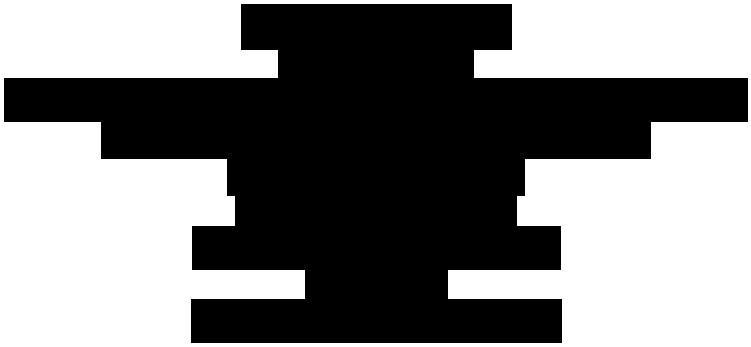


Study Coordinator

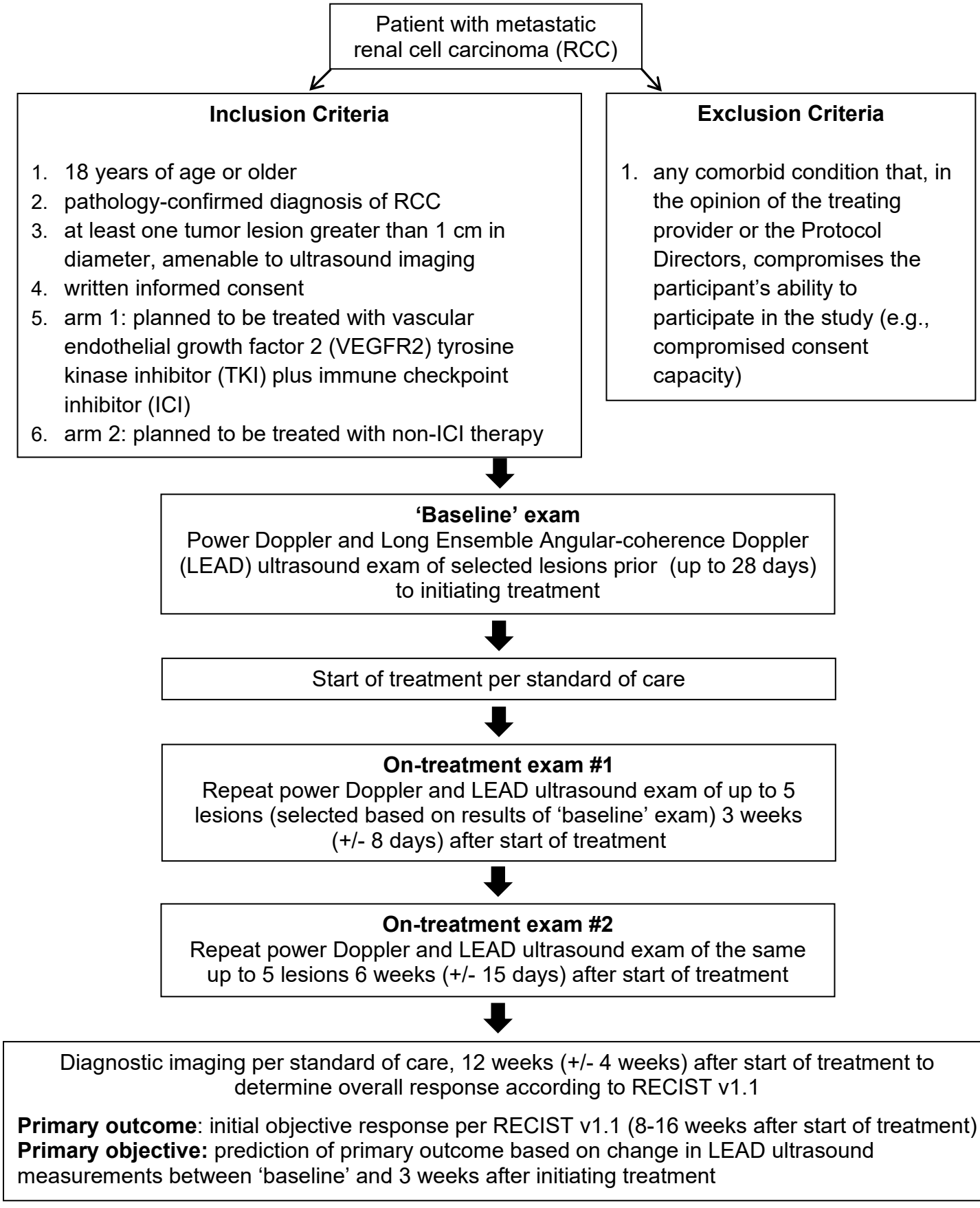
SRC Initial Approval Date: September 10, 2020 (Version 1.0 dated August 19, 2020)

TABLE OF CONTENTS

PROTOCOL SYNOPSIS

TITLE	Early therapeutic monitoring of response to therapy with serial ultrasound in metastatic RCC
STUDY PHASE	Pilot
INDICATION	Metastatic Renal Cell Carcinoma (mRCC)
INVESTIGATIONAL PRODUCT OR PROCEDURE	Study of power Doppler ultrasound for early detection of therapeutic response after initiating therapy with: arm 1: tyrosine kinase (TKI) inhibitor plus immune checkpoint inhibitor (ICI) arm 2: non-ICI therapy (e.g., TKI monotherapy, combination of TKI with other kinase inhibitor, localized tumor therapy such as radiation, chemo- or radioembolization)
PRIMARY OBJECTIVE(S)	To assess whether changes in quantitative tumor perfusion parameters after 3 weeks of treatment, as measured by LEAD ultrasound, can predict initial objective response, defined by current standard-of-care, to therapy at 12 weeks after start of treatment
SECONDARY OBJECTIVE(S)	To evaluate if there is an optimal ultrasound imaging modality (power Doppler or LEAD ultrasound) or optimal time point to predict initial objective response, to assess the correlation of tumor perfusion parameters with change in overall tumor burden, change in diameter on a per-lesion basis and with 12-month progression-free survival (PFS).
STUDY PROCEDURE SUMMARY	power Doppler and LEAD ultrasound of subjects taking targeted therapy medication, measured at treatment ‘baseline’, 3-4 weeks, and 6-8 weeks after initiating treatment
SAMPLE SIZE	arm 1: 20 subjects arm 2: up to 10 subjects
STATISTICAL CONSIDERATIONS	We will evaluate the predictive ability of each model using area under the curve (AUC). For this pilot study, assuming that 59% of patients in arm 1 have an objective response to treatment, a predictive model with a true AUC of 0.7 will have a margin of error of 0.14 so that the estimated AUC can range from 0.56 to 0.84.

SCHEMA


Patient with metastatic renal cell carcinoma (RCC)
Inclusion Criteria
1. 18 years of age or older
2. pathology-confirmed diagnosis of RCC
3. at least one tumor lesion greater than 1 cm in diameter, amenable to ultrasound imaging
4. written informed consent
5. arm 1: planned to be treated with vascular endothelial growth factor 2 (VEGFR2) tyrosine kinase inhibitor (TKI) plus immune checkpoint inhibitor (ICI)
6. arm 2: planned to be treated with non-ICI therapy
Exclusion Criteria
1. any comorbid condition that, in the opinion of the treating provider or the Protocol Directors, compromises the participant's ability to participate in the study (e.g., compromised consent capacity)
'Baseline' exam
Power Doppler and Long Ensemble Angular-coherence Doppler (LEAD) ultrasound exam of selected lesions prior (up to 28 days) to initiating treatment
Start of treatment per standard of care
On-treatment exam #1
Repeat power Doppler and LEAD ultrasound exam of up to 5 lesions (selected based on results of 'baseline' exam) 3 weeks (+/- 8 days) after start of treatment
On-treatment exam #2
Repeat power Doppler and LEAD ultrasound exam of the same up to 5 lesions 6 weeks (+/- 15 days) after start of treatment
Diagnostic imaging per standard of care, 12 weeks (+/- 4 weeks) after start of treatment to determine overall response according to RECIST v1.1
Primary outcome: initial objective response per RECIST v1.1 (8-16 weeks after start of treatment)
Primary objective: prediction of primary outcome based on change in LEAD ultrasound measurements between 'baseline' and 3 weeks after initiating treatment

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

AE	Adverse Event
AUC	Area Under the Curve
CART	Classification And Regression Tree
CEUS	Contrast-Enhanced Ultrasound
CR	Complete Response
CRF	Case Report Form
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTLA-4	Cytotoxic T-Lymphocyte-Associated Protein 4
DSMC	Data and Safety Monitoring Committee
FDA	United States Food and Drug Administration
GCP	Good Clinical Practice
ICI	Immune Checkpoint Inhibitor
IDE	Investigational Device Exemption
IRB	Institutional Review Board
Lasso	Least absolute shrinkage and selection operator
LEAD	Long Ensemble Angular-coherence Doppler
mRCC	Metastatic Renal Cell Carcinoma
OS	Overall Survival
PD	Progressive Disease
PD-1	Programmed Cell Death Protein-1
PD-L1	Programmed Death Ligand-1
PET	Positron Emission Tomography
PFS	Progression-Free Survival
PR	Partial Response
RECIST	Response Evaluation Criteria In Solid Tumors
RCC	Renal Cell Carcinoma
SAE	Serious Adverse Event
SD	Stable Disease
SOP	Standard Operating Procedure
SRC	Scientific Review Committee
TKI	Tyrosine Kinase Inhibitor
VEGFR2	Vascular Endothelial Growth Factor Receptor 2

1. OBJECTIVES

1.1 Primary Objective

Our primary objective is to assess whether in arm 1, changes in quantitative tumor perfusion parameters 3 weeks after start of treatment of metastatic renal cell carcinoma (mRCC) patients with tyrosine kinase inhibitor (TKI) plus immune checkpoint inhibitor (ICI), as measured by LEAD ultrasound, can predict initial objective response to therapy at 12 weeks after start of treatment defined by current standard-of-care Response Evaluation Criteria In Solid Tumors (RECIST v1.1)[1].

1.2 Secondary Objectives

Our secondary objectives are to evaluate in arm 1, if there is an optimal ultrasound imaging modality (power Doppler or LEAD ultrasound), or optimal time point to predict initial objective response. We will assess how well 1) changes in tumor perfusion parameters 6 weeks after start of treatment by imaging modality or 2) tumor perfusion parameters at a single time point (treatment 'baseline', 3 weeks, or 6 weeks after start of treatment), predict initial objective response (at 12 weeks) per RECIST v1.1.

Additional exploratory objectives in both arms are to assess the correlation of tumor perfusion parameters with change in overall tumor burden (defined as sum of the longest diameters of all measurable lesions defined by RECIST v1.1) 12 weeks after start of treatment, with change in diameter on a per-lesion basis (correlation between perfusion parameters of a single lesion with change in diameter of that lesion), and with 12-month progression-free survival (PFS).

2. BACKGROUND

2.1 Study Disease

Renal cancer is the 8th most common cancer in the U.S. and patients with metastatic RCC (mRCC) have a 5-year overall survival (OS) rate of 12%[2]. There are 17 FDA-approved drugs for mRCC, which include TKIs of vascular endothelial growth factor receptor 2 (VEGFR2, a key mediator of angiogenesis), and antibodies that target immune checkpoint proteins such as programmed cell death protein-1 (PD-1), programmed death ligand-1 (PD-L1), or cytotoxic T-lymphocyte-associated protein 4 (CTLA-4)[3–10].

In April 2019, the FDA approved the combinations of a TKI (axitinib) with an ICI (pembrolizumab or avelumab) for first-line treatment of mRCC and additional TKI plus ICI combination therapies (cabozantinib plus nivolumab, lenvatinib plus pembrolizumab) have been approved more recently[11,12]. TKI plus ICI has become the new standard-of-care first-line treatment that improves OS for patients with mRCC[11].

2.2 Imaging Procedure

Power Doppler ultrasound is an ideal non-invasive modality for serial measurements because it can be performed at bedside during routine oncology visits. However, due to the relatively large depths at which some organs such as the kidneys are located and the low frequencies utilized to image deep structures, the ability to detect the smaller vasculature of tumors and the potential changes in tumor vasculature becomes increasingly difficult. Hence, in addition to the commercially available power Doppler imaging mode, we will incorporate advanced power Doppler imaging techniques that improve the sensitivity to slower flow and smaller vessels in our study. Specifically, this advanced power Doppler mode utilizes long Doppler ensembles combined with short-lag angular coherence beamforming to achieve high sensitivity to slow flow and small vessels (Long Ensemble Angular-coherence Doppler [LEAD] ultrasound). The long Doppler ensemble increases the sensitivity to flow by utilizing high frame rates (~300 Hz) to boost the signal-to-noise ratio of the blood signal[13]. Moreover, angular coherence beamforming is utilized in LEAD ultrasound studies to suppress motion/flash artifact over the long ensemble while also suppressing incoherent noise due to reverberation and thermal noise[14,15].

For the ultrasound exams at treatment 'baseline' and 3 and 6 weeks after initiating treatment in this pilot study, lesions are imaged with B-mode and power Doppler ultrasound using a portable Siemens Acuson S3000 ultrasound scanner and a 6C1 or similar transducer (FDA-approved for clinical ultrasound exams but used only for research exams for this study). Measurements are performed in the long axis and short axis planes, if possible by tumor localization. For LEAD ultrasound exams, the same views are obtained using a Verasonics Vantage 256 scanner with a C5-2v or similar transducer (research ultrasound scanner).

2.3 Clinicaltrials.gov

The Verasonics system operates under an abbreviated Investigational Device Exemption (IDE) approved by the Stanford IRB. The device has non-significant risk. The study is registered at clinicaltrials.gov under registration number NCT04508725.

2.4 Rationale

Unfortunately, not all mRCC patients respond to first-line treatment, yet all patients endure the side effects of treatment (fatigue, nausea, diarrhea, and/or liver inflammation) while awaiting standard-of-care imaging (typically computed tomography, CT) to assess response after approximately 12 weeks of therapy[16–22]. Evaluation of efficacy currently requires this time period because molecular and cellular changes induced by therapy require about 12 weeks to result in definitive changes in tumor diameters on CT scans. Patients may respond to subsequent lines of therapy; yet approximately 40% of patients never receive second-line therapy despite 13 additional drugs available[23–25]. Thus, rapid detection of response or resistance to RCC therapy is required to minimize use

of ineffective drugs and to allow patients to cycle through drugs to arrive at effective therapy[22].

Increased angiogenesis and immune system evasion are intrinsic to RCC. Clear cell RCC occurs when the von-Hippel Lindau tumor suppressor is lost, resulting in upregulation of angiogenesis and suppression of immune signaling. In RCC, blood vessels increase in number but have abnormal porosity[26–29]. The abnormal porosity prevents T cells from exiting blood vessels to infiltrate and attack tumors. Immune checkpoint inhibition normalizes blood vessel porosity, allowing T cell egress[27,30,31]. Hence changes in tumor vascularity may be especially important in RCC patients receiving TKI therapy (alone or in combination with other drugs).

We have previously found that imaging-based biomarkers (e.g., perfusion CT, '4D-CT' scans, or novel positron emission tomography [PET] tracers such as F18-FPPRGD2 that binds blood vessels) may have the capability of detecting early response to anti-angiogenic therapy in mRCC[32,33]. However, due to renal dysfunction and often only a solitary remaining kidney after resection of the initial kidney primary lesion, patients are not always eligible for perfusion CT, which requires potentially nephrotoxic iodinated contrast. A limitation of PET imaging is that nuclear imaging does not provide measurements of blood flow. Hence, there is an important clinical need for complimentary, non-nephrotoxic imaging that can be serially repeated without exposure to ionizing radiation, to predict response and resistance to TKI plus ICI and other mRCC therapies.

In addition, not all patients can tolerate TKI plus ICI combination therapy, so we have added an exploratory arm 2 to investigate the ability of Doppler ultrasound to detect changes in tumor perfusion in patients who are planned to receive non-ICI therapies. These include, but are not limited to: TKI monotherapy, combination of TKI with another kinase inhibitor (e.g., mTOR inhibitor), localized tumor therapy such as radiation, radioembolization, chemoembolization. (Patients receiving ICI/ICI combination therapy or ICI monotherapy are enrolled in the parallel ultrasound study 'Serial Ultrasound to Detect Early Response to Immunotherapy in Advanced Renal Cell Carcinoma' (RENAL0045). The choice of treatment is made by the treating medical oncologist based on clinical considerations per standard-of-care (e.g., comorbidities and performance status, need for immediate control over tumor growth versus long-lasting tumor control, response to previous treatments, tolerance of previous treatments). Patients are assigned to a study arm based on the treatment plan at the time of enrollment.

Contrast-enhanced ultrasound (CEUS) has been used for the identification of renal focal lesions and has shown some promise in preliminary studies to monitor changes with therapy[34,35]. However, CEUS has not widely been explored as a methodology to monitor anti-angiogenesis or combination therapy. Furthermore, the use of CEUS to monitor therapeutic response may be unnecessary, as recent advances in power Doppler imaging have yielded non-contrast, high-sensitivity perfusion imaging by using novel filter and ultrasound beamforming design[13,36–

[41]. Such methods have also been applied to monitor cancer therapy response in the liver[39,42]. Doppler ultrasound is an ideal modality for serial measurements because it can be performed at bedside during routine oncology visits, without requiring placement of an intravenous catheter for administration of contrast. In this pilot study, we use conventional power Doppler and LEAD ultrasound imaging to non-invasively assess changes in tumor perfusion parameters performed in real time, with the ultimate goal to accelerate detection of response to combined TKI plus ICI therapy.

2.5 Preliminary results

We have previously demonstrated good performance of LEAD ultrasound in neonates to show highly detailed brain vasculature and suppression of flash- and motion-artifact[15]. In addition, we used this technique for deep imaging within the liver (~22 cm), which overcomes difficulties of imaging large or obese individuals with high body mass index (unpublished results, see Figure).

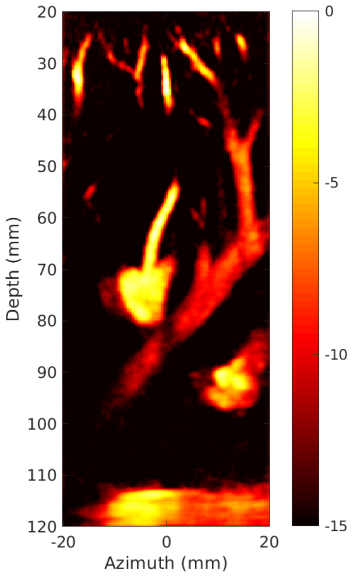


Figure: Demonstration of LEAD applied to the liver of a high BMI individual. This power Doppler-style image shows sensitivity to a wide range of vasculature deep within the liver. The smallest vessels visualized are on the order of 1 mm in diameter.

2.6 Study Design

This is a pilot, single group imaging study, testing a new method of therapeutic response assessment, power Doppler ultrasound, in mRCC patients treated with TKI plus ICI (arm 1) or non-ICI therapy (arm 2). A total of 30 subjects will be enrolled for a two-arm, open-label cohort. No randomization is used. Rather, subjects receiving the treatment as part of their standard of care are followed with the ultrasound studies. Since this is an exploratory pilot study and we are not formally testing a specific research hypothesis, the sample size for each arm has been chosen based on practical considerations and estimated feasibility.

3. PARTICIPANT SELECTION AND ENROLLMENT PROCEDURES

3.1 Inclusion Criteria

The participant population consists of patients treated at Stanford Healthcare or patients referred to Stanford from outside institutions for participation in this study. Patients must meet all of the following general inclusion criteria:

- 18 years of age or older
- pathology-confirmed diagnosis of RCC
- at least one tumor lesion greater than 1 cm in diameter, amenable to ultrasound imaging
- written informed consent.

In addition, patients must meet the following specific inclusion criteria for the respective study arm:

Arm 1: TKI plus ICI Treatment

- planned to be treated with combination of VEGFR2 tyrosine kinase inhibitor (TKI) plus immune checkpoint inhibitor (ICI)*

* prior use of either class of drugs is not necessarily excluded, and patients may be included with the approval of the Protocol Director

Arm 2: Non-ICI Treatment

- planned to be treated with non-ICI therapy**

** including, but not limited to: TKI monotherapy, other kinase inhibitors (e.g., mTOR inhibitors) alone or in combination with TKIs, localized tumor therapy such as radiation, chemo- or radioembolization

Subjects may participate in the study more than once at the discretion of the Protocol Director, for example, if they receive different lines of treatment that all qualify for the study.

3.2 Exclusion Criteria

Any comorbid condition*** that, in the opinion of the treating provider or the Protocol Directors, compromises the participant's ability to participate in the study.

*** Examples: any mental condition that compromises the ability to follow a consent discussion or to make informed decisions (except if represented by a Legally Authorized Representative [LAR]) or to have ultrasound exams.

3.3 Informed Consent Process

No advertisements are utilized. All participants must be provided a consent form describing the study with sufficient information for participants to make an informed decision regarding their participation. Participants are consented by a member of the study personnel at Stanford Healthcare, Stanford University, or remotely. Remote consent discussions will take place via telephone or video conferencing services (e.g., Stanford instance of Zoom, EpiC video visit). The

remote consent process does not waive the requirement for written consent and the investigators must follow the Stanford Cancer Institute's SOP 'Remote Consenting for Adults' to obtain written consent. Participants or their Legally Authorized Representative (LAR) must sign the IRB-approved informed consent prior to participation in any study specific procedure. Written consent may be obtained electronically via Adobe Sign. The participant must receive a copy of the signed and dated consent document. The original signed copy of the consent document must be retained in the medical record or research file.

3.4 Study Timeline

The study will reach primary completion 2 years from the time the study opens to accrual.

3.5 Study Completion

The study will reach study completion 3 years from the time the study opens to accrual.

4. IMAGING PROCEDURE INFORMATION

The ultrasound exams must be performed either by a licensed professional (technologist, fellow, or attending) or by a qualified member of the research staff (e.g., research scholar, fellow), if all of the following criteria (approved by the Stanford School of Medicine Department of Radiology and Stanford Healthcare) are fulfilled:

- delegated individual holds a sonography certification
- task is delegated by the Protocol Director and this is documented on the Delegation of Responsibility Log
- a California-licensed radiologist interprets the sonography (not applicable to this study, as the study does not include any formal interpretation of the ultrasound exams)
- the clinical ultrasound machine is set up with a research function to keep clinical care and research separate (not applicable to this study, as all scanners are exclusively used for research).

Performing the exam includes holding the transducer, providing patient care related to the exam in collaboration with the investigators, and documenting the encounter in the patient's Stanford Healthcare medical record. Investigators with expertise in ultrasound are allowed to be present and provide assistance with operating the instrument to the licensed professional. Assistance may include image review, instrument operation during the scan, but not providing any patient-related care or holding the transducer.

The selected lesions (see section 10.3.2) are imaged in B-mode and with power Doppler ultrasound using a portable Siemens Acuson S3000 ultrasound scanner and a 6C1 or similar transducer, in the long axis and short axis planes, if possible by localization. For LEAD ultrasound exams, the same views using a Verasonics Vantage 256 scanner with a C5-2v or similar transducer are obtained. The ultrasound exams take place during standard-of-care or research-only clinic visits at Stanford Healthcare.

Each participant has three ultrasound exams, each with both scanners: at treatment 'baseline' and 3 and 6 weeks after initiating treatment.

5. STUDY PROCEDURES

5.1 Criteria for Removal from Study

Subjects are removed from active study participation (i.e., no further ultrasound exams), if

- they are non-compliant with study procedures;
- their treatment plan changes before the first ultrasound exam such that the new treatment plan does not qualify for this study;
- they start a different treatment during the active study participation period;
- for participants in arm 1, they receive less than 4 weeks of TKI treatment during the first 12 weeks after initiating TKI plus ICI therapy (i.e. minimum 4 of 12 weeks of TKI treatment and minimum one dose of ICI required for continued study participation);
- for participants in arm 2, they receive less than 4 weeks of systemic therapy druing the first 12 weeks after initiating treatment. (For localized therapies, the minimum treatment requirements are determined by the Protocol Director.)

Subjects removed from active study participation with valid written consent are followed up per study protocol, so that data acquired by study procedures up to that time point can still be used for analysis.

5.2 Alternatives

As an alternative to this study, the patient may elect not to participate. Patients will continue to receive standard care through their oncologist.

6. STUDY CALENDAR

	Pre-Study	Baseline (up to 28 days prior to initiating therapy)[h]	Standard-Of-Care Therapy			
			Week 3 (+/- 8 days)[i]	Week 6 (+/- 15 days)[i]	Week 12 (+/- 4 weeks)[h]	Before Study Completion[j]
Informed consent	X					
Doppler ultrasound exams[a]		X	X	X		
Standard-of-care imaging[b]	X				X	
Adverse event evaluation[c]		X				
Recording of age, race, ethnicity, sex, diagnosis, cancer stage, tumor histology, and drug treatment[d,e]		X (just once during any study timepoint)				
Documentation of lesions selected for ultrasound exam, scanners and modalities used, and imaged axis planes		X	X	X		
Quantification of tumor perfusion parameters[f]						X
Tumor diameter measurements[g]						X
Optional recording of medical history and other diagnoses, concurrent treatment and medication, additional treatment, follow-up, and survival data for future correlation studies[e]		X ---------- X				

a) Doppler ultrasound exams: power Doppler and LEAD ultrasound exams, performed during standard-of-care clinic visit or at research-only visit; if exam can be performed only with one of the two ultrasound scanners, only in one axis, or if one time point is missed entirely, subsequent exams may still be performed in full within their allowed time window.

b) Standard-of-care imaging: The baseline standard-of-care scan is generally within 6 weeks prior to initiating therapy, but may be greater than 6 weeks prior to therapy, if this is the scan that the treating physician chooses to use as reference for response evaluation. Preferably, this is a CT Chest-Abdomen-Pelvis (CAP) scan (with or without contrast), but it may also be an MRI or PET/CT or a combination of MRI and CT. At the discretion of the treating oncologist, the standard-of-care scan at week 12 may be performed outside of the +/- 4-week window.

c) Adverse event (AE) evaluation: Only AEs that are attributed to the study procedures are recorded and reported. During study procedures, study coordinator reminds patient to contact the clinical care team, if they experience any symptoms during the exam or afterwards. If the participant expresses to the coordinator that they have experienced symptoms during or after the exam, the coordinator will alert the clinical care team on the same day to contact the patient for AE evaluation and management. During baseline through week 12, patient will continue with standard-of-care provider visits at or outside of Stanford (generally, every 2-6 weeks, at the discretion of the treating clinical care team) including adverse event management. At week 12, a retrospective chart review will document any undisclosed study-related late adverse events and assure there are no ongoing study-related adverse events. If there is no adequate information in the medical record at week 12, patient will be contacted if needed to complete this documentation. Therefore, at minimum, there is one documented AE evaluation at week 12.

d) to be recorded once between baseline and study completion

e) may be obtained until study completion, even after a participant's death; patients or their outside medical providers may be contacted as needed to obtain the clinico-demographic data, if not available in the Stanford medical record.

f) to be quantified and recorded for all ultrasound exams from this study

g) RECIST measurements to be recorded for all imaging studies used for response evaluation (CT, MRI or PET/CT as described in footnote (b). Performed at minimum at baseline and 12 weeks. RECIST measurements will also be recorded for other subsequent standard-of-care studies that the treating MD obtains at any interval to evaluate treatment response (until 12 months after initiating treatment or until end of the treatment that is being studied, whichever occurs first)

h) For combination treatments, initiation of therapy is typically defined by the day of starting treatment with the second drug, however another day (such as the start of treatment with the first drug) may be defined as start of treatment at the discretion of the Protocol Director; date considered start of treatment will be documented in the research procedure note in the electronic medical record.

i) 10 days is the minimum time period between week-3 and week-6 power Doppler ultrasound exams; similarly, 10 days is the minimum time period between week-3 and week-6 LEAD ultrasound exams; it is not required that power Doppler and LEAD ultrasound exams are performed on the same date.

j) Before end of study (3 years from date of study opening)

7. ADVERSE EVENTS AND REPORTING PROCEDURES

7.1 Potential Adverse Events

There are no known risks from ultrasound as a diagnostic tool in its 60 years of use. The Verasonics system used for LEAD ultrasound exams operates the same way as a regular ultrasound system, except the investigators are performing the acoustic output measurements instead of the manufacturer. The system has also been cleared by Clinical Engineering. Therefore, the risks are no different than a clinical ultrasound scanner such as the FDA-approved Siemens system used for the conventional power Doppler ultrasound exams. We have also previously used the Verasonics system in several IRB-approved protocols in studies involving imaging of adult liver, adult heart, adult thyroid, and neonatal brain. There have been no adverse events in any of these studies or subjects/patients.

Possible adverse events from the procedure include discomfort or tenderness in some cases from slightly pressing the transducer against the tumor site. However, protocol directors are available in the event of an unforeseen adverse event or the need for medical intervention.

7.2 Adverse Event Reporting

For this study, only Adverse Events (AEs) attributed to ultrasound use are reported.

During study procedures, the study coordinator reminds patient to contact the clinical care team, if they experience any symptoms during the exam or afterwards. If the participant expresses to the coordinator that they have experienced symptoms during or after the exam, the coordinator will alert the clinical care team on the same day to contact the patient for AE evaluation and management. During baseline through week 12, patient will continue with standard-of-care provider visits at or outside of Stanford (generally, every 2-6 weeks, at the discretion of the treating clinical care team) including adverse event management. At week 12, a retrospective chart review will document any undisclosed study-related late adverse events and assure there are no ongoing study-related adverse events. If there is no adequate information in the medical record at week 12, patient will be contacted if needed to complete this documentation. Therefore, at minimum, there is one documented AE evaluation at week 12.

AEs are graded according to CTCAE v5.0. Both Serious and Non-Serious study-related AEs are clearly noted in source documentation and listed on study specific Case Report Forms in RedCAP. The Protocol Director or designee assess each AE to determine whether it is unexpected according to the Informed Consent or Protocol Document and related to the investigation. All study-related Serious Adverse Events (SAEs) are tracked until resolution. AEs and SAEs related to standard-of-care procedures or medication, including the studied drug therapy for RCC, are not recorded or reported.

8. REGULATORY CONSIDERATIONS

8.1 Institutional Review of Protocol

The protocol, the proposed informed consent and all forms of participant information related to the study (e.g., advertisements used to recruit participants) must be reviewed and approved by the Stanford IRB and Scientific Review Committee (SRC). Any changes made to the protocol will be submitted as a modification to be approved by the IRB and SRC prior to implementation. The Protocol Director will disseminate the protocol amendment information to all participating investigators.

8.2 Data Management Plan

An alphanumeric study code is selected by the study coordinator for each participant. Source documents and analytic data are used to complete the electronic Case Report Forms (eCRFs) in the Stanford RedCAP database. An eCRFs needs to be completed for each enrolled patient and, at minimum, needs to contain the following data:

- study code
- age, race, ethnicity, sex, diagnosis, cancer stage, tumor histology, and drug treatment
- identification of lesions selected for ultrasound exams and for response evaluation
- identification of time points and modality of ultrasound exams and standard-of-care radiographic imaging
- tumor perfusion parameters quantified from ultrasound studies
- tumor diameter measurements quantified from standard-of-care radiographic imaging studies
- primary and secondary outcomes measures.

The digital ultrasound images including PHI may be stored on the ultrasound scanners for the duration for the study. Ultrasound image files that are transferred off the scanners have coded file names and the files are stored on Dr. Dahl's lab servers or using cloud storage services that are certified for PHI (e.g., Stanford Medicine Box). Other source documents need to be included in the regulatory binder. The Stanford's Data & Safety Monitoring Committee (DSMC) will routinely review the source documentation (incl. the raw imaging files on Dr. Dahl's lab servers) and verify the corresponding data entered in the eCRFs. All entered, changed, and final data shall be available with a validated audit trail or data extract report. The eCRFs shall be considered complete when all expected data has been entered and all discrepancies have been resolved or documented.

Source documents, other documents containing HIPAA identifiers, clinico-demographic or analytic data linked to the participants' identity (i.e., Protected Health Information, PHI), and information linking study codes to participant identities may be additionally maintained in paper form or electronically on co-investigators' computers or using cloud storage services that are certified for PHI

(e.g., OnCore, Stanford Medicine Box) until study completion. All computers need to comply with Stanford University IT security standards. Access to files and folders with PHI (paper or electronic) must be restricted to the study personnel.

Upon study closure, all PHI not stored in the regulatory binder (physical and electronic), regulatory database (i.e., OnCore), or eCRFs must be destroyed, for example by de-identifying the data and files in accordance with the HIPAA Privacy Rule. Ultrasound data must be removed from the ultrasound scanners upon study closure. However, ultrasound images with PHI may be retained in the electronic medical record at Stanford Healthcare at the discretion of the study radiologists as long as they are clearly designated as research data and not associated with a formal interpretation.

The Protocol Directors have ultimate responsibility for collection and reporting of all clinical safety and study data entered in the eCRFs and any other data collection forms (source documents) and ensuring that they are attributable, legible, contemporaneous (timely), original, and accurate as well as complete, consistent, and available when required.

8.3 Data and Safety Monitoring Plan

During the clinical investigation, the Protocol Directors will evaluate the progress of the trial, including periodic assessments of data quality and timeliness, adherence to the data management plan, participant recruitment, accrual and retention, participant risk versus benefit, performance of trial sites, and other factors that can affect study outcome.

The Stanford Cancer Institute Data and Safety Monitoring Committee (DSMC) will audit study-related activities at least annually in accordance with the DSMC SOP to determine whether the study has been conducted in accordance with the protocol, local standard operating procedures, FDA regulations, and Good Clinical Practice (GCP). This may include review of regulatory binders, case report forms, eligibility checklists, and source documents. In addition, the DSMC will regularly review serious adverse events and protocol deviations associated with the research to ensure the protection of human subjects. Results of DSMC audits will be communicated to the IRB and the appropriate regulatory authorities at the time of continuing review, or in an expedited fashion, as needed.

9. MEASUREMENTS

9.1 Primary Outcome Measures

The primary endpoint is initial objective response per RECIST v1.1 assessed 8-16 weeks after start of treatment, using standard-of-care imaging. expressed as a number and proportion without dispersion.

Initial objective response (yes/no): defined as having either Complete Response (CR) or Partial Response (PR) per RECIST v1.1 at first on-treatment response evaluation 8-16 weeks after initiating treatment.

9.1.1 Measurement Methods

Tumor diameter measurements: Where possible, the same lesions that were selected for repeat LEAD ultrasound exams are used as target lesions for response evaluation per RECIST. Different lesions may be selected at the discretion of the radiologist designated to review response evaluation.

9.1.2 Measurement Time Points

At treatment 'baseline' (prior to starting treatment) and 12 weeks after start of treatment (+/- 4 weeks) standard-of-care radiographic imaging is performed for initial response evaluation.
No further follow-up is required for the primary endpoint.

9.1.3 Response Review

Response evaluation per RECIST v1.1 is reviewed by a designated radiologist, who may be the same person that performs the tumor diameter measurements.

9.2 Secondary Outcome Measures

Secondary endpoints are:

1) **initial relative change in tumor burden** (continuous): defined as relative change (i.e., difference between time points divided by value at time point 1, expressed as percentage) in the sum of all tumor diameters between treatment 'baseline' and first on-treatment response evaluation 8-16 weeks after start of treatment, using RECIST v1.1 for tumor diameter measurements, expressed as mean +/- standard deviation and median with interquartile range
2) **initial lesion response** (continuous): defined as the relative change in tumor diameter of a single lesion between treatment 'baseline' and first on-treatment response evaluation 8-16 weeks after start of treatment, using RECIST v1.1 for tumor diameter measurements, expressed as mean +/- standard deviation and median with interquartile range.
3) **12-month progression-free survival (PFS)** (yes/no): defined as not having experienced any PD per RECIST v1.1 within the first 12 months after initiating treatment (day 1 will be treatment start date), as a number and proportion without dispersion.

9.2.1 Measurement Methods

Tumor diameter measurements: same as for primary endpoint
Progression-free survival: determined by medical record review and contacting participants or their outside medical providers as needed.

9.2.2 Measurement Time Points

Initial response evaluation is performed by radiographic imaging at the same timepoints as for the primary endpoint. For repeat response evaluations on treatment, standard-of-care restaging imaging studies will be used. These, as well as determination of 12-month PFS, require follow-up at 12 months after initiation of treatment.

9.2.3 Response Review

Response evaluation per RECIST v1.1 is reviewed by a designated radiologist, who may be the same person that performs the tumor diameter measurements.

10 STATISTICAL CONSIDERATIONS

10.1 Statistical Design

This is a pilot study to evaluate how well changes in tumor perfusion parameters, assessed by LEAD ultrasound 3 weeks after initiating treatment with TKI plus ICI, predict initial objective response, as determined by standard-of-care radiographic imaging 8-16 weeks after start of treatment. All secondary endpoints are exploratory and are intended for generating hypotheses that will require follow-up studies.

10.2 Descriptive Statistics and Exploratory Data Analysis

We will characterize the study population by providing demographic and clinical characteristics. Categorical data will be presented as frequencies and percentages. For continuous data, mean, standard deviation, median, 25^{th} and 75^{th} percentiles will be presented. In addition, we will use graphical techniques to show the distribution of ultrasound parameters by the primary endpoint.

10.3 Primary Analysis

10.3.1 Analysis Population

The population for primary analysis consists of all study participants who have completed the LEAD ultrasound exams at treatment ‘baseline’ and after 3 weeks of treatment, who received at least 4 weeks of TKI and at least one dose of ICI therapy, and for whom initial response can be evaluated per RECIST v1.1. If fewer than 20 of the 20 initially enrolled patients in arm 1 fulfill these criteria, additional patient may be enrolled

at the discretion of the Protocol Directors to have a complete dataset from 20 patients in arm 1 for primary analysis.

10.3.2 Analysis Plan

To address our primary objective, we will conduct a Least absolute shrinkage and selection operator (Lasso) regression as our primary analysis. Specifically, our outcome, initial objective response, will be regressed on the change in each of the LEAD ultrasound tumor perfusion parameters after 3 weeks of treatment and relevant demographic and clinical characteristics. The measurement of LEAD ultrasound tumor perfusion parameters and the definition of their change are described in more detail below. We will not use selection techniques to choose variables. Rather, we will include all factors in our model jointly. We will evaluate the predictive ability of our model using area under the curve (AUC) and calculate a 95% confidence interval for the AUC. Since our sample size is too small to allow for separate training and testing datasets, we will utilize cross-validation techniques for evaluating predictive performance.

Selection of lesions for LEAD ultrasound exams:
Before the first ultrasound exam, the treating medical oncologist, a Protocol Director, or a designated radiologist selects up to 5 lesions per patient (target lesions per RECIST v1.1). At the treatment 'baseline' LEAD ultrasound exam (up to 28 days prior to start of treatment), perfusion of these 5 lesions is measured. Additional lesions may be assessed at the discretion of the investigators, so that in the end up to 5 lesions are selected for repeat ultrasound exams that in the opinion of the investigators lend themselves to reproducible tumor perfusion measurements. LEAD ultrasound tumor perfusion measurements of the up to 5 lesions that were selected for ultrasound studies at the treatment 'baseline' exam are performed again 3 weeks (+/- 8 days) after start of treatment.

Quantification of tumor perfusion parameters:
The following parameters are computed from the LEAD ultrasound measurements for each lesion:

- overall tumor perfusion: the integrated power within the tumor
- average red cell flux: the integrated power within the tumor normalized by tumor size
- tumor-to-background perfusion ratio: the ratio of the integrated power within the tumor to the integrated power in a same sized region in nearby healthy tissue.

Changes in tumor perfusion parameters after 3 weeks of treatment:
Defined as relative change in these parameters, i.e., difference between time points divided by value at time point 1, expressed as percentage. For determining the relative change in several lesions of the same patient, the average of the relative changes in the up to 5 individual lesions is calculated.

10.4 Secondary Analysis

10.4.1 Analysis Population

All patients who have completed at least one LEAD and Doppler ultrasound exam at any time point. For correlation analyses, patients are included for each analysis based on availability of data points for specific analyses. If fewer than 20 patients in arm 1 and 10 patients in arm 2 fulfill these criteria for any secondary analysis, additional patient may be enrolled at the discretion of the Protocol Directors to have a complete dataset from 20 and 10 patients for this analysis, respectively.

10.4.2 Analysis Plan

To address our secondary objective, i.e., evaluating if there is an optimal ultrasound modality to predict initial objective response in arm 1, we will utilize the same analysis approach as outlined above for the primary analysis. Here, we will use change in tumor perfusion parameters at **6 weeks** as our independent variables and conduct two regression analyses: (1) using power Doppler measurements and (2) using LEAD ultrasound measurements. AUCs between the two models will be compared using the nonparametric approach of DeLong for correlated AUC curves[43]. In addition, we will compare AUCs between these models to the predictive model for the primary analysis.

To assess if there is an optimal time point for predicting initial objective response in arm 1, we will conduct Lasso regression models using tumor perfusion parameters for each time point (treatment ‘baseline’, 3 weeks, 6 weeks) separately for each imaging modality using a similar approach as outlined above. Given the exploratory nature of these analyses, we will not adjust for multiple testing.

For each arm, we will conduct correlation analysis for change in perfusion parameters at 3 and 6 weeks and initial relative change in tumor burden (overall and per-lesion), initial overall response, best overall response, and PFS until 12 months after initiating treatment by calculating either the Pearson r or Spearman rank correlation, as appropriate. PFS is estimated using the Kaplan-Meier method and the Kaplan-Meier curves will be presented.

Selection of lesions for ultrasound exams:
The up to 5 lesions that were selected for ultrasound studies for the primary analysis are re-assessed 6 weeks (+/- 15 days) after start of treatment and each lesion is assessed with both LEAD and power Doppler ultrasound at all 3 time points (treatment 'baseline', 3 weeks, and 6 weeks after initiating treatment.

Quantification and change of tumor perfusion parameters: defined in the same way as for the primary analysis for both power Doppler and LEAD ultrasound.

10.5 Sample Size

Since this is an exploratory pilot study and we are not formally testing a specific research hypothesis, the sample size for each arm has been chosen based on practical considerations and estimated feasibility. However, for the primary analysis (arm 1), we consider a predictive model with a true AUC of at least 0.7 promising for predicting initial objective response. With 20 patients and assuming that 59% of patients have an objective response to treatment, a predictive model with a true AUC of at least 0.7 will have a margin of error of 0.14 so that the empirically determined AUC can range from 0.56 to 1.

10.6 Accrual estimates

We estimate that 15 patients yearly are eligible for arm 1 and approximately 10 patients for arm 2. By experience from previous similar imaging studies, patients are motivated to participate in such studies, especially when they do not require extra study visits. However, taking into account attrition, we conservatively estimate that it will take 2 years to accrue 20 patients in arm 1 and up to 10 patients in arm 2 who complete all ultrasound exams, receive treatment over the minimum period, and will have an evaluable response per RECIST.

10.7 Criteria for future studies

An AUC of at least 0.7 will be the minimum needed to design a follow-up study and the predictive model that yields the best AUC will be selected for future studies. If different predictive models give equivalent AUCs, we will consider additional analyses (for example, random forests or Classification and Regression Tree [CART]) to evaluate these models to determine if a specific model can be selected for further studies.

If any predictive model from this pilot study has an AUC of at least 0.7, the next step will be to conduct a larger follow-up study in an independent cohort to validate the predictive model and determine its AUC with a smaller margin of error. The sample size and operating characteristics of the follow-up study will be determined based on findings from this pilot study and other data available at the time of designing the follow-up study. If the larger follow-up study is also deemed a success (AUC at least 0.7), to determine if tumor perfusion measurements by ultrasound are suitable for implementation, an adequately powered randomized clinical trial will be required to

test if an early therapy switch based on ultrasound imaging improves outcomes, compared to current standard of care.

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APPENDIX A: PARTICIPANT ELIGIBILITY CHECKLIST

Protocol Title:	**Early Therapeutic Monitoring of Response to Therapy with Serial Ultrasound in Metastatic RCC**
Protocol Number:	**55742**
Principal Investigators:	**Alice Fan, M.D. and Jeremy Dahl, Ph.D.**

II. Subject Information:

Subject Name & MRN:	
Legal Sex: ☐ Male ☐ Female	

III. Study Information:

SRC Approved ☒ IRB Approved ☒

IV. Inclusion/Exclusion Criteria

General Inclusion Criteria (From IRB approved protocol)	**Yes**	**No**	**Supporting Documentation***
1. 18 years of age or older	☐	☐	
2. Pathology-confirmed diagnosis of RCC	☐	☐	
3. At least one tumor lesion greater than 1 cm in diameter, amenable to ultrasound imaging	☐	☐	
4. Written informed consent	☐	☐	
Specific Inclusion Criteria for Arm 1 (TKI + ICI Treatment)			
Planned to be treated with combination of VEGFR2 tyrosine kinase inhibitor plus immune checkpoint inhibitor	☐	☐	
Specific Inclusion Criteria for Arm 2 (Non-ICI Treatment)			
Planned to be treated with non-immune checkpoint inhibitor	☐	☐	

Exclusion Criteria (From IRB approved protocol)	**Yes**	**No**	**Supporting Documentation***
Any comorbid condition that, in the opinion of the treating provider or the Protocol Directors, compromises the participant's ability to participate in the study.	☐	☐	Initials & Date Treating Provider: _____ _____

*****All subject files must include supporting documentation to confirm subject eligibility. The method of confirmation can include, but is not limited to, laboratory test results, radiology test results, subject self-report, and medical record review.

IV. Statement of Eligibility

By signing this form of this trial, I verify that this subject is [☐ **eligible** / ☐ **ineligible**] for participation in the study. This study is approved by the Stanford Cancer Institute Scientific Review Committee, the Stanford IRB, and has finalized financial and contractual agreements as required by Stanford School of Medicine's Research Management Group.

Treating Medical Provider** Signature:	Date:
Printed Name:	

** Treating Physician or treating Advanced Practice Provider (APP)