SUMMARY OF CHANGES

For Protocol Amendment #4 to: **ACRIN 6690: A Prospective, Multicenter Comparison of Multiphase Contrast-Enhanced CT and Multiphase Contrast-Enhanced MRI for Diagnosis of Hepatocellular Carcinoma and Liver Transplant Allocation**

NCI Protocol #: ACRIN 6690
Local Protocol #: ACRIN 6690

NCI Version Date: February 24, 2014
Protocol Date: February 24, 2014

PARTIAL PROTOCOL—CONTACT ACRIN PROTOCOL DEVELOPMENT AND REGULATORY COMPLIANCE FOR A COMPLETE PROTOCOL

Section	Change
Global	References to the "Adverse Event Expedited Reporting System (AdEERS)" have been changed to "CTEP Adverse Event Reporting System (CTEP-AERS)" throughout the protocol. Additional changes have been made to update language requirements and contact information.
Global	Reference to the Optional Eovist Sub-Study and its Appendices have been removed from the main study protocol with discontinuation of accrual to the sub-study effective as of Friday, February 7, 2014.
Global	References to ACRIN and the ACRIN Imaging Core Laboratory have been clarified to adequately address the roles and responsibilities of ECOG-ACRIN, ECOG-ACRIN Diagnostic Imaging Headquarters and its staff, the Imaging Statistical and Data Management teams, and the ACR Imaging Core Lab.
Cover Pages	New cover pages have been introduced with the transition to ECOG-ACRIN and introduction of CTSU information.
Cover Pages	Have been updated with the current version date and reference to the addition of Amendment 4
Table of Contents	Has been updated
Section 6.2	New section has been added to introduce the CTEP IAM certification requirement and CTSU systems procedures.
Section 7.2	New section has been added to introduce the CTSU system procedures for participant registration.
Section 8.6	Has been added to provide guidance on follow-up for participants on the Eovist sub-study, which ceased accrual as of February 7, 2014. Subsequent sections have been renumbered.
Section 11.7	The AE reporting table has been updated to the current version.
Appendices VII & VIII	DELETED. Former-Appendices VII and VIII related to the Eovist-enhanced MRI sub-study for the trial have been deleted. Accrual to the Eovist sub-study has been halted as of a notification to the sites on Friday, February 7, 2014.
Appendix IX	Appendix IX for the EDRN ancillary study has been renumbered to Appendix VII.

ACRIN 6690

**A Prospective, Multicenter Comparison of Multiphase Contrast-Enhanced CT
and Multiphase Contrast-Enhanced MRI for Diagnosis of Hepatocellular Carcinoma
and Liver Transplant Allocation**

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Version Date: February 24, 2014

Includes Amendments 1 – 4

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Open to NCTN Membership

ORIGINAL DATE
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ACTIVATION DATE
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CONFIDENTIAL

CANCER TRIALS SUPPORT UNIT (CTSU) ADDRESS AND CONTACT INFORMATION

To submit site registration documents:	For patient enrollments:	Data collection will be performed exclusively in Medidata Rave:
CTSU Regulatory Office 1818 Market Street, Suite 1100 Philadelphia, PA 19103 Phone – 1-866-651-CTSU Fax – 215-569-0206	Please refer to the patient enrollment section for instructions on using the OPEN system.	Please refer to the Forms Completion Guidelines for the Forms Submission Schedule.

The **study protocol and all related forms and documents** must be downloaded from the protocol-specific Web page of the CTSU Member Web site located at https://www.ctsu.org. Sites must use the current form version and adhere to the instructions and submission schedule outlined in the protocol.

CTSU sites should follow procedures outlined in the protocol for Site registration, Patient Enrollment, Adverse Event Reporting, Data Submission (including ancillary studies), and Drug Procurement.

For patient eligibility or treatment-related questions Contact the Study PI of the Coordinating Group.

For questions unrelated to patient eligibility, treatment, or data submission contact the CTSU Help Desk by phone or e-mail:

CTSU General Information Line – 1-888-823-5923, or ctsucontact@westat.com. All calls and correspondence will be triaged to the appropriate CTSU representative.

For detailed information on the regulatory and monitoring procedures for CTSU sites please review the CTSU Regulatory and Monitoring Procedures policy located on the CTSU members' website https://www.ctsu.org

The CTSU Web site is located at   https://www.ctsu.org

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A Prospective, Multicenter Comparison of Multiphase Contrast-Enhanced CT
and Multiphase Contrast-Enhanced MRI for Diagnosis of Hepatocellular Carcinoma
and Liver Transplant Allocation

MAIN TRIAL SCHEMA

HCC diagnosis by SOC **baseline** CT or MRI

PATIENT WAITLISTED WITH UNOS

Listing for liver transplantation with HCC-exception points

DECLARE INTENT TO LIST

Patient determined to be potential candidate for waitlisting and trial; Declaration of Intent to List source document completed by site

ENROLLMENT

Study-related (complementary) **baseline** imaging CT or MRI within 60 days after SOC **baseline** CT or MRI

(if 60-day window cannot be accomplished because SOC baseline imaging is too old at time of enrollment, then both complementary and SOC baseline imaging need to be done within 7 days of each other)

Local ablative therapy

(consider pre-treatment biopsy if feasible)

Repeat **serial** imaging every 90 days per UNOS listing update requirements

(CT and MRI completed within 7 days of each other)

Post-ablation Imaging

(SOC + complementary imaging, no less than 28 days and no more than 60 days after completion of ablation)

Transplant surgery

Explant radiology-pathology correlation and histopathology analysis

HCC = hepatocellular carcinoma; CT = computed tomography; MRI = magnetic resonance imaging; SOC = standard of care; UNOS, United Network for Organ Sharing.

The term "SOC imaging" is used in this trial protocol to describe the imaging exam (MR or CT) that is the "first choice/clinical standard of care" at a participating institution to update a patient's HCC-exception MELD points on the liver transplant waitlist every 90-days. The term "complementary imaging" is used in this trial protocol for the "other" modality (MR or CT), which will be considered the protocol-required research scan consistently at a center. At **baseline,** there are two

scenarios to which different timing rules apply: (1) **60-day timeline.** If the complementary imaging can be completed within 60 days of SOC imaging [and the SOC imaging was performed per protocol], then SOC imaging does not need to be repeated; (2) **Within 7 days of each other.** If complementary imaging cannot be completed within 60 days of SOC imaging [or SOC imaging needs to be repeated on an ACRIN-qualified scanner to protocol specifications], then both CT and MR needs to be completed after enrollment, within 7 days of each other. It is permissible to perform both CT and MR on the same day.

Serial imaging (CT and MR) will continue at 90-day intervals according to UNOS waitlisting update requirements.

"Additional imaging" (MR and CT) will need to be performed within 28 to 60 days after completion of ablative therapy to assess for residual or recurrent HCC; this imaging time point may coincide with the next serial imaging time point required for liver transplant waitlist updates, in which case only one pair of imaging exams (MR and CT) needs to be obtained.

STUDY OBJECTIVES/SPECIFIC AIMS

Main trial: We hypothesize that modern imaging technology can accurately diagnose and stage hepatocellular carcinoma (HCC) in patients with chronic liver disease when performed on state-of-the-art multidetector computed tomography (CT) or magnetic resonance imaging (MRI) equipment, with contemporary multiphase contrast-enhanced imaging protocols using non-specific contrast agents. Focal liver lesions can be accurately assigned to pre-malignant and malignant diagnostic categories based on patterns of specific imaging findings. Furthermore, we expect that the false positive rate in the malignant lesion category can be reduced from unacceptable levels by utilizing the new Organ Procurement and Transplantation Network (OPTN) liver imaging policy. This trial compares the accuracy of radiologic staging of HCC by CT and MRI with the reference standard provided by explant pathology workup/staging of participants who undergo liver transplantation for treatment of HCC. It tests the performance of an imaging-based diagnostic algorithm for HCC, which forms the basis of the aforementioned new draft policy.

The Optional EDRN Ancillary Biomarker Study: In coordination with the Early Detection Research Network (EDRN), ACRIN introduces an ancillary biomarker study to the ACRIN 6690 trial with Amendment 3 in order to correlate a minimum of eight biomarkers with imaging and explant pathology results, compared at the patient level, from the ACRIN 6690 trial. Blood (two vials for a minimum of 8 mL of blood in each) will be collected from consenting participants before each study-related imaging time point (baseline, serial imaging, and post-ablation imaging) and at three-months post-transplantation. For additional information, see Appendix VII.

ELIGIBILITY *(see Section 5.0 for details)*

Patients who are diagnosed with HCC and have been listed on the OPTN/UNOS (United Network for Organ Sharing) waitlist for liver transplant surgery with priority MELD (Model for End-Stage Liver Disease) points based on the cancer diagnosis; a given patient may be waiting for a liver from a deceased donor to become available or may be scheduled to undergo a living donor adult liver transplant. Patients must enroll in the trial after initial listing with HCC-exception points to the UNOS waitlist or the principal investigator (PI) at a participating site may complete a Declaration of the Intent to List source document, in which the site PI or designated site co-investigator confirms that there are no known contraindications to waitlisting the patient (see Section 5.3 for additional details).

SAMPLE SIZE

Main trial: A total of 440 patients will be accrued from transplant centers in the 11 OPTN transplant regions across the United States with approximately 25 to 30 centers participating in the study.

The Optional EDRN Ancillary Biomarker Study: A total of 200 patients may be enrolled in the optional Ancillary Biomarker Study. For additional information about the ancillary biomarker study, see Appendix VII.

1.0   ABSTRACT

This protocol for human research study is conducted according to United States and international standards of Good Clinical Practice (International Conference on Harmonisation [ICH] Guidelines), applicable government regulations (e.g. Title 45, Part 46 Code of Federal Regulations), and the Eastern Cooperative Oncology Group (ECOG)-American College of Radiology Imaging Network (ACRIN) research policies and procedures.

Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related deaths with over 500,000 annual deaths worldwide.[1] HCC accounts for approximately 80% of all primary liver cancers and has become the third leading cause of death worldwide according to data published in from the Centers for Disease Control and Prevention.[1,2] With the major clinical risk factor being hepatitis B or hepatitis C virus infection, the prevalence of HCC has increased significantly in the last two decades.[3] The diagnostic algorithm for HCC in clinical practice today has been developed mostly based on the literature and expert consensus rather than analytic *a posteriori* data. The diagnosis is established by serology, cyto-histology, and radiologic characteristics to diagnose and stage the disease.[4,5] As the precise staging of the disease determines prognosis as well as choice of curative, palliative, and/or symptomatic therapy, an accurate assessment of this disease is vital.

The most effective treatment for patients with HCC is liver transplantation, as it removes *in toto* the primary tumor(s), inclusive of any clinically unapparent microscopic disease in the remainder of the liver, and replaces the often-cirrhotic native liver with a graft organ, typically from a deceased donor. In the last two decades, this treatment has yielded survival rates of more than 70% at 5 years and recurrence of disease of less than 15% for patients with HCC undergoing transplant. Once listed for transplant, patients may experience excessive waiting times for organs from deceased donors, especially in regions with an unfavorable ratio of available organs to qualified recipients. Consequently, disease progression while on the waitlist and death drive drop-out rates from the transplant list.[6]

The Organ Procurement and Transplantation Network (OPTN) is the unified transplant network established by the United States Congress under the National Organ Transplant Act (NOTA) of 1984. The United Network for Organ Sharing (UNOS) administers the OPTN under contract with the Health Resources and Services Administration of the U.S. Department of Health and Human Services. To achieve a more timely transplantation for patients in most need, UNOS uses the Model for End-stage Liver Disease (MELD) scoring system to prioritize the transplant waitlist organs to patients with end-stage liver disease (ESLD) based on survival probability. The traditional MELD score is based on serologic metabolic markers of liver function and correlates with a certain mortality risk;[7–9] however, patients with life-threatening HCC often have a MELD score too low to earn them a timely transplant even though their mortality from the cancer equals that of patients with a much higher [metabolic] MELD score. In recognition of this fact, UNOS adopted a new policy in 2002 that allowed liver cancer patients to obtain so-called HCC-exception MELD points. Thus for the first time, priority could be assigned based on survival probability predicated on the diagnosis of HCC only, which was felt to minimize inequities between the two main liver transplant-candidate subsets.

The previous OPTN/UNOS Policy for liver transplantation in the United States specifically allows a pre-transplant diagnosis of HCC based solely upon imaging criteria.[10] A retrospective analysis of the UNOS database comparing the accuracy of radiologic staging with pathologic staging of explant organs found that the performance of imaging under the previous policy was unacceptable. The imaging data did not

portray an accurate staging of the disease, and in many cases resulted in the inappropriate allocation of transplant livers.[11] The previous policy contained no technical and image acquisition requirements for liver imaging and vague qualitative diagnostic imaging criteria, resulting in a high number of false-positive diagnoses. Often, common benign focal abnormalities in the diseased liver were mislabeled as HCC and resulted in misallocation of donor organs. The previous OPTN/UNOS policy requirements are insufficient for imaging-based diagnosis of HCC qualifying the patient for priority on the transplant waitlist. In 2008, UNOS convened an interdisciplinary group of experts including radiologists, hepatologists, transplant surgeons, and pathologists from United States and international transplant centers and developed new policy recommendations to improve the accuracy of the imaging-based diagnosis of HCC.[12] A prospective trial utilizing these revised guidelines comparing diagnostic performance to pathology at time of transplant is warranted to ensure proper allocation of valuable organs from deceased donors. The new OPTN organ allocation policy is now effective/activated as of November 2011. All transplantation centers should be following the new policy guidelines, making the procedures for the ACRIN 6690 trial standard practice procedures at participating institutions. For more information about the policy, visit http://optn.transplant.hrsa.gov/policiesAndBylaws/policies.asp; see Section 3.6 for information about liver allocation for transplantation and Section 3.6.4.4 for information about the criteria for waitlisting of patients with HCC.

2.0   BACKGROUND AND SIGNIFICANCE

The significance of HCC in public health is of two-fold importance, as there is a large at-risk population and increased incidence nationwide. Deceased donor liver transplantation and living donor adult liver transplantation (LDALT) are the best currently available treatment options for HCC. However, patients with ESLD and patients diagnosed with other liver disease compete for the same scarce pool of transplant organs from deceased donors. As all of these conditions are life limiting, the timely assignment of graft livers to the appropriate patients based on individual mortality risk is of vital importance. Additionally, as liver transplantation is associated with significant healthcare expenditure related to postoperative care and long-term immunosuppression therapy, the allocation of transplant livers to unsuitable patients should be avoided for economic reasons.

The presence and severity of ESLD are diagnosed by a combination of clinical and laboratory data, as well as imaging tests and, ultimately, tissue sampling where appropriate. The imaging-based diagnosis of HCC is used to allocate priority on the liver transplant waitlist to those patients who suffer from liver cancer. An expert panel recently developed a draft policy for liver imaging in context with liver transplant allocation in the United States.[12] Minimum technical and protocol requirements, expert interpretation by transplant center radiologists, and a new classification and diagnosis system were created in 2008 in an attempt to increase the specificity of imaging-based liver cancer diagnosis and staging in patients with ESLD. Due to lack of robust clinical data, the proposed policy had to be based on expert consensus rather than scientific evidence generated from clinical trials. It is therefore imperative to test the performance of this new policy in clinical practice to evaluate its impact early on during implementation.

2.1   HCC and Liver Transplantation in the United States
Liver cancer, primarily HCC, is the third leading cause of cancer-related death worldwide and the ninth leading cause of death in the United States.[1] The incidence of HCC has increased by 70% from 1.4 per 100,000 in 1976 to 1980 to 2.4 per 100,000 in 1991 to 1995. Additionally, patients' age at initial diagnosis has decreased during this period. Still, only a marginal improvement in survival has occurred,

with a five-year survival rate of 5%.[13] This poor prognosis is partly due to the advanced tumor stage at time of diagnosis, which precludes effective treatment.[14] Furthermore, the number of HCC cases are expected to increase in the next three decades, as a major risk factor for the development of HCC is another condition increasing in incidence—chronic viral hepatitis B or C.[15]

Liver transplantation for early-stage HCC is more likely to provide a potential cure and has shown improved survival over other less radical techniques.[16] Transplantation is associated with a 75% four-year survival compared to a 25% three-year survival for untreated, small HCCs.[17,18] HCC was the primary indication for liver transplantation, which accounted for 18.4% of U.S. liver-transplant recipients in 2007 to 2008.[19] With a rising number of patients in need of liver transplantation and essentially stagnant organ availability, appropriate organ allocation is a growing concern.

The OPTN is the unified transplant network established by the United States Congress under NOTA, established in 1984. UNOS administers the OPTN under contract with the Health Resources and Services Administration of the U.S. Department of Health and Human Services. Previous OPTN liver allocation policy is based on objective, verifiable measures of disease severity with minimal emphasis on waiting time.[20] The MELD score predicts survival probability in patients with ESLD.[7–9] The score is calculated by a formula using routine lab test results. The MELD score is used to tie priority on the transplant waitlist to quantitation of disease severity and, thus, predicted length of survival. In 2002, the U.S. liver transplant allocation system was revised, henceforth granting HCC-exception MELD points to ESLD patients on the transplant waitlist who are diagnosed also with liver cancer and meet the so-called Milan criteria.[21]

2.2   Previous Imaging-Based Assignment of Priority and Management of Patients on the Transplantation Waitlist for HCC Diagnosis

A total of 6103 liver transplants were performed in the United States between November 1, 2007, and October 31, 2008. The number of patients transplanted with an approved HCC exception (MELD points assigned for liver cancer diagnosis) was 1293 (21.9%). Of these 1293 patients, the vast majority (n=1143, 88.3%) of diagnoses were based on imaging alone. In 145 patients (11.6%) a combination of biopsy data and imaging findings contributed to the diagnosis.[22] Prerequisite conditions for receiving extra priority for candidates on the waiting list with TNM stage T2 HCC previously include an imaging assessment of the candidate's liver with ultrasound, computed tomography (CT), or magnetic-resonance imaging (MRI) scan that documents the HCC. Few specific imaging criteria are listed in the previous policy, which states that patients must have a "vascular blush corresponding to the area of suspicion seen on the above imaging studies," or "an arteriogram confirming a tumor, a biopsy confirming HCC, chemoembolization of [the] lesion[s], radiofrequency, cryo-ablation or chemical ablation of the lesion".[10]

After initial HCC diagnosis, management of patients on the regional transplant waitlists differed significantly: 1287 (54%) out of 2377 individual patients submitted in 2008 for HCC exception had some form of adjuvant local treatment between time of listing and transplantation. Radiofrequency ablation (RFA) accounted for 420 (32.6%); chemoembolization, 966 (75.1%); chemical ablation, 59 (4.6%); cryo-ablation, 4 (0.3%). Some patients underwent multiple types of local adjuvant therapy; for example, 11% of patients had a combination of RFA and chemoembolization.[22]

2.3 Shortcomings of Previous Policy

Freeman et al[11] performed a retrospective analysis of the UNOS database comparing the accuracy of radiologic staging with pathologic staging of explant organs in 789 liver transplant recipients. This study found that the performance of MR or CT imaging under the previous policy was unacceptable. The imaging data did not portray an accurate staging of the disease, and in many cases resulted in the inappropriate allocation of transplant livers. In 2008, UNOS convened an interdisciplinary group of experts including radiologists, hepatologists, transplant surgeons, and pathologists from the United States and international transplantation centers which developed policy recommendations to improve imaging of HCC.[12] The experts agreed that the previous OPTN/UNOS policy requirements were insufficient for imaging-based diagnosis of HCC qualifying the patient for priority on the transplant waitlist. The previous policy contained no technical and protocol requirements for liver imaging and only vague qualitative diagnostic imaging criteria. The inaccurate imaging findings allowed a high number of false-positive diagnoses labeling common benign focal abnormalities in the diseased liver as HCC, resulting in misallocation of donor organs.

The new OPTN organ allocation policy is now effective/activated as of November 2011. All transplantation centers should be following the new policy guidelines, making the procedures for the ACRIN 6690 trial standard practice procedures at participating institutions. For more information about the policy, visit http://optn.transplant.hrsa.gov/policiesAndBylaws/policies.asp; see Section 3.6 for information about liver allocation for transplantation and Section 3.6.4.4 for information about the criteria for waitlisting of patients with HCC.

2.4 Shortcomings of Imaging After Local Adjuvant Therapy

Imaging criteria for the detection of residual or recurrent tumor after local adjuvant therapy are not well established. The diagnostic accuracy of imaging for the detection of residual or recurrent liver cancer after local ablative therapy has not been formally compared to explant pathology workup in a prospective multicenter trial.

2.5 New Proposed Policy

A new policy for the imaging diagnosis of HCC in context with liver transplant allocation has been made effective for standard practice at transplantation centers in the United States. It incorporates minimum technical requirements for CT and MRI, presents imaging protocol parameters, and proposes a new system for diagnosis, classification, and reporting of liver lesions in this specific clinical context. Apart from introducing a reporting class for technically-inadequate examinations, the new lesion classification was written with the intent to reduce false-positive image diagnoses of liver cancer leading to inappropriate organ allocation. Specific qualitative imaging findings and size criteria were introduced in order to establish the diagnosis of HCC and to set it apart from suspicious lesions not meeting criteria for definitive liver cancer diagnosis (Tables 1 and 2). The American College of Radiology is sponsoring a workgroup to create the "Liver Imaging Reporting and Data System" (LI-RADS).[1] Liver lesion category 5 in the LI-RADS system "Definitely HCC" matches OPTN Class 5. In its new liver imaging policy, OPTN/UNOS will include Classes 0 and 5 and will defer to the LI-RADS group for the future definitions of Classes 1 through 4. The performance of the new DRAFT UNOS/OPTN policy will have a significant impact on priority allocation for transplantation based on liver cancer diagnosis.

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[1] LI-RADS (Liver Imaging Reporting and Data System). Available at: www.acr.org/Quality-Safety/Resources/LIRADS. Accessed December 18, 2012.

Performance of the previous OPTN policy was never prospectively assessed in a multicenter study. This older policy lacks standardization of minimum imaging equipment specifications, imaging protocols, and diagnostic criteria across participating institutions. Therefore, it is difficult to quantify the absolute change/improvement to clinical practice expected to occur when the new policy goes into effect. The proposed trial will require uniformity in the imaging approach across numerous institutions and will help define the sensitivities and specificities of the two imaging modalities in this specific clinical context.

It should be noted that when the new, now-active UNOS/OPTN classification was created, only those imaging features of HCC that were broadly supported by literature and expert consensus were included in the final specific diagnostic criteria. These criteria can be observed on multiphasic contrast-enhanced CT or MRI with conventional non-specific contrast agents. The aim was to simplify and, if anything, increase specificity of the imaging-based HCC diagnosis across clinical practice. However, the trial team recognizes that there may be image observations and considerations beyond mere application of the diagnostic criteria listed in Tables 1 and 2 and Figure 1, which may influence a radiologist's opinion on whether a nodule represents HCC or not.

Specifically, certain morphologic features of a nodule on CT and, for example, certain appearance of a nodule on T2-weighted or diffusion-weighted MRI may compel the interpreting radiologist to believe that HCC is more or less likely present than they were forced to state simply based on the stringent application on the new UNOS/OPTN criteria. Therefore, in addition to adjudicating a lesion based on the UNOS/OPTN criteria, we will ask that site readers indicate:

- The binary presence of HCC and the probability of HCC on a 0 to 100 point scale; and

- Which imaging sequences aided in these decisions.

This purely observational data shall inform the trial team about patterns of diagnostic consideration in clinical practice and may prove to be useful in modeling potential future sets of criteria by taking into account prevailing clinical practice patterns of the collective readers in the participating centers.

Tables 1 and 2 and Figure 1 follow on the subsequent pages for easy reference and printing.

Table 1: Classification system for liver imaging modified after proposed OPTN policy

OPTN Class*	Description	Comment
OPTN 0	*Incomplete or technically inadequate study*	*Repeat study required for adequate assessment; automatic priority MELD points cannot be assigned based on a OPTN Class 0 classified imaging study*
OPTN 1	*Observation is definitely benign*	
OPTN 2	*Observation is probably benign*	
OPTN 3	*Observation is of intermediate probability for HCC*	
OPTN 4	*Observation is probably but not definitely an HCC*	*Only isolated qualitative features of HCC are present: Lesion < 2 cm?* - *Only later arterial enhancement, only washout, only pseudocapsule, or any combination of 2 out of 3 of these options, but not all* *Lesion ≥ 2 cm?* - *Only late arterial enhancement* ***OR*** - *Only washout, only pseudocapsule, or both (washout and pseudocapsule), but no late arterial enhancement*

OPTN 4-g	*Observation is probably but not definitely an HCC* ***AND*** *Growth (maximum diameter increase) by 50% or more documented on serial MRI or CT obtained ≤ 6 months apart*	*Only isolated qualitative features of HCC **AND** growth are present:*
		- *Only late arterial enhancement*
		  OR
		- *Only washout, only pseudocapsule, or both (washout and pseudocapsule), but no late arterial enhancement*
		  AND
		- *Growth*
		Could represent iso- or hypovascular HCC, consider biopsy if imaging remains inconclusive in growing lesions

OPTN 5 (See subclasses in Table 2)	*Meets qualitative radiologic criteria for HCC, definite HCC*	*Patient may be eligible for automatic priority MELD points based on this imaging study. Please refer to definitions for Classes 5A, 5A-g, 5B, 5B-g, and 5T criteria in Table 2*

* *UNOS defers to LI-RADS (see Section 2.5) for definitions of OPTN Classes 1 through 4.* Available online at: www.acr.org/Quality-Safety/Resources/LIRADS. Accessed December 18, 2012.

Table 2: Proposed imaging criteria for <u>OPTN Class 5 lesions</u>
(compatible with imaging diagnosis of HCC, modified after UNOS proposed policy)

OPTN Class 5	Lesion Size	Appearance	Comment
5A	*Maximum diameter of lesion ≥1cm and <2cm, measured on late arterial or portal vein phase images*	*Increased contrast enhancement on late hepatic arterial phase (relative to hepatic parenchyma)** *AND* *Washout during later contrast phases* *AND* *Peripheral rim enhancement (capsule/pseudocapsule) on delayed phase*	*This category describes a **Stage I** HCC which meets **<u>stringent</u> qualitative imaging criteria** diagnostic of HCC* *Class 5A lesions **do not** qualify for automatic HCC-exception MELD points*
<u>OR</u>[†]			
5A-g	*See 5A*	*Increased contrast enhancement on late hepatic arterial phase (relative to hepatic parenchyma)** *AND* *Growth (maximum diameter increase) by 50% or more documented on serial MRI or CT obtained ≤ 6 months apart. Growth criteria do not apply to ablated lesions*	*A **rapidly growing Stage I** HCC with **some (arterial only!) qualitative imaging features** diagnostic of HCC*
5B	*Maximum diameter of lesion ≥2cm, measured on late arterial or portal vein phase images*	*Increased contrast enhancement on late hepatic arterial phase (relative to hepatic parenchyma)** *AND* *Either washout[‡] during later contrast phases <u>or</u> peripheral rim enhancement (capsule/pseudocapsule) on delayed phase*	*This category describes a **Stage II** HCC which meets **qualitative imaging criteria** diagnostic of HCC*
<u>OR</u>[†]			
5B-g	*See 5B*	*Increased contrast enhancement on late hepatic arterial phase (relative to hepatic parenchyma)** *AND* *Growth (maximum diameter increase) by 50% or more documented on serial MRI or CT obtained ≤ 6 months apart. Growth criteria do not apply to previously ablated lesions*	*A **rapidly growing Stage II** HCC with **some (arterial only!) qualitative imaging features** diagnostic of HCC* *Class 5B lesions **qualify for automatic HCC-exception MELD points***
5T	*Prior local regional treatment for HCC*	*Past local regional treatment (e.g., TACE or thermal ablation or combination therapy) for HCC (OPTN Class 5 or biopsy-proven prior to ablation)*	*This category describes previously-ablated focal liver lesion*

*   Iso- and hypovascular HCC may occur which do not exhibit this feature, consider biopsy if suspected.

†   When faced with a choice between qualitative imaging criteria for cancer versus growth criteria for cancer, the former "trumps" the latter, i.e., if a lesion has ALL qualitative characteristics for a Class 5B lesion AND meets the growth criteria, it should be classified as 5B. Only use the "–g" growth classes if growth was in fact the decisive factor for classification.

‡   "Washout" is defined as hypointensity/hypoattenuation of a nodule in venous or more delayed phases compared to background liver parenchyma. This "Washout" indicates a difference in the vascular and extravascular spaces of a tumor compared to background liver parenchyma.

Figure 1. Application of OPTN/UNOS criteria for cirrhosis and focal liver lesion(s)

The flow diagram below illustrates how the above criteria for OPTN/UNOS Classes 4 and 5 lesions apply to patients with underlying hepatic cirrhosis and at least one focal liver lesion.
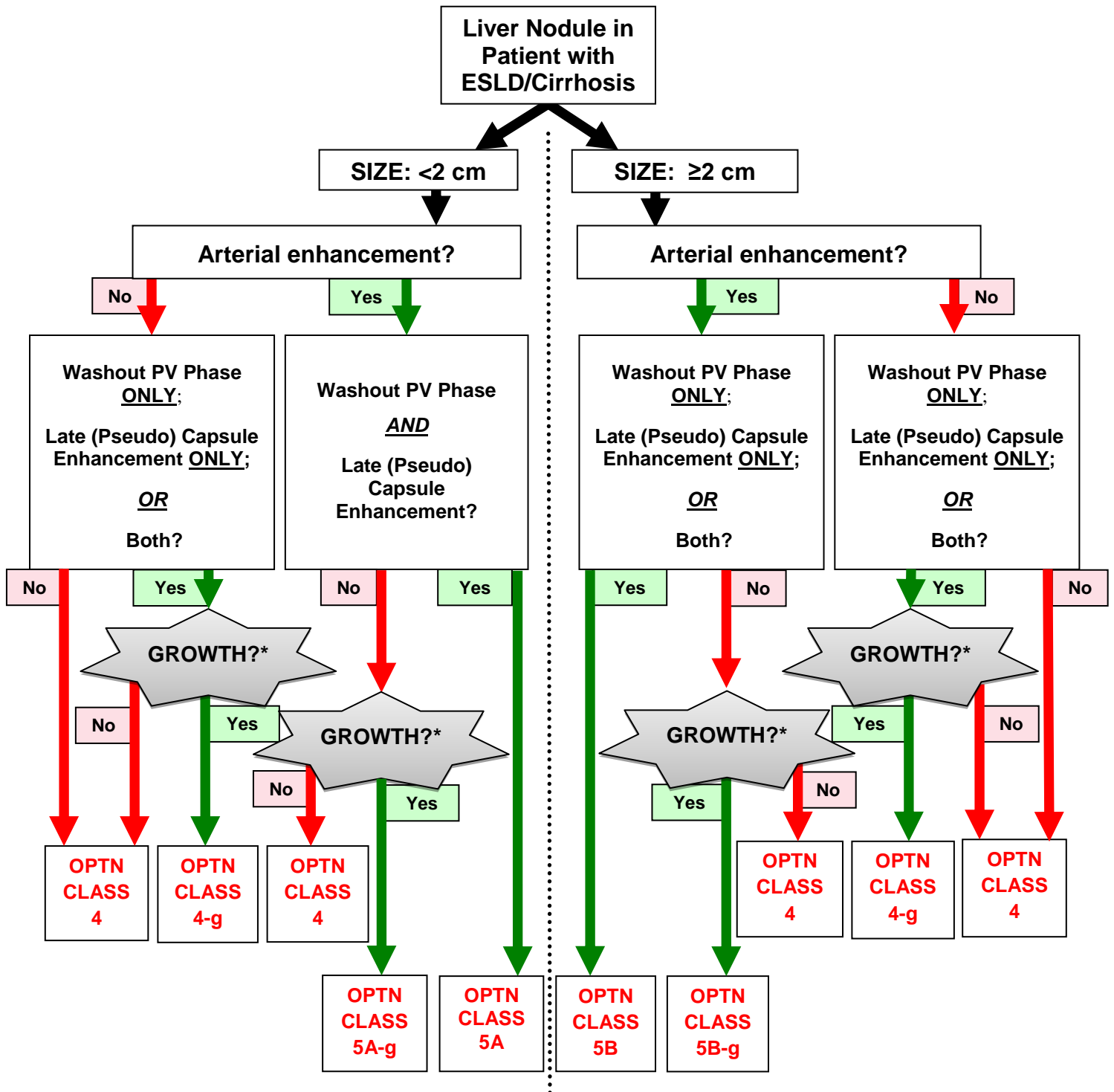
* **Growth** is defined as: *Maximum diameter increase by 50% or more documented on serial MRI or CT obtained ≤ 6 months apart.*

2.6 Proposed Research

Multiphase contrast-enhanced CT and MRI will be compared to explant pathology liver workup to establish the respective performance characteristics of these imaging modalities to accurately detect, diagnose, and stage hepatocellular cancer in patients with chronic liver disease. The previous clinical decision-making relative to the treatment of patients with ESLD depends upon the presence, number, and location of HCC lesions. Thus, our primary approach is to study imaging performance at the lesion level. By comparing these imaging modalities and their interpretation by both local and central readers to the pathological explant results, the proposed research will help identify optimal conditions for the diagnosis and staging of HCC at lesion and patient levels. It is hypothesized that the combination of state-of-the-art multidetector CT or MRI minimum equipment specifications, contemporary multiphase contrast-enhanced imaging protocols, and new diagnostic criteria will reduce false positive image diagnoses of liver cancer and ultimately lead to more informed treatment decisions and appropriate organ allocation and associated priority transplantation in the United States. The diagnostic imaging involved in this particular trial is routine multiphase contrast-enhanced MRI and CT, not to be confused with high-temporal resolution dynamic perfusion MRI or CT. The imaging protocols in this trial can be readily accomplished at UNOS-accredited transplant centers in the United States, which represent the pool of potential enrolling institutions for this trial. This study protocol defines a highly standardized approach to collecting and interpreting cross-sectional images aimed at the detection and evaluation of HCC.

2.7 Specific Hypotheses

1. Modern imaging technology can accurately diagnose and stage HCC in patients with chronic liver disease when performed on state-of-the-art multidetector CT or MRI equipment, with contemporary multiphase contrast-enhanced imaging protocols.

2. Focal liver lesions can be accurately assigned to pre-malignant and malignant diagnostic categories based on patterns of specific imaging findings. The false positive rate in the malignant lesion category can be reduced from previous, unacceptable levels by utilizing the criteria active as standard practive in the new OPTN/UNOS liver imaging policy (http://optn.transplant.hrsa.gov/policiesAndBylaws/policies.asp).

3. MRI is the superior cross-sectional imaging method for diagnosing HCC in patients with ESLD due to its inherently higher tissue contrast resolution and tissue characterization properties.

4. Imaging with CT or MRI can diagnose residual or recurrent viable HCC after focal ablative therapy in patients listed for liver transplant.

3.0 STUDY OBJECTIVES/SPECIFIC AIMS

3.1 Primary Aim

To compare the sensitivity of multiphase contrast-enhanced CT to that of multiphase contrast-enhanced MR for diagnosing HCC. The primary analysis for this comparison will be performed at the lesion level using core laboratory interpretations of the imaging studies. A secondary analysis will be performed at the patient level.

3.2 Secondary Aims

3.2.1 To compare the positive predictive value (PPV) of CT to that of MRI for diagnosing HCC. The primary analysis for this comparison will be performed at the lesion level using core laboratory interpretations. A secondary analysis will be performed at the patient level;

3.2.2 To compare the lesion-level sensitivity and PPV of CT and MRI as interpreted by radiologists at the respective transplant centers;

3.2.3 To compare the sensitivity and specificity of multiphase contrast-enhanced CT versus MRI for diagnosing residual or recurrent HCC after local ablative therapy in patients listed for liver transplant. The reference standard for this analysis will be based on pathologic diagnosis at time of explantation;

3.2.4 To determine the accuracy of imaging-based diagnosis and staging of HCC in clinical practice using the new OPTN liver imaging criteria compared with the reference standard of pathologic diagnosis and staging at time of explantation;

3.2.5 To explore whether the comparisons of sensitivity and PPV are affected by stratifying patients by AFP level (elevated vs normal).

3.3 Exploratory Aim
3.3.1 To assess the sensitivity and PPV of MRI and CT interpreted at the participating sites on the basis of all available information and sequences and compare to the sensitivity and PPV of the two modalities interpreted using the main study criteria.

4.0 STUDY OVERVIEW

The new OPTN organ allocation policy is now effective/activated as of November 2011. All transplantation centers should be following the new policy guidelines, making the procedures for the ACRIN 6690 trial standard practice procedures at participating institutions. For more information about the policy, visit http://optn.transplant.hrsa.gov/policiesAndBylaws/policies.asp; see Section 3.6 for information about liver allocation for transplantation and Section 3.6.4.4 for information about the criteria for waitlisting of patients with HCC.

4.1 Enrollment
ACRIN 6690 is not a screening trial for imaging diagnosis of HCC in an at-risk patient population; rather, it focuses on whether imaging can correctly identify HCC based on the new OPTN/UNOS policy's criteria. Sites need to ensure eligibility of patients, who must have at least

one OPTN Class 5 lesion(s) per study-defined imaging criteria in <u>Section 10.0</u>, and with overall disease stage meeting OPTN Stage 2, which is Milan criteria.

OPTN Class 5 lesion(s) [=HCC] need to meet one of the following definitions:
EITHER … OPTN Class 5B: at least 1 focal liver lesion(s) ≥ 2 cm diameter compatible with imaging diagnosis of stage II HCC on contrast-enhanced CT imaging and/or contrast-enhanced MRI;

OR … OPTN Class 5A: 2 or 3 focal liver lesions, each between >1 and <3 cm diameter, if each is compatible with imaging diagnosis of HCC on contrast-enhanced CT imaging and/or contrast-enhanced MRI.

Patients with ESLD in transplant centers typically undergo routine imaging for detection of HCC. Most centers image these patients with either multiphase contrast-enhanced CT or multiphase contrast-enhanced MRI at least once a year as their standard of care (SOC) imaging. The term "SOC imaging" is used in this trial protocol to describe the imaging modality (MR or CT) that is the "first choice" or "standard of care" at a participating institution to diagnose HCC de-novo or update a patient's HCC-exception MELD points on the liver transplant waitlist every 90-days. If a patient is found to have at least one OPTN Class 5B liver nodule (HCC) on SOC imaging and meets the so-called Milan criteria,[21] this patient may be eligible for listing for liver transplantation with HCC-exception points (either waiting for a liver from a deceased donor to become available or scheduled to undergo an LDALT). Predicated on such enrollment on the OPTN transplant waiting list with HCC-exception points, the patient becomes eligible for participation in the ACRIN 6690 liver imaging study. Patients must be enrolled to the trial after OPTN transplant waitlisting with HCC-exception points or the site PI or designated site co-investigator must complete a Declaration of Intent to List source document stating that, as far as the investigator can determine at the time of this declaration, the patient meets the criteria for waitlisting (see <u>Section 5.3</u>).

This study will enroll a total of 440 participants and will be open to all UNOS accredited liver transplant centers in the United States. The United States is divided currently into 11 transplant regions, which vary in area and number of organ transplant procedures. ECOG-ACRIN plans to enlist approximately 25 to 30 centers to participate in the study. Using a conservative estimate, recruitment of one participant per month per site would result in 20 participants per month fulfilling our accrual goal of 440 participants within a two-year timeline. This study will not enroll equal numbers from all regions but rather, the quota of participants recruited from a particular UNOS region will be kept proportional to the overall contribution of the region to the national total of patients transplanted with HCC-exception points. Historic UNOS data are used to determine the regional quotas. Each site will be notified of their regional accrual as well as the number of sites in their region that will be accruing to the trial.

4.2   Trial Design

Baseline imaging. After enrollment, the participating center must acquire multiphasic contrast-enhanced imaging with the complementary modality, at the expense of the trial, within 60 days of the initial SOC diagnostic scan (if initial diagnosis was made on CT, then MRI or if initial diagnosis was made on MRI, then CT). However, if both MRI and CT scans have already been obtained per protocol on an ACR core lab-qualified scanner within 60 days of each other, neither imaging needs to be repeated. If the **baseline** SOC imaging used for the purpose of UNOS listing is older than 60 days before the complementary scan can be scheduled, it will need to be repeated at the expense of the trial (that is, both MR and CT will need to be completed for **baseline**). In this case, both SOC and complementary imaging need to be

completed within 7 days of each other. It is acceptable for centers to schedule and obtain both exams on the same day for participant convenience, preferably in the order CT followed by MRI.

Serial imaging. Subsequently, participants will undergo **serial** (SOC and complementary) imaging scheduled in accordance with the 90-day intervals required for cyclical update of the HCC-exception points with UNOS. Both CT and MR scans must be completed within 7 days of each other. It is permissible to perform both imaging tests on the same day, preferably in the order CT followed by MRI. **The ultimate goal of this imaging schedule is to have a set of images from both imaging modalities available that is 90 or fewer-days-old when transplantation occurs (at a date that is unknown during the wait time period).** Results from this last imaging time point prior to transplant will be used for correlation with explant pathology analysis. In the rare event of an unexpectedly early transplantation, the most recent available imaging will be used for correlation with explant analysis, which may result in a time interval longer than 90 days for the complementary modality imaging in a few cases.

4.3   Ablative Therapy and Trial Imaging

Post-ablation imaging. If the decision is made that participants should undergo local ablative therapy after transplant listing and enrollment into this study, they will receive both CT and MRI no less than 28 days and no more than 60 days after the last ablative therapy session. One of the post-ablation imaging studies is considered clinically indicated and will be covered by the participant's insurance, while the study-related complementary imaging will be performed at the expense of this trial. If several consecutive sessions of transcatheter arterial chemoembolization (TACE) are planned, or combination therapy with TACE and thermal ablation is conducted, participants need to first complete the entire treatment scheme per institutional SOC before undergoing imaging with both modalities. In some participants, the post-ablation imaging time point may occur closer than 90 days to the next **serial** imaging time point required for liver transplant waitlist updates. Sets of **post-ablation** imaging studies that are less than 90 days old at the time of the next scheduled UNOS HCC-exception point update (**serial**) images do not have to (but may) be repeated at the time of exception point update and may count towards the **serial** imaging time point for the purpose of the trial.

For the remainder of the trial, these participants continue to be imaged according to the OPTN/UNOS schedule for updating HCC-exception points. Should another round of ablative treatment become necessary, the above rules apply for all post-ablation imaging sets and subsequent **serial** time points.

4.4   Expected Drop Out Rates

Historically, the drop-out rate on the transplant waitlist has been approximately 10% for HCC patients. These participants become ineligible for transplant either due to disease progression beyond Milan criteria, becoming "too sick to transplant," or dying while on the waitlist, either related or unrelated to the HCC. The sample size in this trial has been adjusted to compensate for dropout. Rates of dropout per region will be monitored. The regional pace of accrual will be adjusted to obtain a nationally-representative study sample should substantial variation across regions be identified during monitoring of the trial. The sample size has been adjusted to account for an expected number of participants who will have no available explant pathology, unless the site successfully obtains a post-mortem liver pathology analysis.

5.0 PARTICIPANT SELECTION/ELIGIBILITY CRITERIA

5.1 Inclusion Criteria

5.1.1 Must be able to provide a written informed consent;

5.1.2 Must be 18 years or older;

5.1.3 **Must be listed for liver transplantation with HCC exception points based on the imaging diagnosis of at least one OPTN Class 5 HCC lesion(s) per study-defined imaging criteria in Section 10.0.**

(Participating institutions may <u>not</u> enroll patients in whom the HCC diagnosis is solely based on biopsy and who do not have at least one liver lesion that meets imaging criteria for OPTN Stage 2, Class 5 HCC.)

Patients must meet one of the following descriptions based on imaging findings:

EITHER OPTN Class 5B: at least 1 focal liver lesion(s) ≥ 2 cm diameter compatible with imaging diagnosis of Stage II HCC on contrast-enhanced CT imaging and/or contrast-enhanced MRI;

OR OPTN Class 5A: 2 or 3 focal liver lesions, each between >1 and <3 cm diameter, if each is compatible with imaging diagnosis of HCC on contrast-enhanced CT imaging and/or contrast-enhanced MRI.

Imaging findings at the patient level in both situations must be within the UNOS Stage 2, which is Milan criteria[17] (see Appendix IV);

5.1.4 Must have been listed on the regional OPTN/UNOS liver transplant waitlist with HCC-exception MELD points prior to enrollment in this trial (up-to-date UNOS Policy requirements to determine HCC-exception are available at www.unos.org/policiesandbylaws/policies.asp, see Section 3.6 under Allocation of Livers).

OR

Site PI or designated site co-investigator determines whether patient is likely to meet all criteria for being listed on the regional OPTN/UNOS liver transplant waitlist with HCC-exception MELD points, but has not yet been listed with UNOS UNet. Investigator has completed and signed the Declaration of Intent to List source document declaring that the patient will likely meet all waitlist criteria.

Participants listed with the intent to undergo either deceased donor transplant or LDALT are eligible for this trial.

5.2 Exclusion Criteria

5.2.1 Tumors beyond Milan criteria (see Appendix IV).[17] This trial does not enroll patients with tumors beyond Milan criteria even from region(s) where transplant listing might still be permissible due to a special regional arrangement. Any of the following will exclude the patient from the trial:

5.2.1.1 Evidence of extrahepatic tumor;

5.2.1.2 Unifocal HCC > 5 cm in diameter;

5.2.1.3 Multifocal HCCs, 4 or more in number;

5.2.1.4 Multiple (2 or more) HCCs with at least one tumor ≥ 3 cm;

5.2.2 History of having undergone any local ablative therapy to liver <u>prior to</u> enrollment on the trial;

5.2.3 History or current use of sorafenib treatment (or comparable antiangiogenic therapy) **PRIOR** to enrollment (sorfenib treatment initiated after completion of baseline imaging is permissible);

5.2.4 Not suitable to undergo MRI with an extracellular gadolinium-based contrast agent that does not have dominant hepatobiliary excretion because of:

5.2.4.1 Claustrophobia, unless patient agrees to sedation measures per institutional standard practice during MR imaging;

5.2.4.2 Presence of metallic objects or implanted medical devices in body per institutional safety standards;

5.2.4.3 Sickle cell disease;

5.2.4.4 Weight greater than that allowable by the MR table;

5.2.5 Not suitable to undergo CT with an iodinated contrast agent:

5.2.5.1 Weight greater than that allowable by the CT table;

5.2.6 Renal failure, as determined by estimated glomerular filtration rate (eGFR) < 30 mL/min/1.73 m^2 by the Modification of Diet in Renal Disease (MDRD) model based on a serum creatinine level obtained within 28 days prior to enrollment;

5.2.7 Renal insufficiency at the time of enrollment, as determined by eGFR 30 to 60 mL/min/1.73 m^2 by the MDRD model based on a serum creatinine level obtained within 28 days prior to enrollment, unless permitted by the institution's policy and/or American College of Radiology (ACR) guidance for risk reduction strategies (see www.acr.org/SecondaryMainMenuCategories/quality_safety/contrast_manual.aspx for guidance on contrast selection and pre-treatment strategies);

5.2.8 Known allergy-like reaction to contrast media (iodinated or extracellular gadolinium that does not have dominant hepatobiliary excretion) or moderate or severe allergic reactions to one or more allergens as defined by the ACR, and unwillingness to undergo pre-treatment as defined by the institution's policy and/or ACR guidance (see www.acr.org/SecondaryMainMenuCategories/quality_safety/contrast_manual.aspx for reaction definition and premedication guidance);

5.2.9 Unable to give informed consent;

5.2.10 Unable to comply with breathing or other imaging related instructions resulting in inability to obtain diagnostic quality CT or MRI studies (OPTN Class 0);

5.2.11 Pregnancy (if a female is of childbearing potential—defined as a premenopausal female capable of becoming pregnant—a pregnancy test should be done);

5.2.12 Does not meet OPTN Class 5 imaging criteria for HCC, even if they have biopsy-proven HCC.

NOTE: Patients enrolled to the trial under the "Declaration of Intent to List" mechanism who fail to be listed with HCC MELD/PELD Score Exception history data on the UNOS UNET

Web site within 60 days from enrollment will come off trial and will not be counted towards target accrual.

5.3 Patient Selection: Declaration of Intent to List Source Documentation

To enable flexibility of treatment options for patients joining the ACRIN 6690 trial, the Declaration of Intent to List source documentation procedures have been developed as of Amendment 2 to the ACRIN 6690 main trial protocol. Figure 2 outlines the procedures. The Declaration of Intent to List source document is available online at www.acrin.org/6690_protocol.aspx. The original document, signed by the site PI or designated site co-investigator, must be kept in the patient's trial record. All **baseline** imaging must be completed prior to ablative treatment. No **serial** imaging may be completed until waitlisting submission has been finalized with UNOS UNet and ECOG-ACRIN has received confirmation of waitlisting. The original application date displayed on UNOS UNet will need to be provided to ECOG-ACRIN within 60 days after enrollment as the final step to complete the Declaration of Intent to List procedures.

Figure 2. Declaration of Intent to List Source Documentation Procedures

STANDARD OF CARE. MR or CT shows HCC per Milan criteria.

↓

ELIGIBILITY. Site PI or designated site co-investigator determines patient radiographically-eligible for HCC-exception MELD points; site moves forward with process of waitlisting.

↓

DECLARE INTENT TO LIST. Site PI or designated site co-investigator completes Declaration of Intent to List source document (see www.acrin.org/6690_protocol.aspx), ensures all other eligibility criteria for the trial are met, and places the signed original form in participant research folder.

↓

CONSENT AND ENROLLMENT. Patient consents to ACRIN 6690.
Patient is enrolled to ACRIN 6690 trial under Declaration of Intent to List procedures.
Enrollment marks day 1 of 60-day limit to provide ACRIN with confirmation of waitlist status.

↓

COMPLETE ACRIN 6690 BASELINE IMAGING. Site completes baseline imaging (MR or CT or both if outside 60-day window from SOC prior to enrollment) for ACRIN 6690 per main trial protocol and continues to TACE/ablation if part of treating physicians' plan.

↓

CONFIRM WAITLIST ELIGIBILITY. Site obtains all data points required for UNOS/OPTN liver transplant waitlisting with HCC-exception MELD points.

↓ (branches)

YES,
site lists participant with exception points by completing
UNOS UNet Web entry form

↓

NO MORE TRIAL-RELATED SCANS SHOULD BE CONDUCTED UNTIL ACRIN RECEIVES CONFIRMATION OF WAITLISTING

↓

Site provides UNOS MELD/PELD score exception original application date to ACRIN *within 60 days after enrollment*.

↓ (branches)

YES

↓

Participant continues on trial per protocol

NO

↓

PATIENT OFF STUDY

NO,
contraindication for transplantation, patient not qualified for exception point listing

↓

PATIENT OFF STUDY

5.4   Recruitment

The investigative team at each participating site will typically include the radiologist, transplant surgeon, hepatologist and/or oncologist, and pathologist. The site will identify a corresponding site PI who will coordinate efforts at the site and be the primary contact for all site-related matters. We encourage that the site PI be a radiologist (but this is not a requirement) as the cyclical recurring imaging and site reader image interpretations constitute a major portion of the work contributed to this trial. The site PI works closely with the other interdisciplinary members of the site investigative team. Of particular concern for this trial is a close collaboration between the radiology and pathology departments. Preparation by the radiologist of lesion identification (ID) correlation to identify same-lesions by ID on CT and MRI is strongly encouraged prior to the macroscopic explant liver analysis. Direct participation and physical presence of a radiologist at the time of and during the macroscopic explant liver analysis is strongly encouraged to help with identification of the Class 4 and 5 nodules for the purpose of the radiologic-pathologic correlation. This study aims to enroll all suitable participants in a consecutive fashion to exclude any selection bias beyond inclusion/exclusion criteria.

As patients are listed on the OPTN transplant list with HCC-exception MELD points, a review of eligibility criteria will take place. Patients become eligible for enrollment when the diagnosis of one OPTN Stage 2, Class 5 HCC is made <u>on either CT or MRI</u>, and patients are listed for transplant with HCC-exception points. Patients will be approached to participate in the main trial, ideally as soon as they have been listed for liver transplantation, with HCC exception points, in their respective region.

ECOG-ACRIN will develop a trial communications plan that will describe the production of materials to aid participant recruitment. All materials used for participant recruitment will be reviewed and approved by each institution's Institutional Review Board (IRB).

ECOG-ACRIN will obtain written consent from participating sites and their local IRBs to obtain de-identified notifications directly from UNOS administration of all site-specific patients listed for liver transplantation with HCC-exception points. Receiving these de-identified notifications from UNOS will allow ECOG-ACRIN staff to help sites recognize patients who are potentially eligible for the trial and to communicate directly with the site investigative team about opportunities to enroll such patients.

5.5   Inclusion of Women and Minorities

Both men and women and members of all ethnic groups are eligible for this trial. In conformance with the National Institutes of Health (NIH) Revitalization Act of 1993, with regard to inclusion of women and minorities in clinical research, the projected gender and minority accruals are shown below:

Table 3: Gender and Minority Accrual Estimates

Ethnic Category	Sex/Gender		
	Females	Males	Total
Hispanic or Latino	17	40	57
Not Hispanic or Latino	115	268	383
Ethnic Category: Total of all subjects	132	308	**440**
Racial Category			
American Indian or Alaskan Native	1	3	4
Asian	8	18	26
Black or African American	15	34	49
Native Hawaiian or other Pacific Islander	0	0	0
White	108	253	361
Racial Category: Total of all subjects	132	308	**440**

6.0 SITE SELECTION

6.1 Institution Requirements

The potential sites for this study are ECOG-ACRIN-participating institutions that meet qualifications for participating in this study. Qualification will include the submission of anonymized, retrospective, multiphase MR and CT liver images from patients with cirrhosis and HCC at each site. Sagittal and coronal reconstructed images derived from the dynamic post-contrast imaging series from both modalities will be required from each site. Each site interested in participation in this trial must submit anonymized DICOM images of three complete exams of each, multiphasic contrast-enhanced CT and MRI, of patients with HCC obtained during the previous 12 months on the scanners intended for use in the trial. The CT and MRI data need not have been obtained in the same patient. If more than one such CT and/or MRI system is anticipated for use in the course of this trial, then qualifying scans for each substantially different vendor/model/platform combination must be submitted. If 1.5T and 3T scanners are to be used for the trial, example images will need to be submitted for both. The images will be reviewed centrally for technical adequacy (timing of contrast enhancement relative to image acquisition, biologic motion, MRI artifacts, etc) and to provide feedback to the site. If the site successfully demonstrates the ability to acquire high-quality liver images, and meets other criteria set forth in this paragraph, the site becomes eligible for participation in the trial.

If several qualified sites from an OPTN/UNOS region apply for participation in this trial, the ECOG-ACRIN trial team will select sites based on the following criteria:
- Transplant case volume three years prior to application, by year;
- Accreditation by OPTN/UNOS;
- Research track record in (up to four) participating subspecialties in the centers; we will request bio-sketches from site investigators;
- Letter of commitment from participating departments;
- Occurrence of regular interdisciplinary case conferences featuring HCC/transplant patients at respective institutions.

NOTE: This study will not enroll equal numbers from all regions but rather, the quota of participants recruited from a particular UNOS region will be kept proportional to the overall contribution of the region to the national total of patients transplanted with HCC-exception points. The number of sites needed for participation will depend on the regional estimated accrual needed. If accrual in one or several regions fails to meet the required number of participants, the trial leadership reserves the option to abandon the concept of proportional accrual and enroll additional patients from any of the participating sites.

Each institution must complete a Protocol Specific Application (PSA) and have the MRI and CT scanners qualified by the ACR Imaging Core Laboratory, prior to the institution participating in the study. Detailed information for MRI and CT Qualification Procedures and its application to become qualified, as well as the PSA, can be accessed at www.acrin.org/6690_protocol.aspx. All regulatory documentation must be submitted to ECOG-ACRIN Diagnostic Imaging Headquarters (via fax: 215-717-0936, ATTN: ACRIN Protocol Development and Regulatory Compliance Department).

6.2 Access Requirements
Site personnel will be required to obtain CTEP-IAM (Clinical Therapy Evaluation Program-Identity and Access Management) log-in credentials to access the portals for site and roster profiling within the Cancer Trials Support Unit (CTSU), site approval for enrollment (CTSU RSS-the Regulatory Support System), and participant registration (via OPEN-the Oncology Patient Enrollment Network). For more information about CTEP-IAM credentialing, see the CTSU Web site FAQs: https://www.ctsu.org/readfile.aspx?fname=public/ctep-iam_factsheet.pdf. Data collection will continue according to the outline under Data Management in Section 7.0.

6.3 IRB Approval and Informed Consent Form
All institutions must have study-specific Institutional Review Board (IRB) approval for the protocol and informed consent form (ICF). The investigator and the investigator-designated research staff must follow OHRP-approved consent procedures (Title 45, Part 46 Code of Federal Regulations), as well as those set by the local IRB at the institution. A copy of the IRB approval letter and a copy of the IRB-approved, site-specific ICF must be delivered to the ACRIN Monitor for the trial to review the approved form and to keep on file at ECOG-ACRIN Diagnostic Imaging Headquarters (fax: 215-717-0936, ATTN: Protocol Development and Regulatory Compliance Department) prior to registering the first participant.

6.4 Accrual Goals and Monitoring
The ECOG-ACRIN Biostatistics and Data Management Center (BDMC) will monitor participant accrual. Total target accrual for this study is 440 participants. During the first year, accrual will be reviewed monthly with the intention of discovering and resolving any recruitment barriers. Efforts to increase accrual will be made throughout the trial. The OPTN/UNOS board has recognized this trial as an important collaborative research effort and intends to support planning and execution of this trial by making staff resources and data available to ECOG-ACRIN as appropriate.

ECOG-ACRIN regularly reviews the overall trial accrual and may request information about a trial's accrual performance to better understand general accrual barriers or issues. Accrual and safety information will be presented to the ECOG-ACRIN Data and Safety Monitoring Committee (DSMC) at regularly scheduled meetings thereof; the DSMC may, at its discretion, re-evaluate the study with respect to feasibility or the need for additional participating institutions.

7.0 DATA MANAGEMENT/ONLINE REGISTRATION

7.1 General

7.1.1 The ACRIN web address is www.acrin.org.

7.1.2 Data collection and management will be performed by the Imaging Biostatistics and Data Management Center (BDMC) of ECOG-ACRIN under the direction of Dr. Constantine Gatsonis. The Biostatistics Center (BC) is located at Center for Statistical Sciences at Brown University in Providence, RI, and the Data Management Center (DMC) is located at ECOG-ACRIN Diagnostic Imaging Headquarters in Philadelphia, PA.

7.1.3 Data collection and management for the Optional EDRN Ancillary Study of Multiplexed Biomarkers will be coordinated by the ECOG-ACRIN BDMC and EDRN DMCC. Briefly, all biologic specimens collected will be delivered to EDRN's Core Biomarker Testing Facility (EDRN's CBTF) for quality assurance testing. A subset of randomly selected specimens containing either serum or plasma will be sent to EDRN participating laboratories for analysis of eight (8) biomarkers listed in Appendix VII, Section 10.0. Remaining samples will be used to establish a biorepository containing training and validation reference sets that will be used in subsequent research studies. Test results from the eight (8) biomarkers will be provided to EDRN's Data Management Coordinating Center (DMCC) for subsequent statistical analysis. Results from DMCC's biomarker analysis and biomarker-related data elements will then be provided to the ECOG-ACRIN BDMC for inclusion into the ACRIN trial database and subsequent analysis of image features and biomarker findings correlated to pathology and immunohistochemical results. A transparent, open data exchange with accessibility to image and biomarker data will be ensured by policies and procedures adopted by ECOG-ACRIN and EDRN.

7.2 Participant Registration and Clinical Data Submission

7.2.1 Patient registration can occur only after eligibility criteria have been met and the study site is listed as 'approved' in the CTSU RSS. Patients must have signed and dated all applicable consents and authorization forms.

All site staff (Lead Group and CTSU Sites) will use OPEN to enroll patients to this study. OPEN can be accessed at https://open.ctsu.org or from the OPEN tab on the CTSU members' side of the website at https://www.ctsu.org.

Prior to accessing OPEN site staff should verify the following:

- All eligibility criteria have been met within the protocol stated timeframes. Site staff should use the registration forms provided on the group or CTSU web site as a tool to verify eligibility.

- All patients have signed an appropriate consent form and HIPAA authorization form (if applicable).

Access requirements for OPEN:

- Site staff will need to be registered with CTEP and have a valid and active CTEP-IAM account. This is the same account (user id and password) used for the CTSU members' web site.

- To perform registrations, the site user must have been assigned the 'Registrar' role on the relevant Group or CTSU roster.

- To perform registrations on protocols for which you are a member of the Lead Group, you must have an equivalent 'Registrar' role on the Lead Group roster. Role assignments are handled through the Groups in which you are a member.

- To perform registrations to trials accessed via the CTSU mechanism (i.e., non-Lead Group registrations), you must have the role of Registrar on the CTSU roster. Site and/or Data Administrators can manage CTSU roster roles via the new Site Roles maintenance feature under RSS on the CTSU members' web site. This will allow them to assign staff the "Registrar" role.

- Once the patient is successfully registered to the OPEN system, sites will receive an e-mail notification from ECOG-ACRIN containing the 6690 case number and calendar.

Further instructional information is provided on the OPEN tab of the CTSU members' side of the CTSU website at https://www.ctsu.org or at https://open.ctsu.org. For any additional questions contact the CTSU Help Desk at 1-888-823-5923 or ctsucontact@westat.com.

7.3   Clinical Data Submission Via ACR CTMS Portal

7.3.1   Upon successful participant registration in OPEN, sites transition to the ACRIN.ORG web site to submit study data. The investigator-designated research staff may download the participant specific data submission calendar, which lists all forms and designated reports required by protocol, along with the form due dates at the DMC. These calendars will be updated as the study proceeds to reflect data that have been received, reply deadlines for queries about unclear data, deadlines for follow-up reports of adverse events, or changes in the protocol that change the data being collected or the timeframe. Updated calendars for each participant can be obtained 24 hours a day from the ACRIN web site. The research associate (RA) may use the calendar as a case management tool for data submission and follow-up scheduling.

7.3.2   The investigative site is required to submit data according to protocol as detailed on each participant's calendar, as long as the case status is designated as open/alive or until the study is terminated. The case is closed when all data have been received, reviewed, and no outstanding data query exists for the case.

7.3.3   To submit data via the ACRIN web site, the appropriate investigator-designated research staff will log onto the ACRIN web site and supply the pre-assigned user name and password. Case report forms will be available on the web site through a series of links. Each web form is separated into modules; each module must be completed sequentially in order for the internal programming to be accurate. The user selects the link to the appropriate form and enters data directly into the web-based form. As information is entered into the web form application, various logic checks will be performed. These logic checks look for data that are missing, data that are out of range,

and data that are in the wrong format (e.g. character data in a field requiring numeric responses). Such errors will be detected as soon as the user attempts to either submit the form or move to the next data element. They must be corrected before the form is transmitted to the DMC. The user will not be able to finalize form transmission to the DMC until all data entered pass these logic checks. Forms that are not completed in one sitting can still be submitted and completed at a later date. The form will remain available on the web until the "Complete Form Submission" button is depressed.

7.3.4   Once data entry of a form is complete, and the summary form is reviewed for completeness and accuracy, the investigator or the research staff presses the "Complete Form Submission" button on the form summary screen and the data are transferred into the clinical database. No further direct revision of the submitted data is allowed after this point. E-mail confirmation of web data entry is automatically generated and sent to the site investigator or research associate listing all of the data generated and just submitted. Should a problem occur during transmission and the e-mail confirmation of data submission is not received, the investigator or research associate should contact the DMC for resolution of the submission.

7.3.5   If a temporary problem prevents access to the Internet, all sites are notified of the event and estimated down time through an ECOG-ACRIN broadcast message. The investigative site should wait until access is restored to submit data. The site RA or investigator should notify the DMC of the problem and the DMC will give an estimated time when access will be restored. If access will be unavailable for an extended period, sites must seek another Internet Service Provider (ISP). On a short-term basis, the ACR can serve as an ISP.

7.4   Data Security

The registration and data collection system has a built-in security feature that encrypts all data for transmission in both directions, preventing unauthorized access to confidential participant information. Access to the system will be controlled by a sequence of identification codes and passwords.

7.5   Electronic Data Management
7.5.1   Data received from the web-based forms are electronically stamped with the date and time of receipt by the ACR server. The data are then entered into the database. A protocol-specific validation program is used to perform more extensive data checks for accuracy and completeness. Complementary validation programs are initiated at the Brown BC and the DMC. The logic checks performed on the data at this point are more comprehensive than those built into the web-based data entry screens. They include checking that answers are logical, based on data entered earlier in the current form and the more thorough checks. Data elements that fail validation are followed up by the DMC RA. The validation program generated by BC produces a log of errors, which is sent to the DMC for resolution. The program is frequently updated to incorporate exceptions to rules so that subsequent validity checks minimize the time the DMC needs to spend resolving problems. Additional data review will take place once the data are transferred to the BC. The BC will run thorough cross-form validations, frequency distributions to look for unexpected patterns in data, and other summaries needed for study monitoring. Any errors found at the BC will be reported to the DMC for

resolution. All BDMC communication with the participating sites is normally done through the DMC.

7.5.2 If checks at DMC or BC detect missing or problematic data, the DMC personnel assigned to the protocol sends a Request for Information (Z1 query letter) to the site RA or investigator specifying the problem and requesting clarification. The DMC updates the participant's data submission calendar with the due date for the site RA or investigator's response.

7.6   Missing and Delinquent Data Submission

In addition to providing the investigator a data collection calendar for each case, the DMC periodically prompts institutions for timely submission of data through the use of a Forms Due Report. Distributed at intervals via the electronic mail system directly to both the RA and the investigator at each site, this report lists data items (e.g. forms, reports, and images) that are delinquent and those that will be due before the next report date. In addition to prompting clinicians to submit overdue data, the Forms Due Report helps to reconcile the DMC's case file with that of the RA and/or investigator. Future Due Forms Report may be sent on an as needed basis in addition to past due reports. The site investigator or RA may use the Forms Due and Future Due Reports as a case management tool.

7.7   Data Quality Assurance

7.7.1 The BC at Brown University will maintain a study database at its site for monitoring data quality and for performing analyses. These data are drawn directly from the permanent database of the DMC. The transfer of data between the DMC and the BC has been validated through a series of checks consisting of roundtrip data verification in which data are sent back and forth to verify that the sent data are equivalent to the received data. These checks are repeated at random intervals during the course of a given study. Any discrepancies and other data quality issues will be referred to DMC for resolution, since only the DMC can correct the data file. No changes to the data will be made at the BC.

7.7.2 A goal of the monitoring of data is to assess compliance with the protocol and to look for unforeseen trends that may be indicative of procedural differences among clinical sites. If patterns are discovered in the data that appear to arise from causes specific to an institution, the BDMC will apprise the ECOG-ACRIN Diagnostic Imaging Headquarters and the site of the problem, and work with the site, along with ACRIN Protocol Development and Regulatory Compliance (PDRC) department, until the problem has been resolved. If the BDMC, along with the PDRC, cannot find a resolution to the problem, it will be brought to the ACRIN Quality Assurance (QA) Committee for further discussion and resolution.

7.7.3 In addition, the ACRIN QA Monitor will review case report forms and source documents at several different time points: after first few participants enrolled and during the conduct of the trial, including staff changes at the participating sites. In addition, the QA Monitor will review the initial and annual regulatory documents and any revised regulatory documents. This monitoring process ensures protocol and regulatory compliance, participant's welfare and safety, and provides resources to sites for clarification to the protocol and guidance in completion of the case report forms.

8.0   STUDY PROCEDURES

ACRIN 6690 is not a screening trial for HCC; it focuses on the difference between CT and MRI with regard to their ability to correctly identify HCC based on the new OPTN/UNOS policy's criteria. Sites need to ensure eligibility of patients, who must have at least one OPTN Class 5 lesion(s) [=HCC] per study-defined imaging criteria in per Sections 5.1.3 and 10.0, with overall disease stage meeting Milan criteria (OPTN Stage 2).

For the main trial, baseline SOC and study-related complementary imaging (MR and CT) for each participant will need to have been executed per protocol on an ACR core lab-qualified scanner. **Baseline** complementary imaging with the alternate modality may be obtained within 60 days of the SOC scan. Should the SOC scan be executed off-protocol, on a scanner that is not ACR core lab-qualified, or outside of the 60-day window, then both imaging modalities will need to be completed after enrollment at the expense of the trial to achieve the study objectives. If both scans are completed after enrollment, then they must be completed within 7 days of each other (same-day is acceptable). These scans may be used for the subsequent UNOS/OPTN update. No ablative therapy may be performed between **baseline** MR and CT scans. Again, **the ultimate goal is to have a set of CT and MR images that is 90 days or younger when transplantation occurs.** See Section 8.2 for additional details.

Serial MR and CT imaging will be performed at distinct time points as dictated by OPTN/UNOS until transplantation. Timing of **serial** SOC imaging in waitlisted patients (and those eligible for the Declaration of Intent to List per Section 5.3) is dictated by OPTN/UNOS HCC-exception point update requirements (90 day intervals). Waitlisted patients are defined as those eligible for/scheduled to undergo LDALT as well as those waiting for livers from deceased donors to become available. **Serial** imaging will need to be completed (both MR and CT) within a 7-day time window if scans are performed on different days; no ablation may be performed between the MR and CT scans.

Participants with disease staged within Milan criteria who subsequently progress beyond Milan criteria while on trial may be kept in the trial and proceed to explantation under two "special" circumstances: if they happen to either already be living in or be moving to a UNOS/OPTN region that allows listing for deceased donor transplantation at a higher stage and thus may progress to transplant per regional practice OR if the participant is slated to undergo LDALT.

If a participant undergoes local ablative therapy after enrollment, this study protocol requires imaging with both CT and MRI 28 to 60 days after completion of that treatment. Ablative therapy must not be conducted prior to completion of **baseline** imaging with both modalities nor between **serial** imaging for the trial (that is, ablation must not occur until both **baseline** scans are completed or within the specified 7-day time limit between **serial** scans if CT and MR are completed on separate days). Sets of **post-ablation** imaging studies that are less than 90 days old at the time of the next scheduled UNOS HCC-exception point update (**serial**) images do not have to (but may) be repeated at the time of exception point update and may count towards the serial imaging time point for the purpose of the trial**.** One of the post-ablation imaging studies is considered clinically indicated and will be covered by the participant's insurance, while the study-related complementary imaging will be performed at the expense of this trial.

Serial SOC and complementary imaging will be performed to support the 90-day intervals required for cyclical update of the HCC-exception points with UNOS. The objective is to have a set of serial images, no older than 90 days prior to transplant in [the majority of] trial participants, available to allow for a comparison of imaging findings with the explant liver pathology findings.

For participants enrolled in the EDRN Multiplexed Ancillary Biomarker Study, all main trial procedures should be completed as described below, with the addition of blood collection and processing at participating sites. Blood (two vials for a minimum of 8 mL of blood in each) will be collected from consenting participants before each study-related imaging time point (baseline, serial imaging, and post-ablation imaging) and at three-months post-transplantation. Blood will need to be processed within 4 hours after collection. For additional information, see Appendix VII.

NOTE: All CT and MRI scans should be performed on the same ACR core lab-qualified scanners; it is encouraged that the same model scanner should be consistently used for a participant throughout the trial if at all possible.

8.1 Eligibility/Enrollment Visit
(After HCC-Exception Point Liver Transplant Waitlisting or Completion of Declaration of Intent to List Source Documentation)

Patients must be enrolled to participate in the trial prior to ablation after being waitlisted with HCC-exception points **OR** the site PI's or designated site co-investigator's completion of the Declaration of Intent to List source documentation. Procedures for the Declaration of Intent to List are provided in Section 5.3, describing patient selection, and Figure 2 outlining the process.

At the registration visit, the potential participant will be confirmed for eligibility by the appropriate study-team designee prior to electronic enrollment:

8.1.1 Obtain written informed consent from the patient to participate in the trial;

8.1.2 Confirm eligibility (Section 5.0), which includes:

- Review of the inclusion and exclusion criteria;

- Confirm transplantation waitlist status or complete Declaration of Intent to List source document;

- Collect CT and/or MR images for submission;

- Review medical history;

   NOTE: If the Declaration of Intent to List source document is used, then the original application date must be supplied to ECOG-ACRIN Diagnostic Imaging Headquarters **within 60 days after enrollment** to trigger subsequent **serial** imaging time points.

- Review eGFR levels if assessed 28 days prior to enrollment (if creatinine has not been assessed within 28 days of enrollment, additional laboratory results may need to be obtained to confirm renal status);

8.1.3 Collect data from routine laboratory studies, including basic liver enzyme panel (i.e., aspartate aminotransferase [AST], alanine aminotransferase [ALT], alkaline phosphatase [AlkPhos], internal normalized ratio [INR], bilirubin, albumin), and alpha fetoprotein (AFP), and assessment for ascites and hepatic encephalopathy;

8.1.4 Confirm images used to determine participant eligibility for transplantation waitlist are available for submission to ECOG-ACRIN Diagnostic Imaging Headquarters, including, if possible, the most-recent prior MRI and/or CT images. These images may be used as SOC and/or complementary **baseline** scans if they were taken within 60 days of each other. If not, most-recent prior images are still required for submission and both CT and MR scans will need to be completed for **baseline** within 7-days of each other as described below (see also Sections 4.2 and 10.2);

8.1.5 In women of childbearing potential, conduct a pregnancy test as per institution's SOC. Should a participant become pregnant at any time during the trial, the woman will be off-study;

8.1.6 Enroll patient electronically to the trial via the ACRIN Web site (www.acrin.org).

8.2 BASELINE: Initial UNOS HCC-Exception MELD Points Waitlisting Standard-of-Care Imaging Plus Study-Related Complementary Imaging OR Repeat Imaging (Either: Complementary Imaging Within 60 Days of Pre-Enrollment Protocol-Appropriate SOC Imaging, Or Both MR and CT Repeated After Enrollment Per Protocol and Within 7-Days of Each Other)

Both MR and CT images will need to be completed and collected at **baseline** for all participants; ablative therapy must not be performed prior to completion of these two imaging studies. If both MR and CT scans were completed **per protocol on an ACR core lab-qualified scanner** to determine initial UNOS waitlist eligibility, they are eligible as **baseline** images for the trial provided they were completed within 60 days of each other (regardless of enrollment at waitlist confirmation or using Declaration of Intent to List procedures [see Section 5.3]). Otherwise, if **baseline** SOC was not completed per protocol, not completed on an ACR core lab-qualified scanner, or was completed outside of 60 days before the complementary scan can be performed, then both CT and MR scans need to be performed after enrollment to complete **baseline** imaging requirements. The scans will need to be completed within 7 days of each other. It is acceptable for centers to schedule and obtain both exams on the same day for participant convenience. In that case it is preferable that the CT be performed first, if at all possible.

NOTE: Should a registered participant undergo ablative therapy prior to completion of **baseline** imaging (MRI **and** CT), then the participant cannot continue on the trial. Note that patients who are enrolled in the optional Eovist sub-trial will need to complete an additional Eovist-enhanced MRI at baseline **prior to any ablative therapy**.

8.2.1 Complementary CT With Iodinated Contrast Agent

- In women of childbearing potential, conduct a pregnancy test per the institution's SOC; should a participant become pregnant at any time during the trial, the woman will be off-study;

- Evaluate eGFR levels for renal failure only (via participant record review or special testing if necessary) if not assessed within 28 days prior to CT scan;

- Place one (1) IV catheter in the participant's arm vein to inject the contrast bolus;

- Administer iodinated contrast agent per protocol requirements;

- Perform a multiphase contrast-enhanced CT scan according to requirements outlined in Section 10.0 and online at www.acrin.org/6690_imagingmaterials.aspx;

- Assess for adverse events (AEs) prior to departure from imaging suite; participants will be encouraged to call the research staff to report any adverse reactions.

OR

8.2.2 Complementary MR With Extracellular Gadolinium Contrast Agent Without Dominant Hepatobiliary Excretion

- In women of childbearing potential, conduct a pregnancy test per the institution's SOC; should a participant become pregnant at any time during the trial, the woman will be off-study;
- Evaluate eGFR levels for renal failure only (via participant record review or special testing if necessary) if not assessed within 28 days prior to MR scan;
- Place one (1) IV catheter in the participant's arm vein to inject the contrast bolus;
- Administer extracellular gadolinium contrast agent that does not have dominant hepatobiliary excretion per protocol requirements;
- Perform a multiphase contrast-enhanced MR scan according to requirements outlined in Section 10.0 and online at www.acrin.org/6690_imagingmaterials.aspx;
- Assess for AEs prior to departure from imaging suite; participants will be encouraged to call the research staff to report any adverse reactions.

8.3 Study-Related SERIAL Imaging: Timing Per UNOS Listing Update Requirements (Every 90 Days)

Transplantation centers will perform SOC imaging (CT or MR) to assess disease status and are required to submit the radiologist report (and other clinical information) to UNOS no later than every 90 days for patients to remain on transplant waitlist with updated HCC-exception MELD points. This SOC imaging should occur per customary intervals required by OPTN/UNOS to maintain transplant listing and update HCC-exception points. This trial requires that complementary imaging also be completed at approximately the same time SOC imaging is performed; if study-related **serial** imaging (CT and MRI) cannot be performed the same day, they must be completed within 7 days of each other.

IMPORTANT: Ablative therapy **must not** interfere with the study-related serial imaging sequence; in other words, centers **must not** perform elective local ablative therapy **in between** the SOC and complementary imaging examinations, for which a maximum 7-day window is allowable as described below. This should not be a problem since most centers may perform both exams on the same day for participant convenience.

8.3.1 SOC MRI or CT scans will be completed at-least every 90 days per institutional and UNOS listing update requirements to assess participant disease and transplant waitlist status;

8.3.2 Study-related complementary imaging (MRI or CT) will be completed within 7 days of the SOC imaging;

8.3.3 In women of childbearing potential, conduct a pregnancy test per the institutional SOC; should a participant become pregnant at any time during the trial, the woman will be off-study;

8.3.4 Review participant's basic liver enzyme panel (AST, ALT, AlkPhos, INR, bilirubin, albumin) and serum AFP level;

8.3.5 Review assessment for ascites and hepatic encephalopathy.

NOTE: ALL images obtained with CT and MRI at a specific imaging time point will need to be submitted to ACR Imaging Core Laboratory. This includes all sequences, series, and reconstructions that are completed for participant imaging, e.g., also those MR sequences/images not directly applicable to the criteria used for HCC diagnosis in this trial.

8.4 Post-Ablation Imaging
(Re-Imaging 28 to 60 Days After Completed Ablation)

8.4.1 Should a participant undergo local ablative therapy while on the waitlist for liver transplantation (see Section 4.3), biopsy of the ablative area prior to ablation is strongly encouraged, although not mandated of the sites; results of the biopsy must be submitted to ECOG-ACRIN Diagnostic Imaging Headquarters. Biopsy-based diagnosis of the nodule may be used as a surrogate endpoint in case such a nodule undergoes complete necrosis during ablative therapy and would be considered non-diagnostic during eventual explant pathologic workup;

8.4.2 Additional study-related **post-ablation** imaging will be required within 28 to 60 days after the completion of ablative therapy. Imaging will comprise SOC and study-related complementary imaging, completed within 7 days of each other; as a result, all participants undergoing local ablative therapy will undergo CT and MRI within this timeframe to assess for residual or recurrent HCC;

8.4.3 If no additional ablative therapy is necessary, the participant will return to study-related serial imaging per UNOS listing updates and protocol-specific requirements (see Section 8.3). Should additional ablative therapy be necessary at any time, imaging will be obtained as per Section 8.4;

8.4.4 If several consecutive sessions of transcatheter arterial chemoembolization (TACE) are planned, or combination therapy with TACE and thermal ablation is conducted, participants need to first complete the entire treatment scheme per institutional SOC before imaging with CT and MRI as described above for post-local ablative therapy;

8.4.5 Additional ablation should not be conducted such that it would interfere with the post-ablation imaging (i.e., during the 7-day allowance to complete the post-ablation serial MR and CT imaging);

8.4.6 Sets of **post-ablation** imaging studies that are less than 90 days old at the time of the next scheduled UNOS HCC-exception point update (**serial**) images do not have to (but may) be repeated at the time of exception point update and may count towards the serial imaging time point for the purpose of the trial.

8.5 Off-Study Criteria
- Death without explant pathology analysis and report for submission to ECOG-ACRIN Diagnostic Imaging Headquarters;
- Removal from the waitlist at any time;

- Renal failure during the trial as defined in Exclusion Criteria Section 5.2.6;
- Ablative therapy prior to completion of baseline imaging (both MRI and CT);
- Changing care facilities to a facility not involved in the trial;
- Participants who develop contrast-induced nephropathy following iodinated contrast administration for CT may undergo contrast-enhanced MRI only if their renal function recovers within 30 days of CT, otherwise, they will be excluded because of risk of developing nephrogenic systemic fibrosis (NSF) after gadolinium administration;
- Patients enrolled to the trial prior to completion of OPTN/UNOS waitlisting with HCC-exception MELD points who are <u>not</u> accepted for waitlisting will need to be replaced to meet target accrual;
- If UNOS UNet original application date is not provided to ECOG-ACRIN Diagnostic Imaging Headquarters within 60 days of Declaration of Intent to List source document completion, then the patient will be off-study and will need to be replaced;
- If a patient is waitlisted by multiple transplant centers (in more than one UNOS region) and transplantation occurs at a site which is not participating in the trial, that patient will need to be replaced to meet target accrual.

8.6   Management of Eovist-enhanced Sub-study Participants

As of February, 7, 2014, the Eovist sub-study ceased accrual. All participants who consented and completed the Eovist-enhanced MRI studies will continue with the main study and continue to complete Eovist scans. See Amendment 3's (protocol version September 13, 2013) Eovist appendices for full instructions for sub-study conduct and radiology reader instructions.

8.7 Study Procedures Table

Study Procedure	Eligibility/ Enrollment (After HCC-Exception Point Waitlisting or Declaration of Intent to List)	BASELINE: CT and MR Images Within 60 Days of Each Other (Or Both Scans Completed With 7-Days of Each Other After Enrollment)	Study-Related SERIAL Imaging‡ (CT and MR Every 90 Days Per UNOS Listing Update Requirements)	POST-ABLATION Imaging (CT and MR Within 28 to 60 Days After Completed Ablation)
Informed Consent Form	X			
Review Inclusion/Exclusion Criteria	X			
Confirm Transplant Waitlist Status **OR** Declaration of Intent to List (Need to Provide UNOS UNet Original Application Date Within 60 Days of Declaration, Prior to 1st **Serial** Imaging Time Point)	X			
Submit Diagnostic MR and/or CT Images	X			
Review Medical History	X			
Review Routine Lab Results, Including eGFR Levels	X	X	X	X
Review Assessment for Ascites and Hepatic Encephalopathy	X		X	
Confirm Diagnostic Images Available for Submission to ECOG-ACRIN*	X			
Conduct Pregnancy Test for Women of Childbearing Potential	X	X	X	X
Web Registration	X			
Place IV Catheter for Contrast Bolus		X	X	X
Inject Contrast (Extracellular Gadolinium Without Dominant Hepatobiliary Excretion for MR or Iodinated for CT)		X	X	X
Standard-of-Care Imaging (MR or CT)†‡		X§	X	
Study-Related Complementary Imaging (MR or CT)†‡		X§	X	
Post-Ablation MRI and CT†				X
AE Assessment		X	X	X

* Institutions are requested to not only upload this baseline imaging study to ECOG-ACRIN Diagnostic Imaging Headquarters but also the most-recent prior imaging study (same-modality—MR and/or CT), if available.

† All images obtained with CT and MRI at a specific imaging time point will need be submitted to ECOG-ACRIN Diagnostic Imaging Headquarters. This includes all sequences, series, and reconstructions that are completed for participant imaging.

‡ Each site images patients (with CT or MRI) no later than every 90 days (or earlier) to adhere to the UNOS waitlist update guidelines. The due date for the HCC-exception point update is explicitly stated in UNOS UNet, the UNOS web portal that can be accessed online by transplant center staff. This imaging to evaluate disease is considered standard of care. This research trial requires that complementary imaging (CT or MRI, whichever imaging was not done as the standard of care) be completed at each 90-day UNOS waitlist update interval. **At each serial imaging time point, CT and MR need to be completed within 7 days of each other.** It is permissible to perform both imaging tests on the same day; the order of CT prior to MR is preferred.

§ Standard-of-care **baseline** imaging may be necessary (at the cost of the trial) if the most recent previous scan was completed more than 60 days prior to participant being able to complete the **baseline** complementary imaging. No study-related baseline imaging is necessary if both MR and CT were completed per protocol on an ACR core lab-qualified scanner for initial UNOS waitlist eligibility requirements within 60 days of each other prior to trial enrollment and both imaging sets are submitted to the ACR core lab. Should the SOC imaging scan used for initial HCC-exception point waitlisting have been completed more than 60 days before the complementary baseline scan can be completed for the trial, then both MR and CT scans will need to be completed after enrollment at the expense of the trial to complete the baseline imaging time point. Depending on the timing of these scans, they may be used for the 90-day UNOS update serial scan time point. Ablative therapy should not be started before both **baseline** CT and MR scans are completed.

9.0   EXPLANT PATHOLOGY

9.1   Patient Identification

Not all patients transplanted in a participating center will be part of this study cohort. In fact, in most centers, approximately 75% of patients are transplanted with regular MELD points rather than HCC-exception points. Therefore, the majority of explant livers received by a pathology department in a participating center will not belong to a participant from this study cohort. Upon receipt of an explant liver, the local pathologist or a designee familiar with this protocol will check with the transplant team and/or trial study coordinator to see whether the patient is enrolled in this trial so the explant workup can be performed according to the specifications of this trial protocol. Alternatively, the study site investigator and study coordinator will inform the local pathologist of when the study participant went to transplant.

Details of explant pathology processes for correlation with imaging are available in the Pathology Manual online at www.acrin.org/6690_imagingmaterials.aspx. Digital photographs of all relevant macroscopic lesions and each side of all cut gross specimen liver sections will need to be submitted to ECOG-ACRIN Diagnostic Imaging Headquarters. Submission details are available in the Pathology Manual.

9.2   Explant Liver Workup in Local Pathology Lab

9.2.1   Goals
Of particular concern for this trial is a close collaboration between the radiology and pathology departments. Preparation by the radiologist of lesion ID correlation to identify same-lesions by ID on CT and MRI is strongly encouraged prior to the macroscopic explant liver analysis. Direct participation and physical presence of a radiologist during the macroscopic explant liver analysis is strongly encouraged to help with identification of the Class 4 and 5 nodules for the purpose of the 1:1 radiologic-pathologic correlation. Availability of the images at time of explant pathology workup is key; this would best be accomplished in respective centers by having either PACS access or other image display available during the organ dissection. Radiologists are familiar with the localization of nodules on imaging, should review the relevant report of Class 4 and 5 nodules in a given participant, and may be able to directly communicate the location of a specific nodule and verify that the correlative tissue sampling at the time of explant pathology workup matches the location of the respective nodule seen on imaging.

9.2.1.1 Identify and sample all OPTN Class 4 and 5 lesions described on imaging and obtain 1:1 macroscopic and histopathologic correlation; summary reports of most recent CT and MRI performed in the participant will be provided to the pathologist by the local site trial designated radiologist and/or the study coordinator. As well, corresponding images will be available through the online trial portal/database and should be reviewed to guide lesion search and 1:1 correlation in the pathology lab;

9.2.1.2 Identify and sample all other suspicious focal liver lesions and report/record those which turn out to be OPTN Class 5 lesions but were not recorded as such (false negative imaging findings);

9.2.1.3 Report pathologic staging on a per patient basis based on Sections 9.2.1.1 and 9.2.1.2 above;

9.2.1.4 Provide macroscopic digital photos of all relevant nodules for correlative purposes;

9.2.1.5 Provide systematic digital macroscopic images of each side of all gross specimen liver slices/sections. Indicate on slices where sampling took place.

10.0 IMAGING PROTOCOL

The required images must be submitted to the ACR Imaging Core Laboratory. The protocol-required radiographic images must be in DICOM format on CD/DVD-ROM or submitted via the internet using TRIAD software. For all TRIAD submissions, Imaging Transmittal Worksheets must be completed electronically online and submitted to ECOG-ACRIN Diagnostic Imaging Headquarters for each image (C0 Form for CT scan transmission and MW Form for MR scan transmission).

The electronic forms can be accessed via the ACRIN 6690 web site (www.acrin.org). All CD/DVD-ROM submissions must be accompanied by the appropriate worksheet(s), as well; paper copies can be downloaded from www.acrin.org/6690_imagingmaterials.aspx.

10.1 Imaging Acquisition

This study will be open to all UNOS-accredited transplantation centers in the United States. Participating centers will need to comply with minimum technical requirements for CT and MR as shown in Tables 4 and 5. Multiphase contrast-enhanced CT and multiphase contrast-enhanced MRI will be used for imaging in this trial. Additional imaging parameter details are available in the ACRIN 6690 Imaging Manual, found online at www.acrin.org/6690_imagingmaterials.aspx. In cases of discrepancy between the protocol and Manual, sites should defer to the Manual contents.

Physical image acquisition may be performed at the participating transplant center or at a different site as long as technical and protocol requirements, including appropriate DICOM-format image submission, are met and the scanners used have been vetted through the ACR core lab-scanner qualification process for this trial. Interpretation for the purpose of this trial has to be performed according to reporting requirements specified in the protocol by participating transplant center radiologists. If patients listed for transplant with priority points for HCC consent to participate into the study, **baseline** CT and MR need to be completed within 60 days of each other using the original submission images and/or being able to schedule the patient to return to complete the complementary study-related scan. Otherwise, both CT and MR will need to be completed after enrollment within 7 days of each other.

Serial imaging will then be performed no later than every 90 days as per OPTN/UNOS requirement for updating priority MELD points. Under the main trial protocol, MR and CT will occur within 7 days of each other for assessment of disease while on the waitlist.

All imaging will be transferred to the ACR Imaging Core Lab in DICOM format per directions in Section 10.2.

10.1.1 Importance of High-Quality, Carefully Timed Multiphasic Contrast-Enhanced Imaging

It is well known in the imaging community that optimal detection of liver nodules with

predominant arterial vascular supply (such as HCC) on cross-sectional imaging (CT or MRI) requires careful timing of image acquisition to take place during **late arterial phase** of contrast enhancement. At that point in time there is maximal signal-to-background contrast between capillary enhancement in the lesion and surrounding hepatic parenchyma. In most patients, early arterial phase imaging does not improve tumor conspicuity by either quantitative or subjective analysis.[23,24]

There is a relatively small time window for acquisition of the late arterial phase, which persists for approximately 10 seconds in most patients and explains the need for careful timing.

While also important for diagnostic purposes, the time window of opportunity to acquire images of the hepatic parenchyma during portal vein and equilibrium (delayed) phase is much wider. Therefore it is permissible to use fixed-time delays (approximately 60 to 75 seconds post injection and 120 to 180 seconds post injection) for the later contrast phase imaging. These numbers are suggestions, and portal vein and delayed phase imaging should be performed per institutional preference and standard of care.

10.1.2 CT Imaging

10.1.2.1 General CT imaging parameters are outlined in the table and text below.

- Helical CT scanning is required; axial serial scanning cannot be used.
- Multi-detector scanning must be performed, using a scanner with a minimum of 8 detector rows.
- Pitch should be based on institutional routines. Reconstruction should be performed at ≤ 5 mm intervals.
- Scanner settings (kV, mAs) should be per institutional routine procedures.
- The use of radiation dose-savings strategies offered on a given scanner platform is encouraged. For instance, dose-modulation should be turned on if available to adapt dose to patient shape throughout the scan. Technologists should pay careful attention to limiting coverage of multiphasic scans to the [anatomic] area of interest.
- Choice of contrast agent should be according to local institutional routine.
- Contrast dose should be 300 mg I/mL or higher concentration, for dose of 1.5 mL/kg body weight.
- Injection rates should be no less than 3 mL/sec of contrast, better 4 to 6 mL/sec. 18G IV is preferred for bolus injection rates.
- Central lines need not be used unless absolutely required due to lack of acceptable peripheral IV access. Central lines should not be used with power injector unless specifically approved for that indication.

10.1.2.2 Abdominal CT

Abdominal imaging should be tailored for multiphase liver imaging techniques. Optional pre-contrast and then late arterial-phase, portal vein phase, and equilibrium/delayed phase post-contrast imaging provides optimal evaluation of the diseased liver for presence of HCC. Each vascular phase scan (expiration

preferred) of the liver must be obtained in a single breathhold helical acquisition.

HCC has a range of presentations on CT. The most diagnostic images are the properly timed multi-phase contrast-enhanced images. The following section covers the key elements necessary to achieve optimal diagnostic sensitivity and specificity, as well as an optional pre-contrast imaging sequence recommended especially after ablative therapy.

10.1.2.3 Guidelines for Multiphasic Contrast-Enhanced CT Imaging

10.1.2.3.1 Pre-contrast: Recommended but not required

Non-contrast imaging through the liver prior to contrast-enhanced imaging is optional and not required for the purpose of this protocol. However, note that pre-contrast imaging is strongly encouraged for CT studies performed after local ablative therapy, especially after chemoembolization with densely radiopaque materials such as ethiodol. This will help the reader distinguish contrast-enhancing residual or recurrent HCC from radiopaque tissue bound embolization material.

10.1.2.3.2 Late arterial phase

Imaging characteristics include the following:

- Fully enhanced hepatic artery and branches;
- Early contrast enhancement of portal vein;
- Lack of enhancement of the hepatic venous system.

Time to peak enhancement in abdominal aorta at celiac axis level can be determined either by timing bolus injection or through use of triggering facility provided on newer scanners. Some scanners have an "auto-triggering" feature that commences the scan when a pre-defined threshold (typically 100 HU) is reached in the target area; some scanners will display a time-density curve at the pre-defined anatomic location to the technologist and require a manual start of the exam. Either mechanism optimizes timing of the scan to the cardiac output and circulatory time of the individual participant and is **strongly preferred** over a fixed-time delay exam. Late arterial phase scanning should typically commence 5 to 10 seconds after peak enhancement in the upper abdominal aorta at the level of the celiac axis. In the unlikely event that fixed time delay needs to be used, an empirical delay of 25 to 30 seconds may work for most participants.

10.1.2.3.3 Portal vein phase

Imaging characteristics include the following:

- Fully enhanced portal vein;
- Peak liver parenchymal enhancement;
- Early contrast enhancement of hepatic veins.

The time window of opportunity to acquire images of the hepatic parenchyma during portal vein and equilibrium (delayed) phase is relatively wide. Portal vein phase images should typically be acquired 35 to 55 seconds <u>after initiation</u> of late arterial phase.

10.1.2.3.4 Equilibrium/Delayed phase
Imaging characteristics include the following:

- Variable appearance;
- >120 seconds after initial injection of contrast.

The time window of opportunity to acquire images of the hepatic parenchyma during portal vein and equilibrium (delayed) phase is relatively wide. Equilibrium phase images should typically be acquired 120 to 180 seconds post initial contrast injection.

Table 4: Minimum technical specifications
for multiphasic contrast-enhanced CT of the liver

Feature	Specification	Comment
Scanner type	*Multidetector row scanner*	
Detector type	*Minimum of 8 detector rows*	*Need to be able to image entire liver during brief late arterial phase time window*
Reconstructed slice thickness	*Minimum of 5 mm reconstructed slice thickness*	*Thinner slices are preferable, especially if multiplanar reconstructions are performed*
Injector	*Power injector, preferably dual chamber injector with saline flush*	*Bolus tracking desirable*
Contrast injection rate	*No less than 3mL/sec of contrast, better 4–6 mL/sec with at least 300 mg I /mL or higher concentration, for dose of 1.5 mL/kg body weight*	
Dynamic phases on contrast-enhanced MDCT (comments describe typical hallmark image features)	*0) OPTIONAL: Pre-contrast*	*Strongly encouraged after use of ethiodol in context with ablation*
	1) MANDATORY: Late arterial phase	*1) Artery fully enhanced, beginning contrast enhancement of portal vein*
	2) MANDATORY: Portal venous phase	*2) Portal vein enhanced, peak liver parenchymal enhancement, beginning contrast enhancement of hepatic veins*
	3) MANDATORY: Equilibrium/Delayed phase	*3) Variable appearance, >120 sec after initial injection of contrast*
Dynamic phases (timing)	*Bolus tracking preferred over timing bolus for accurate timing*	

10.1.3 MR Imaging

10.1.3.1 General MRI parameters are outlined in the table and text below.

- Field strength of 1.5 Tesla or greater.

- Imaging must be performed with a specialized torso array coil or other local coil combinations appropriate for body imaging. Body coil for signal reception is not acceptable.

- Image slice thickness should be ≤ 10 mm.

- Field of view (FOV) as appropriate for given patient body habitus.

- Matrix for T1 and T2 weighted images should be no less than 256 (frequency) x 128 (phase).

- Diffusion-weighted imaging may be used by sites per institutional protocol but is not required by this trial protocol. If sites perform this type of imaging, the use of lower resolution matrices is acceptable.

- For axial imaging, phase encoding should be anterior–posterior.

- For contrast-enhanced scanning, standard extracellular gadolinium chelates that do not have dominant hepatobiliary excretion should be used at a dose of 0.1 mmol/kg to a maximum of 20 mL.

- Injection rate should be 2 cc/sec, and all injections must be followed by a saline flush of 30 cc. Peripheral IV access is preferred.

10.1.3.2 Abdominal MRI

Contrast-enhanced imaging with a standard extracellular gadolinium chelate that does not have dominant hepatobiliary excretion is required for MRI. Scanning protocol should be per institutional standards, but should include at a minimum: pre-contrast (mandatory) and dynamic post-extracellular-gadolinium T1-weighted (T1W) gradient echo sequence (3D preferable), T2W (with and without FAT SAT), T1W in and out of phase imaging. The inclusion of other imaging techniques/planes is acceptable per institutional/imaging center's standard, and all imaging performed will be collected for the purpose of this trial.

10.1.3.3 Guidelines for Multiphasic Contrast-Enhanced MR Imaging

HCC has a range of presentations on MRI. The most common is a circumscribed mass that may be inconspicuous on pre-contrast T2W and T1W imaging. The strongest diagnostic images are the multiple contrast-enhanced timed T1W images. The key elements necessary to achieve optimal diagnostic sensitivity and specificity are the following:

- 3D Gradient Echo (GRE) fat-suppressed acquisitions acquired with identical parameters throughout the pre- and post-contrast series. 3D volumetric imaging is preferred, but multiplanar 2D imaging is acceptable.

- Pre-contrast T1W images:
  - 2D or 3D in- and opposed-GRE;

o 3D GRE (depending on scanner platform used: Vibe; Lava-xv; Thrive) with fat suppression.
- Parameters identical to the post-contrast 3D GRE sequence:
  o Avoid misinterpreting a nodule intrinsically with high T1W signal as an enhancing mass, as can be seen in regenerating nodules or dysplasia. HCC rarely has high T1 signal, but can. Comparison must always be made between the pre-contrast and arterial phase images.
  o Examine the in/out-of-phase images for fat. Occasionally the T1 signal may be lower than adjacent liver on fat-suppressed 3D GRE due to lipid, < 10% incidence.

10.1.3.3.1 Pre-contrast: Mandatory
Non-contrast imaging through the liver prior to contrast-enhanced imaging is **mandatory**.

10.1.3.3.2 Arterial phase
- Imaging characteristics include the following:
  o Fully enhanced hepatic artery and branches;
  o Early contrast enhancement of portal vein;
  o Lack of enhancement of the hepatic venous system.
- Acquisition of a properly timed late arterial phase is the most technically challenging and diagnostically critical element of the dynamic liver examination.
- Both the MRI system and the technologist training must be considered for optimized arterial phase imaging.
- Technologists are reminded to carefully go over patient instructions prior to scanning, especially as they pertain to breathing instructions, to minimize artifact on the study.
- Using set (empirical) timing delays from the start of the injection will be associated with a large range of contrast arrival times (from < 12 to > 30 seconds range timed from the start of the contrast injection to the arrival in the hepatic artery) and **will not provide the most optimized method.**
- HCC will transiently enhance over a period of 5 to 10 seconds above the adjacent liver parenchyma signal, therefore the timing is critical and is optimized if:
  o The gadolinium bolus is injected in as short a time as possible;
  o Peak HCC enhancement must be aligned in time with the time during the 3D GRE acquisition that accumulates low k-space frequencies (e.g., linear order = align at middle of breath hold; low to high ordering = align at beginning of breath hold, which means adding a longer delay time to account for this).

- **The following is required for optimized timing in order to achieve an arterial-phase breath hold liver examination (ABLE):**
  - Dual chamber power injector;
  - Injection of contrast at 2 cc/sec (measured to the recommended dose by weight; standard extracellular gadolinium chelates that do not have dominant hepatobiliary excretion should be used at a dose of 0.1 mmol/kg to a maximum of 20 mL);
  - Chase with saline at 2 to 3 cc/sec x 30 cc;
  - Start a real-time reconstruction high–speed, low-quality coronal (suggested) GRE (e.g., care-bolus) at the start of the infusion for bolus monitoring;
  - Field of view on coronal set to allow visualization of the heart, mediastinum, and centered on the diaphragm to visualize the celiac axis;
  - Technologist trained to recognize filling of the right side of heart, pulmonary artery, left heart, aorta, in preparation for recognizing bolus arrival;
  - Stop the bolus imaging and start timing when the contrast arrives at the celiac axis (diaphragm);
  - Count 8 sec if using a linear ordered 16 to 18 sec breath hold acquisition time 3D GRE (based on data looking at perfusion kinetics of arterial enhancing tumors);
  - During this time give the breathing commands and train the technologists to provide adequate time for the participant to complete the breath hold maneuver 2 to 3 sec prior to initiation of the sequence to allow the participant to complete following the command and stop all voluntary movements;
  - Start the arterial phase acquisition.
- An approximate guide to show that an ideal acquisition was obtained usually shows the hepatic artery fully enhanced and the portal veins centrally just enhancing to well enhanced; hepatic veins show no enhancement.

10.1.3.3.3 Portal venous phase (AKA venous and blood pool phase)
- Imaging characteristics include the following:
  - Fully enhanced portal vein;
  - Peak liver parenchymal enhancement;
  - Early contrast enhancement of hepatic veins.
- Images captured just after the hepatic veins have filled with contrast. Timing is less critical and can be acquired (35 to 55 sec after initiation of late arterial phase scan). Typically the portal venous phase is started one or two breathing cycles after completion of late arterial phase.

- This provides adequate time for the participant to regain their breath before being asked to perform the next breath hold and reduce motion effects from poor breath holding due to rushing this second enhanced acquisition.
- This acquisition provides optimal visualization for portal or superior mesenteric vein (SMV) thrombosis and varices.

10.1.3.3.4 Equilibrium phase (AKA extracellular, interstitial, or delayed phase)
- Imaging characteristics include the following:
  - Variable appearance;
  - >120 seconds after initial injection of contrast.
- Timing less critical and can be acquired at 120 to 180 sec post injection as a third breath hold. This provides adequate time for so-called HCC "wash-out".
- The signal in the HCC is lower in this phase due to a combination of lower vascular volume and interstitial uptake than in the adjacent liver.
- The margins of the HCC enhance, forming an apparent thin pseudo-capsule.

**Table 5: Overview technical specifications
for multiphase contrast-enhanced MRI of the liver**

Feature	Specification	Comment
Scanner type	*1.5 T or greater magnetic field strength*	*Low-field magnets not suitable*
Coil type	*Phased-array multichannel torso coil*	*Unless patient-related factors precludes use (e.g., body habitus)*
Gradient type	*Current generation high speed gradients (providing sufficient coverage)*	
Slice thickness	*5 mm or less for dynamic series; 8 mm or less for other imaging*	
Injector	*Dual chamber power injector recommended*	*Bolus tracking desirable*
Contrast injection rate	*2–3 mL/sec of extracellular gadolinium chelate that does not have dominant hepatobiliary excretion*	*Preferably resulting in vendor-recommended total dose*
Required non-dynamic sequences	*T1W in and out of phase imaging* *T2W (per institutional standard, not STIR)*	*Optional diffusion imaging*
Dynamic phases on contrast-enhanced MRI (comments describe typical	*0) MANDATORY:* *Pre-contrast T1W*	*0) Do not change scan parameters for post contrast imaging*
	1) MANDATORY:	*1) Artery fully enhanced, beginning*

hallmark image features)	*Late arterial phase*	*contrast enhancement of portal vein*
	2) MANDATORY: Portal venous phase	*2) Portal vein enhanced, peak liver parenchymal enhancement, beginning contrast enhancement of hepatic veins*
	3) MANDATORY: Equilibrium/delayed phase	*3) Variable appearance, > 120 sec after initial injection of contrast*
Dynamic phases (timing)	*The use of a bolus tracking method for timing contrast arrival for late arterial phase imaging is preferable. Portal venous phase (35–55 sec after initiation of late arterial phase scan), equilibrium/delayed phase (120–180 sec after initial contrast injection)*	
Breath holding	*Max length of series requiring breath hold should be about 20 sec with a minimum matrix of 128 x 256*	*Compliance with breath hold instructions very important, technologists need to understand the importance of participant instruction before and during scan*

10.2   Images Preparation for Submission

10.2.1 For TRIAD Submission: The preferred image transfer method is via TRIAD, a software application that ACR provides for installation on a site's PC. One or several computers of choice within the institutional "firewall" and on the institutional network may be equipped with TRIAD software; Internet access is also required. The TRIAD application can then be configured as a DICOM destination on either scanner(s) and/or PACS system for direct network transfer of study related images into the TRIAD directory. When properly configured, the TRIAD software anonymizes, encrypts, and performs a lossless compression of the images before they are transferred to the ECOG-ACRIN image archive in Philadelphia. Once equipment-readiness has been determined, imaging personnel from the ACR Imaging Core Laboratory will coordinate installation and training for the software.

For more information, contact: TRIAD-support@phila.acr.org or call 215-940-8820.

10.2.2 For Submission Via Media: In the event that the transfer of image data is not available via TRIAD, images may also be sent on a CD/DVD-ROM to the ACR core lab for transfer to the image archive. All image data submitted to the ACRIcore lab must be in DICOM format.

The C0 Form (for all CT images) and MW Form (for MR images) must accompany media submissions. PDF versions of the transmission worksheets are available for downloaded at www.acrin.org/6690_imagingmaterials.aspx

10.2.3 Images may be mailed to:

American College of Radiology Imaging Core Laboratory
MR/CT Core Laboratory
Attn: ACRIN 6690
1818 Market Street 16th floor

10.3   Quality Control at ACR Core Lab

The ACRIN 6690 protocol explicitly requires participating centers to meet technical specifications for uniformity to the CT and MR scanners used to obtain images. Additionally, specific parameters for image acquisition are outlined in the protocol and provided on the ACRIN web site. This routine imaging will occur only at UNOS-accredited transplantation centers that have successfully demonstrated their competence during the site qualification process. ECOG-ACRIN will provide ongoing quality control through the ACR core lab. Specifically, the ACR core lab will receive and conduct quality control evaluations on images to help centers maintain trial grade quality. The ACR core lab specialists will provide feedback to sites, especially during early trial imaging to ensure high-quality imaging per protocol. However, re-imaging will not be requested once the trial is under way. Furthermore, the protocol contains specific language for image capture (how to scan) and diagnostic (how to read), with specific reporting requirements.

10.4   Provision of Multiplanar (Sagittal or Coronal) Images

As the primary aims of this study necessitate the recognition of borders of liver segments, which are important for lesion localization, axial plane imaging will be mandatory since most readers are most familiar with that imaging plane. Dynamic imaging in the axial plane is customary in most radiology departments. However, the trial team recognizes that (secondary) sagittal or coronal reconstructions may be of value in particular during the radiology-pathology correlation. Primary image acquisition in the sagittal plane may pose challenges due to inherent physics of image acquisition (MRI), possibly increasing likelihood of wrap-around artifacts, etc. The trial protocol therefore asks that sagittal or coronal reconstructions (per preference of local pathologist) of the dynamic images be performed on the scanner consoles or separate 3D workstations. Sagittal and/or coronal images should be provided to ECOG-ACRIN Diagnostic Imaging Headquarters/ACR Imaging Core Lab together with the other required images for each participant. The reconstructed images are made available to the pathologist for correlation at time of explant pathology analysis through institutional PACS or other image display, depending on departmental preference. The quality of such secondary sagittal and coronal reconstructions is expected to be very good when based on isotropic CT data and reasonable when based on (an)isotropic dynamic 3D MRI data.

10.5   Image Interpretation by Local and Central Reader Studies

For the main trial: Image interpretation will be performed by local site radiologists in accordance with new standardized diagnostic class reporting as established by expert consensus at the HCC Consensus Conference, Chicago, IL, 2008 (please refer to Tables 1 and 2, as well as Figure 1, under Section 2.5), which has been adapted for use in this trial. These "local interpretations" will be used to establish the performance of the new diagnostic criteria in clinical practice ("in the field"), as per the study's Secondary Aim 3.2.4. Local interpreting radiologists also record which modality was used to make the (initial) diagnosis used to obtain priority MELD points. They will record whether prior imaging was available at the time of initial diagnosis and are asked to submit these images to ECOG-ACRIN since several of the lesion classes are based on growth criteria that can only be accurately assessed in comparison to prior studies.

Radiologists who typically interpret clinical multiphasic contrast-enhanced liver CT and MR images at an UNOS-accredited transplant center are considered competent to interpret imaging studies for this trial. In order to minimize or avoid "cross-contamination" of the reads, centers need to internally designate "CT" and "MR" readers for the purpose of this trial: the same radiologists must not interpret

CT *and* MR images of the **same** patient over time, much less of the same patient for a single trial imaging time point. In order to accomplish this goal, centers would typically need a minimum of 2 different radiologists as readers for this trial. Should a clinical need for retrospective comparison with complementary imaging from a previous time point arise during interpretation of the SOC imaging, the radiologist should strive to complete the reader form for the SOC modality first before such comparison is undertaken.

Lesions will be measured and growth will be assessed based on comparison between most-recent prior imaging (within 90 to 180 days before) and current time point. Prior imaging (180 days and less) must be available at the time of local radiologist assessment.

No more than five (5) Class 4 lesions will be reported for each participant per time point of this trial. Readers are asked to report the five (5) **most prominent or most concerning** Class 4 nodules. No other specific rules about how to make that determination are dictated; this is left up the best judgment of the (experienced) reader. Radiologists will be asked to note if and when more than five (5) Class 4 lesions are present. An actual precise count of remaining/additional Class 4 lesions will not need to be reported.

The study-related complementary imaging will be interpreted and reported to ECOG-ACRIN Diagnostic Imaging Headquarters via the data collection forms and, depending on site standard operating procedures, an official radiology report may be furnished. The local site will use the results of this interpretation of the complementary imaging for clinical care per the determination and judgment of the transplant team.

All imaging studies will be transferred to the ACR core lab, and the matching pair of imaging studies closest in time to the explant date will be interpreted by a minimum of two blinded, expert, central readers. A consensus approach will be used in cases of discrepant expert interpretations, and this consensus diagnosis will be entered into the database. Expert readers also will record image quality and compliance with protocol specifications.

NOTE: Institutions are requested to not only upload this baseline imaging study to ECOG-ACRIN Diagnostic Imaging Headquarters but also the most-recent prior imaging study (same-modality—MR and/or CT), if available as noted above.

10.6   Reporting of Data

Imaging findings will be recorded on modality-specific (CT and MRI) reader forms on a per lesion basis (reporting every Class 5 lesion and up to five [5] Class 4 lesions). There will be separate, individual reporting required for all OPTN Class 5 lesions (HCC) or OPTN Class 4 lesions (dysplastic nodules, small atypical HCC) by location, size, and specific imaging characteristics. If the participant has undergone prior local ablative therapy, reporting will be done on a per-nodule basis using post-ablation forms for those nodules located in the treated liver (whole liver = all nodules; partly treated liver = some nodules; see Appendix VI for further explanation).

11.0   ADVERSE EVENTS REPORTING

Prompt reporting of AEs is the responsibility of each investigator, clinical RA, and/or nurse engaged in clinical research. Anyone uncertain about whether a particular AE should be reported should contact the ECOG-ACRIN Diagnostic Imaging Headquarters at 215-574-3183 for assistance.

Adverse events (AEs) meeting the criteria in the tables below, including all serious adverse events (SAEs) will be reported to the CTEP Adverse Event Reporting System (CTEP-AERS) and to Cancer Imaging Program (CIP) as directed in this section.

CTEP-AERS is an electronic, internet based expedited Adverse Event reporting system operated by NCI/CTEP. It is generally used to capture and disseminate information on relatively significant Adverse Events, based upon trial stage, expectedness, severity, and attribution. However, it may be used to report adverse events of all types if CTEP-AERS reporting is required per protocol.

The electronic-CTEP-AERS AE system is to be used for all 'expedited reporting' events as defined herein. If the system is temporarily unavailable, a paper and telephone/FAX based process is provided herein. Expedited AE data is to be re-submitted via the electronic CTEP-AERS system as soon as is possible in cases where temporary e-CTEP-AERS unavailability has necessitated manual capture and submission.

11.1   General Definitions

Adverse Event (AE): For the purpose of this study, an Adverse Event is an untoward medical condition experienced by a study participant during the Adverse Event reporting period defined in Section 11.7 Table A of the protocol, or by applicable guidance, regulation, or policy. An AE is any unfavorable or unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with participation in the study, regardless of exposure to an agent or procedure, and regardless of whether it is considered to be caused by the agent, device, or process under investigation.

If there is thought to be a conflict between the protocol and a regulatory or guidance source, consult the CIP Clinical Trials Branch. If a decision must be made pending final clarification, the stricter requirement should be applied.

Serious Adverse Event (SAE): An SAE is defined as any untoward medical occurrence that meets any one of the following criteria:
- Results in death or is life-threatening at the time of the event
- Requires inpatient hospitalization, or prolongs a hospitalization

  NOTE: Hospitalization for expedited AE reporting purposes is a medically required inpatient hospital stay equal to or greater than 24 hours. Hospitalization is used as an indicator of the seriousness of the adverse event and should be reserved for situations where the adverse event truly fits this definition, and not for hospitalizations associated with less serious events. For example, a hospital visit where a subject is admitted for observation or minor treatment (e.g., hydration), and released in less than 24 hours, generally is not intended, in and of itself, to qualify as an SAE. Furthermore, hospitalization for pharmacokinetic sampling, is not an AE, and therefore is not to be reported either as a routine AE or in an expedited report. As in all cases, if there is any doubt as to reporting an event, the CIP SAE reporting desk help line is to be consulted promptly.

- Results in a persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect (in a participants offspring)
- Requires intervention to prevent any of the above, per the investigator/sponsor

Life-Threatening Adverse Event: A life-threatening AE is any adverse event that places the study participant, in the clinical opinion of the investigator, at immediate risk of death.

All SAEs are to be followed by the investigator until resolution, stabilization, scientifically and clinically satisfactory explanation as to attribution and etiology or until subject is lost to follow up.

CTEP ADVERSE EVENT REPORTING SYSTEM (CTEP-AERS): CTEP-AERS is a web-based system created by NCI for electronic submission of SERIOUS and/or UNEXPECTED AE reports & is to be used in this study. All CIP trials must use CTEP-AERS for expedited reporting of AEs.

Commercial Agent: A commercial agent is any agent marketed and obtained from a commercial source, and used under approved label indication. For example, the extracellular gadolinium contrast agent without dominant hepatobiliary excretion used in this study is commercial agent.

11.2   AE Reporting Requirements

The list of AEs, and the characteristics of an observed AE [see Section 11.4], will determine whether the event requires expedited (via electronic-CTEP-AERS) reporting **in addition** to routine reporting. For this study CTEP-AERS reporting will be done electronically.

11.3   Adverse Event List(s) for Study Procedures

11.3.1   Expected Adverse Events Associated With Standard of Care Practice

Any AE that is a result of standard-of-care practice will be reported and managed per the institution's policies and procedures.

11.3.2 Expected Adverse Events Associated With CT Scan

- Discomfort;
- Claustrophobia.

NOTE: As of July 14, 2008, FDA released a preliminary public health notification of possible malfunction of electronic medical devices caused by CT scanning. Site should use CT scout views to determine if implanted or externally worn electronic medical devices are present and if so, their location relative to the programmed scan range.

11.3.3   Expected Adverse Events Associated With Oral and IV Iodine Contrast

A history of contrast allergy or asthma excludes potential participants from this study unless pre-treated per institutional standard or ACR guidance. The injection may cause discomfort and irritation. The iodine-containing contrast used for CT scanning may cause significant contrast reactions in about one in a thousand participants. Severe reaction is seen in as low as 4/10000 to as high as 2/1000 depending on the type of contrast used. Fatal reactions are exceedingly rare and have been reported in 1:170,000 irrespective of the type of contrast used. The most common reactions are nausea, vomiting, hives, or rash. The risk of death is less than 1 in 10,000.

11.3.4   Expected Adverse Events Associated With MRI

- Anxiety/stress;
- Claustrophobia;

- Discomfort.

11.3.5   Expected Adverse Events Associated With Extracellular Gadolinium Contrast Agent that Does Not Have Dominant Hepatobiliary Excretion

- Nausea;
- Headache;
- Hives;
- Temporary low blood pressure;
- Allergic reaction;
- *Rare, but Serious:* Kidney impairment, details follow.

Precautions should be exercised for patients with severely impaired renal function or hemolytic anemia. The very unlikely possibility of a reaction, including anaphylactic or cardiovascular reactions, should be considered especially for patients with a known sensitivity to gadolinium or history of asthma.

Nephrogenic Systemic Fibrosis (NSF) or Nephrogenic Fibrosing Dermopathy (NFD), kidney disorders, may occur in patients with moderate to end-stage kidney disease (glomerular filtration rate < 30 mL/min/1.73m^2) and in patients with renal dysfunction due to the hepatorenal syndrome or in the perioperative liver transplantation period after they have had a MRI scan with extracellular gadolinium-based MR contrast agents (GBMCA) that do not have dominant hepatobiliary excretion.

NSF causes fibrosis of the skin and connective tissues throughout the body. Patients develop skin thickening that may prevent bending and extending joints, resulting in decreased mobility of joints. NSF usually starts in the lower extremities. Fibrosis can also develop in the diaphragm, muscles in the thigh and lower abdomen, and lung vessels. Reference: FDA/Center for Drug Evaluation and Research. May 23, 2007 www.fda.gov/cder/drug/infopage/gcca/qa_200705.htm

11.3.6   Expected Adverse Events Associated With IV Needle Placement

- Hemorrhage (hematoma at the injection site);
- Phlebitis;
- Minor discomfort;
- Bleeding;
- Infection;
- Bruising.

11.3.7   Expected Adverse Events Associated With Radiation Exposure From CT Scan
While the radiation dosage for CT scanning varies with the part of the body being scanned, the exposure (effective dose) for a multiphasic CT of the upper abdomen is typically in the range of 15 to 25 mSv. Actual exposure during a given examination depends on many factors, especially individual patient size; therefore, radiation exposure for these examinations can vary from patient to patient and be smaller or larger than the average dose range provided above. The CT examinations used for this trial are limited to the upper abdomen and typically do not directly irradiate organs with

the highest radiosensitivity. However, there is some bone marrow exposure and some exposure to other radiosensitive organs (lung, breast) from scattered radiation.

The total [cumulative] radiation exposure depends on the dose given during any single examination and the number of serial imaging time point update scans required until the patient reaches transplantation. The following table provides individual and cumulative doses broken down by average number of CT examinations expected by UNOS region based on historic data of time from listing to transplant:

Table 6: Single and cumulative dose/exposure ranges
for CT examinations during ACRIN 6690

Research CT scan time point (total # of CT examinations)	Dose range per exam [mSv]	Cumulative dose range* [mSv]	UNOS region[†] (based on typical time to transplant)
Baseline (1)	15–25	15–25	ALL
90-day update (2)	15–25	30–50	Regions 1, 2, 4, 5, 7, 8, 9
180-day update (3)	15–25	45–75	Region 1

* *If a patient has ablative therapy while on the waitlist, a CT scan is required post-treatment. If this CT scan does not fall in line with the 90-day time points, an additional CT scan will be performed per protocol guidelines. The dose range for this additional CT scan would be equivalent to 15 to 25 mSv.*

† *Based on historic UNOS data from 2008, the longest time point from HCC-exception point listing to transplant is 208 days across all regions.*

Participating sites are strongly encouraged to use all reasonable methods available to lower radiation exposure while maintaining adequate image quality, such as lowering kVp settings in suitably small patients, use of dose modulation technology provided on many scanners, and tight limit on anatomic coverage of the body area of interest.

The maximum exposure expected from a single scan is well below a dose where direct [deterministic] effects of radiation, such as erythema or hair loss, would be observed. Such effects are not expected to occur in this trial. Overall risk from radiation exposure needs to be considered in the clinical context, i.e., the disease a patient is suffering from and the associated limitation in life expectancy. Patients included in this trial typically suffer from advanced-stage chronic liver disease as well as a T2-stage liver cancer. These conditions can significantly limit the patient's life expectancy, even if transplantation occurs. Based on post-transplant survival data for recipients of deceased donor liver transplants, with an approved T2 HCC exception covering the time period from 2/28/02 to 12/31/08, approximately 32% of patients (approximate 95% confidence interval, 31% to 34%) had died 5 years post-transplant (see Figure 3; data based on personal communication of Dr. Christoph Wald with Erick Edwards (UNOS), 8/20/10).

Figure 3:

Deceased Donor Liver Transplants: 2/28/02-12/31/08
Approved Stage T2 HCC Exceptions
Post-Transplant Patient Survival
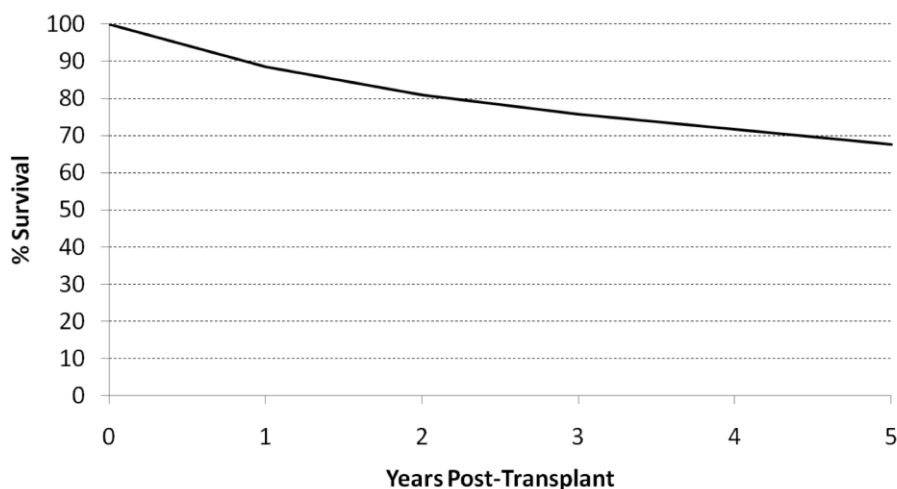
*Based on OPTN data as of 8/13/2010. Deaths supplemented with data from Social Security Death Master File.
N=6003 Stage T2 HCC transplants

Considering the significant mortality of the trial patient population, the added lifetime [cancer] risk to the patient from the radiation exposure in this study would appear to be negligible. It should be noted that the type of CT scanning required for this trial is considered standard of care in many institutions in the United States.

11.4   Adverse Event Characteristics

Expected Adverse Event: An expected AE is an event that is listed in the protocol or the Investigator's Brochure.

Unexpected Adverse Event: An unexpected AE is an event that is NOT listed in the protocol or the Investigator's Brochure.

Attribution: Attribution is a clinical determination, by the investigator, as to whether an AE is related to a medical treatment or procedure. Attribution categories are:

- **Definite:** The AE is **clearly related** to a treatment or procedure
- **Probable:** The AE is **likely related** to a treatment or procedure
- **Possible:** The AE **may be related** to a treatment or procedure
- **Unlikely:** The AE is **likely unrelated** to a treatment or procedure
- **Unrelated:** The AE is **clearly not related** to a treatment or procedure

<u>Note</u>: For this study, attributions are in terms of the study related procedures (i.e. study imaging, contrast injection, etc.)

Grade: Grade denotes the **<u>severity</u>** of the AE. An AE is graded using the following categories:

- **Mild**
- **Moderate**
- **Severe**
- **Life-threatening or disabling**
- **Fatal**

   NOTE: Severity is graded on a Common Terminology Criteria for Adverse Events (CTCAE) based scale for each CTCAE event. For example, an abnormal hemoglobin value is graded for severity from 1 to 5 [death] based upon where that value falls on the CTCAE scale of abnormal Hemoglobin values. "Severity" is NOT the same as "Seriousness," which is an overall assessment that determines reporting requirements.

11.5   CTCAE Term (AE description and grade)
The descriptions and grading scales found in the NCI CTCAE version 4.0 will be utilized for AE reporting. All appropriate clinical areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov).]

11.6   Expedited Adverse Event Reporting
Expedited AE reporting for this study must use electronic CTEP-AERS, accessed via the CTEP home page (http://ctep.cancer.gov). Site personnel will be trained in required AE identification and reporting procedures. These requirements are briefly outlined in Section 11.7 of the protocol.

24 Hour Telephone Reporting Instructions
Any AE/SAEs that require 24-hour notification are reported as follows:

   1. **AEMD Help Desk at 301-897-7497**

   2. **CIP–SAE Reporting Line: (301) 897-1704**
      - The CIP-SAE reporting line is staffed Monday through Friday from 7:30am – 7:30pm ET (Eastern Time).
      - AE/SAEs may be reported via voicemail during off hours.
      - A TRI contact for AE/SAE reporting will return your call within 24 hours.

   Generally the following details are essential to initiate an AE/SAE report:
      - Name of person reporting the AE/SAE and telephone number
      - Institution name and institution number
      - Protocol title and number
      - Participant's case number and initials
      - Site principal investigator name and telephone number
      - Date and time of the AE/SAE
      - Date and time you learned of the AE/SAE
      - Brief description of the AE/SAE
      - Site principal investigator's assignment of the grade of the adverse event
      - Site principal investigator's assignment of the attribution of the adverse event (do not delay initial report if not available)

   2. **ACRIN–AE/SAE Reporting Line: (215) 717-2763**

- The ACRIN–AE/SAE reporting line is monitored by the ACRIN AE Coordinator: Monday through Friday from 8:30am – 4:30pm ET.
- AE/SAEs may be reported via voicemail during off hours.
- The ACRIN AE Coordinator will return your call within 24 hours.

Generally the following details are essential to initiate an AE/SAE report:
- Name of person reporting the AE/SAE, telephone number
- Institution name and institution number
- Protocol title and number
- Participant's case number and initials
- Site principal investigator's name and telephone number
- Date and time of the AE/SAE
- Date and time you learned of the AE/SAE
- Brief description of the AE/SAE
- Site principal investigator's assignment of the grade of the adverse event
- Site principal investigator's assignment of the attribution of the adverse event (do not delay initial report if not available)

IMPORTANT: After the 24 hour contact to CIP and ACRIN-AE/SAE reporting lines, an electronic CTEP-AERS must be submitted per the protocol-specific requirements or the regulatory reporting timelines, if not specified in the protocol.

In the rare event when Internet connectivity is disrupted a 24-hour notification is to be made to NCI by telephone at: 301-897-7497, or 301-897-7402 for CIP studies. An electronic report **MUST** be submitted immediately upon re-establishment of internet connection.

11.7 Late Phase 2 and Phase 3 Studies: Expedited Reporting Requirements for Adverse Events [1]

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)
NOTE: Investigators **MUST** immediately report to the sponsor (NCI) **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)
An adverse event is considered serious if it results in **ANY** of the following outcomes:
1) Death
2) A life-threatening adverse event
3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

ALL SERIOUS adverse events that meet the above criteria **MUST** be immediately reported to the NCI via CTEP-AERS within the timeframes detailed in the table below.

Hospitalization	Grade 1 Timeframes	Grade 2 Timeframes	Grade 3 Timeframes	Grade 4 & 5 Timeframes
Resulting in Hospitalization ≥ 24 hrs	10 Calendar Days			24-Hour 5 Calendar Days
Not resulting in Hospitalization ≥ 24 hrs	Not required		10 Calendar Days	

> **NOTE**: Protocol specific exceptions to expedited reporting of serious adverse events are found in the Specific Protocol Exceptions to Expedited Reporting (SPEER) portion of the CAEPR
>
> **Expedited AE reporting timelines are defined as:**
> - "24-Hour; 5 Calendar Days" - The AE must initially be reported via CTEP-AERS within 24 hours of learning of the AE, followed by a complete expedited report within 5 calendar days of the initial 24-hour report.
> - "10 Calendar Days" - A complete expedited report on the AE must be submitted within 10 calendar days of learning of the AE.
>
> [1]Serious adverse events that occur more than 10 hours (10 radioactive half-lives of the agent) after the single administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:
>
> **Expedited 24-hour notification followed by complete report within 5 calendar days for:**
> - All Grade 4, and Grade 5 AEs
>
> **Expedited 10 calendar day reports for**:
> - Grade 2 adverse events resulting in hospitalization or prolongation of hospitalization
> - Grade 3 adverse events
>
> Effective Date: May 5, 2011

11.8   Expedited AE Reporting Timelines Defined:

- "24 hours; 5 calendar days" – The investigator must initially report the AE via a telephone report to NCI/CIP and ACRIN within 24 hours of learning of the event, followed by a complete CTEP-AERS report within 5 calendar days of the initial 24-hour report.

- "10 calendar days" - A complete CTEP-AERS report on the AE must be submitted within 10 calendar days of the investigator learning of the event.

  Use the NCI protocol number and the protocol-specific patient ID assigned during trial registration on all reports.

11.9   Routine Adverse Event Reporting

The following adverse events **must** be reported in routine study data submissions (i.e. ACRIN AE case report form).

- Grade 3 Expected and Unexpected AEs with an attribution of **possible, probable or definite** require routine reporting. [See Section 11.7 for CTEP-AERS reporting requirements].

- Grade 4 Expected and Unexpected AEs with an attribution of **possible, probable or definite** require routine reporting. [See Section 11.7 for CTEP-AERS reporting requirements].

- Grade 5 Expected and Unexpected AEs with an attribution of **possible, probable or definite** require routine reporting. [See Section 11.7 for CTEP-AERS reporting requirements].

AEs reported through CTEP-AERS must also be reported in routine study data submissions.

11.10   Local Institutional Review Board (IRB) Reporting

Refer to the IRB policies and procedures for AE reporting.

12.0   ETHICAL CONSIDERATIONS

This study is to be conducted according to US and international standards of Good Clinical Practice (International Conference of Harmonisation [ICH] guidelines), applicable government regulations, and ECOG-ACRIN research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Ethics Committee (EC) or Institutional Review Board (IRB) for formal approval of the study conduct. The decision of the EC/IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided before implementation of the study.

The investigator will provide the institution's Federalwide Assurance (FWA) number, along with the IRB approval letter and copy of the IRB-approved ICF. The investigator will provide a copy(s) of IRB approval letter(s) for any amendment(s), and copy(s) of annual renewal(s).

All study participants in this study will be given an IRB-approved, site-specific ICF describing the study and providing sufficient information for participants to make informed decisions about their participation in this study (see accompanying document for an ICF template). The ICF will be submitted along with the protocol for review and approval by the EC/IRB. The study participant MUST be consented with the EC/IRB-approved ICF before the participant is subjected to any study procedures. The approved ICF MUST be signed and dated by the study participant or legally acceptable representative and the investigator-designated research staff obtaining the consent. Any revisions to the ICF at any time during the trial will need to be submitted to the IRB for approval with submission to ECOG-ACRIN Diagnostic Imaging Headquarters, Protocol Development and Regulatory Compliance Department.

13.0   CONFLICT OF INTEREST

Any investigator and/or research staff member who has a conflict of interest with this study (such as patent ownership, royalties, or financial gain greater than the minimum allowable by their institution) must fully disclose the nature of the conflict of interest in accordance with ACRIN Conflict of Interest policies and applicable federal, state, and local laws and regulations.

14.0   PUBLICATION POLICY

Neither complete nor any part of the results of the study obtained under this protocol, nor any information provided to the investigator for the purposes of performing the study, will be published or passed on to any third party without the consent of ACRIN. Any investigator involved in this study is obligated to provide ACRIN with complete test results and all clinical data obtained from the participants in this protocol. Investigators will follow the ACRIN Publication Policy (available online at www.acrin.org/PublicationsPolicy.aspx).

15.0   INSTITUTIONAL MONITORING AND AUDITS

The investigator will permit study-related monitoring, auditing, and inspections of all study-related documents by the EC/IRB, government regulatory agencies, and ECOG-ACRIN Diagnostic Imaging Headquarters. The investigator will ensure the capability for inspection of all participating sites' study-related facilities (e.g. imaging centers, satellite sites). The investigator will allocate adequate time for these activities, allow access to all study-related documents, and provide adequate space to conduct these visits.

15.1   Monitoring

Monitoring ensures data integrity and quality, as well as that the rights, safety, and well-being of the participants are protected. Monitoring also makes certain that the trial is in compliance with the currently approved protocol/amendments, with GCP and applicable regulatory requirements. It ensures the reported trial data are accurate, complete, and verifiable from source documents. Institutional monitoring will be implemented at several different time points during the conduct of the study. Case report forms (CRFs) and source documents of study participants enrolled at each site will be reviewed. In addition, the initial regulatory documents and any revised regulatory documents will also be monitored.

15.2   Audits

All participating institutions with study participants will be audited. The timing of initial on-site audit will depend upon several factors, including the rate of accrual (both study-wide and site enrollment), the number of evaluable participants at an individual site, the status of the protocol and pending amendments, and status of the site monitoring.

Generally, audits will be conducted after the number of evaluable participants reaches 20% of targeted accrual, either study-wide and/or at the site level. Audits are typically scheduled to occur at least 3 months after an institution has been monitored, providing that monitoring did not identify issues that mandate an immediate audit visit. This schedule may be altered in the event of pending protocol amendments. Closure of the study to accrual will trigger auditing of all participating institutions not yet audited which may affect the conduct of the trial. Additionally, site-specific circumstances may prompt an audit visit at any time.

Subsequent audits will be scheduled per the outcome of the initial audit. Audits can be conducted more frequently at the discretion of the protocol team. The audits will be conducted per procedures established by the NCI/CIP. Instructions for preparation for the audit visit will be sent to the site prior to the scheduled audit visit. CRFs and study-related source documents of study participants enrolled at each site will be audited. Major discrepancies will be forwarded to the appropriate oversight body within ECOG-ACRIN and NCI/CIP.

IRB procedures, approvals, and ICFs may also be reviewed at the time of the audit visit. The ACRIN Audit Manual is available online at www.acrin.org/pdrc.aspx.

To help sites prepare for monitoring and audit visits and to assure that the investigator and the research staff maintain appropriate study-related documents, ECOG-ACRIN Diagnostic Imaging Headquarters will offer training to any participating sites. The training will include all aspects of data collection and special instructions to obtain, file, and maintain the various source documents for verification of submitted trial data. **Please refer to the study-specific protocol audit guidelines for details.**

15.3   Source Documents

Source data are found in all information, original records of findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Source documents represent the first recording of any observations made or data generated about a study participant while he or she is enrolled in a clinical trial. Source documents for each study participant substantiate the data that are submitted to ECOG-ACRIN through the ACR CTMS data collection portal.

Source documents must verify the eligibility criteria and data submitted on all CRFs. If an item is not mentioned (e.g., history and physical examination alluding to a condition, but no mention of a psychological condition), it will be assumed it is not present.

Research records for each case should contain copies of the source documents for the data collected and reported to ECOG-ACRIN. If data are abstracted from medical charts that are not filed at the investigative sites (e.g. hospital charts), copies of these records should be filed in the research chart. Every attempt must be made to obtain all records/charts that were used to abstract any study data for this protocol. This will prevent any discrepancies and the inability to verify the document and the data reported.

15.4   Case Report Forms (CRFs)

CRFs, both web-based and paper forms, are the primary data collection instruments for the study. All data requested on the CRFs must be recorded, and any missing data must be explained. If a space is left blank on paper CRFs because the procedure was not done or the question was not asked, "N/D" must be noted. If the item is not applicable to the individual case, "N/A" must be noted. All entries on paper CRFs must be printed legibly in black ink on the paper CRFs. In the event of any entry errors, corrections must be made by drawing a **single straight line** through the incorrect entry, writing **the initials of the person making the correction, recording the date** when the correction is being made, and entering the correct data above the strike through. Do not use white out or an eraser. Please refer to [ICH Good Clinical Practice Guidelines](#).

Data elements that are extracted from the medical record (such as participant history or official clinical interpretations of images, pathology, or surgery results) and recorded on the CRFs will be reviewed against the appropriate component of the medical record. Data elements gathered from signed participant questionnaires must be available for review. Required study image interpretation data that are more detailed in information than the image and not typically documented in the standard radiology report may be documented on the CRF and are acceptable source documentation **if signed by the Investigator**.

At the time of audit, the auditor will verify the occurrence of the imaging examination, the reader, and the date of the exam(s) from the medical record(s). Any use of approved CRFs as source documentation require a signature and date on the CRF with a reference to the information source (participant questionnaire, CT, MR, etc.). Any use of CRFs as source documentation when the protocol has designated the source data will be medical record documentation will be considered a major protocol deficiency.

15.5   Institutional Review Board

Sites must obtain initial local IRB approval to participate in ECOG-ACRIN trials. Prior to participant registration, a copy of the IRB approval letter for the protocol and the ICF must be sent to ECOG-ACRIN Diagnostic Imaging Headquarters, along with a copy of the IRB-approved, site-specific ICF. Investigator will provide a copy(s) of IRB approval letter(s) for any amendment(s), and copy(s) of annual renewal(s).

16.0   STATISTICAL CONSIDERATIONS

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APPENDIX I

ACRIN 6690

SUPPLEMENTAL MATERIALS AVAILABLE ONLINE

Supplemental materials that support the conduct of the trial are available on the ACRIN Web site at the ACRIN 6690 Protocol Web page (www.acrin.org/6690_protocol.aspx). Types of materials posted include:

➢ Application and protocol activation documents (General Qualifying and Protocol Specific Applications, Form FDA 1572, ACRIN Statement of Investigator form, protocol activation checklist, etc.);
➢ Data forms;
➢ Imaging materials (Imaging Manual, Image Transmittal Worksheets (C0 and MW Forms), Pathology Manual, Pathology Transmittal Worksheet, and scanning and image qualification instructions);
➢ Recruitment and education materials;
➢ Regulatory resources;
➢ Participating site list.

For more information related to the trial, contact the ACRIN 6690 Contact Personnel link on the above-mentioned Web page for a list of protocol team members at ACRIN Headquarters and their roles.

ACRIN 6690

UNOS REGIONAL MAP
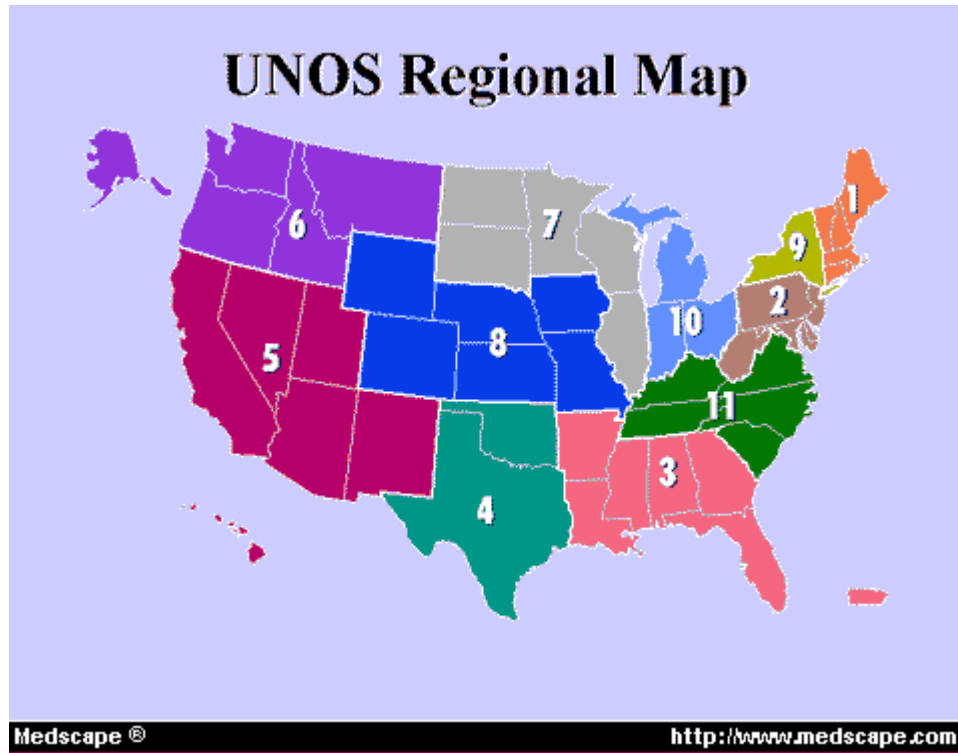
Region 1: CT, MA, ME, NH, RI, VT (eastern)
Region 2: DE, DC, MD, NJ, PA, WV
Region 3: AL, AR, FL, GA, LA, MS, PR
Region 4: OK, TX
Region 5: AZ, CA, NM, NV, UT
Region 6: AK, HI, ID, MT, OR, WA
Region 7: IL, MN, ND, SD, WI
Region 8: CO, KS, IA, MO, NE, WY
Region 9: NY, VT (western)
Region 10: IN, MI, OH
Region 11: KY, NC, SC, TN, VA

APPENDIX III

ACRIN 6690

OPTN/UNOS: LIVER TRANSPLANTS PERFORMED—BY DIAGNOSIS (U.S. NATIONAL DATA FROM 2007–2008)

OPTN: Organ Procurement and Transplantation Network

http://optn.transplant.hrsa.gov/latestData/rptData.asp

| Policy Management | Members | About OPTN | Donation & Transplantation | Data | News | Resources |

Transplant : Transplant Year (2007 - 2008) by Diagnosis
U.S. Transplants Performed : January 1, 1988 - August 31, 2009
For Organ = Liver, Format = Portrait
Based on OPTN data as of November 27, 2009

		2008	2007
All Diagnosis	#	6,319	6,494
	%	100.0%	100.0%
Ahn: Type C	#	30	36
	%	0.5%	0.6%
Ahn: Type D	#	1	0
	%	0.0%	0.0%
Familial Cholestasis: Byler'S Disease	#	7	6
	%	0.1%	0.1%
Familial Cholestasis: Other Specify	#	6	16
	%	0.1%	0.2%
Alcoholic Cirrhosis	#	592	673
	%	9.4%	10.4%
Graft Vs. Host Dis Sec To Non-LI Tx	#	2	9
	%	0.0%	0.1%
Alcoholic Cirrhosis With Hepatitis C	#	332	357
	%	5.3%	5.5%
Metdis: Alpha-1-Antitrypsin Defic A-1-A	#	84	67
	%	1.3%	1.0%
Metdis: Glyc Stor Dis Type I. (Gsd-I)	#	12	5
	%	0.2%	0.1%
Metdis: Glyc Stor Dis Type II (Gsd-Iv)	#	3	1
	%	0.0%	0.0%
Metdis: Hemochromatosis -. Hemosiderosis	#	29	26
	%	0.5%	0.4%
Metdis: Maple Syrup Urine Disease	#	6	9

12/5/09 10:49 PM

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OPTN/UNOS: LIVER TRANSPLANTS PERFORMED—BY DIAGNOSIS
(U.S. NATIONAL DATA FROM 2007–2008)

OPTN: Organ Procurement and Transplantation Network   http://optn.transplant.hrsa.gov/latestData/rptData.asp

		2008	2007
	%	0.1%	0.1%
Metdis: Other Specify	#	30	30
	%	0.5%	0.5%
Metdis: Primary Oxalosis/Oxaluria, Hyperoxaluria	#	9	21
	%	0.1%	0.3%
Metdis: Tyrosinemia	#	1	2
	%	0.0%	0.0%
Metdis: Wilson'S Disease, Other Copper Metabolism	#	34	28
	%	0.5%	0.4%
Neonatal Cholestatic Liver Disease	#	0	4
	%	0.0%	0.1%
Neonatal Hepatitis Other Specify	#	5	7
	%	0.1%	0.1%
Plm: Cholangiocarcinoma (Ch-Ca)	#	41	42
	%	0.6%	0.6%
Plm: Fibrolamellar (Fl-Hc)	#	1	3
	%	0.0%	0.0%

Data subject to change based on future data submission or correction.

ACRIN 6690

OPTN/UNOS: LIVER TRANSPLANTS PERFORMED—BY DIAGNOSIS
(U.S. NATIONAL DATA FROM 2007–2008)

OPTN: Organ Procurement and Transplantation Network   http://optn.transplant.hrsa.gov/latestData/rptData.asp

Transplant : Transplant Year (2007 - 2008) by Diagnosis
U.S. Transplants Performed : January 1, 1988 - August 31, 2009
For Organ = Liver, Format = Portrait
Based on OPTN data as of November 27, 2009

		2008	2007
Plm: Hemangloendothelioma, Hemanglosarcoma, Anglo	#	7	8
	%	0.1%	0.1%
Plm: Hepatoblastoma (Hbl)	#	43	30
	%	0.7%	0.5%
Plm: Hepatoma (Hcc) And Cirrhosis	#	917	685
	%	14.5%	10.5%
Plm: Hepatoma -. Hepatocellular Carcinoma	#	249	231
	%	3.9%	3.6%
Plm: Other Specify (I.E., Klatzkin Tumor, Lelomys	#	8	26
	%	0.1%	0.4%
Primary Biliary Cirrhosis (Pbc)	#	180	206
	%	2.8%	3.2%
Psc: Crohn'S Disease	#	39	34
	%	0.6%	0.5%
Benign Tumor: Hepatic Adenoma	#	2	3
	%	0.0%	0.0%
Psc: No Bowel Disease	#	79	88
	%	1.3%	1.4%
Psc: Other Specify	#	28	50
	%	0.4%	0.8%
Psc: Ulcerative Colitis	#	108	134
	%	1.7%	2.1%
Benign Tumor: Other Specify	#	7	4
	%	0.1%	0.1%
Benign Tumor: Polycystic Liver Disease	#	25	26
	%	0.4%	0.4%
Sec Biliary Cirrhosis: Caroll'S Disease	#	8	7

ACRIN 6690

OPTN/UNOS: LIVER TRANSPLANTS PERFORMED—BY DIAGNOSIS
(U.S. NATIONAL DATA FROM 2007–2008)

OPTN: Organ Procurement and Transplantation Network   http://optn.transplant.hrsa.gov/latestData/rptData.asp

		2008	2007
	%	0.1%	0.1%
Sec Biliary Cirrhosis: Choledochol Cyst	#	2	0
	%	0.0%	0.0%
Sec Biliary Cirrhosis: Other Specify	#	34	37
	%	0.5%	0.6%
Secondary Hepatic Malignancy Other Specify	#	7	1
	%	0.1%	0.0%
Bile Duct Cancer: (Cholangioma, Biliary Tract Car	#	5	3
	%	0.1%	0.0%
Biliary Atresia Or Hypoplasia: Other, Specify	#	14	14
	%	0.2%	0.2%
Tpn/Hyperalimentation Ind Liver Disease	#	60	76
	%	0.9%	1.2%
Trauma Other Specify	#	5	4

Data subject to change based on future data submission or correction.

ACRIN 6690

OPTN/UNOS: LIVER TRANSPLANTS PERFORMED—BY DIAGNOSIS
(U.S. NATIONAL DATA FROM 2007–2008)

OPTN: Organ Procurement and Transplantation Network   http://optn.transplant.hrsa.gov/latestData/rptData.asp

Transplant : Transplant Year (2007 - 2008) by Diagnosis
U.S. Transplants Performed : January 1, 1988 - August 31, 2009
For Organ = Liver, Format = Portrait
Based on OPTN data as of November 27, 2009

		2008	2007
	%	0.1%	0.1%
Biliary Atresia: Extrahepatic	#	177	177
	%	2.8%	2.7%
Other, Specify	#	328	414
	%	5.2%	6.4%
Not Reported	#	4	8
	%	0.1%	0.1%
Biliary Hypoplasia: Alagille's Syndrome (Paucity	#	13	13
	%	0.2%	0.2%
Ahn: Drug Other Specify	#	44	66
	%	0.7%	1.0%
Biliary Hypoplasia: Nonsyndromic Paucity Of Intra	#	3	1
	%	0.0%	0.0%
Budd-Chiari Syndrome	#	21	24
	%	0.3%	0.4%
Ahn: Etiology Unknown	#	78	64
	%	1.2%	1.0%
Choles Liver Disease: Other Specify	#	33	50
	%	0.5%	0.8%
Cirrhosis: Autoimmune	#	139	155
	%	2.2%	2.4%
Cirrhosis: Chronic Active Hepatitis: Etiology Unk	#	27	20
	%	0.4%	0.3%
Cirrhosis: Cryptogenic (Idiopathic)	#	327	362
	%	5.2%	5.6%
Ahn: Other, Specify (E.G., Acute Viral Infection,	#	138	150
	%	2.2%	2.3%

OPTN/UNOS: LIVER TRANSPLANTS PERFORMED—BY DIAGNOSIS
(U.S. NATIONAL DATA FROM 2007–2008)

OPTN: Organ Procurement and Transplantation Network

http://optn.transplant.hrsa.gov/latestData/rptData.asp

		2008	2007
Cirrhosis: Cryptogenic- Idiopathic	#	1	0
	%	0.0%	0.0%
Cirrhosis: Drug/Indust Exposure Other Specify	#	12	10
	%	0.2%	0.2%
Cirrhosis: Fatty Liver (Nash)	#	322	295
	%	5.1%	4.5%
Cirrhosis: Other, Specify (E.G., Histiocytosis, S	#	128	103
	%	2.0%	1.6%
Cirrhosis: Type A	#	3	1
	%	0.0%	0.0%
Cirrhosis: Type B. And C	#	22	23
	%	0.3%	0.4%
Cirrhosis: Type B. And D	#	4	0
	%	0.1%	0.0%

Data subject to change based on future data submission or correction.

ACRIN 6690

OPTN/UNOS: LIVER TRANSPLANTS PERFORMED—BY DIAGNOSIS
(U.S. NATIONAL DATA FROM 2007–2008)

OPTN: Organ Procurement and Transplantation Network   http://optn.transplant.hrsa.gov/latestData/rptData.asp

Transplant : Transplant Year (2007 - 2008) by Diagnosis
U.S. Transplants Performed : January 1, 1988 - August 31, 2009
For Organ = Liver, Format = Portrait
Based on OPTN data as of November 27, 2009

		2008	2007
Cirrhosis: Type B- Hbsag+	#	109	126
	%	1.7%	1.9%
Cirrhosis: Type C	#	1,282	1,352
	%	20.3%	20.8%
Cirrhosis: Type D	#	2	2
	%	0.0%	0.0%
Ahn: Type A	#	5	3
	%	0.1%	0.0%
Congenital Hepatic Fibrosis	#	9	17
	%	0.1%	0.3%
Ahn: Type B. And C	#	2	4
	%	0.0%	0.1%
Cystic Fibrosis	#	12	16
	%	0.2%	0.2%
Ahn: Type B- Hbsag+	#	22	29
	%	0.3%	0.4%

Data subject to change based on future data submission or correction.

MILAN CRITERIA

The Milan criteria were proposed by Mazzaferro et al[17] to afford staging of patients with hepatocellular carcinoma (HCC) with the intent to identify those patients at low risk for recurrence after surgical treatment (transplantation) for this disease.

<u>Patients are considered to be within the Milan Criteria with:</u>

- A single HCC ≤ 5cm diameter

OR

- Multiple (3 or less) HCC, each < 3 cm in diameter

ACRIN 6690

GFR MDRD CALCULATORS FOR ADULTS (CONVENTIONAL UNITS)

In adults, the best equation for estimating glomerular filtration rate (eGFR) from serum creatinine is the Modification of Diet in Renal Disease (MDRD) Study equation, according to the National Kidney Disease Education Program (a subdivision of the National Institutes of Health). **NOTE: This equation should be used <u>only</u> with those creatinine methods that have <u>not been recalibrated</u> to be traceable to isotope dilution mass spectroscopy (IDMS).** For more information about recalibration, visit the <u>National Kidney Disease Education Program's (NKDEP's) Laboratory Professionals</u> section.

Original MDRD Study Equation (Conventional Units)
eGFR (mL/min/1.73 m^2) = 186 x (serum creatinine)$^{-1.154}$ x (age)$^{-0.203}$ x (0.742 if female) x (1.212 if African-American) (conventional units)

IDMS-Traceable MDRD Study Equation (Conventional Units)
eGFR (mL/min/1.73 m^2) = 175 x (serum creatinine)$^{-1.154}$ x (age)$^{-0.203}$ x (0.742 if female) x (1.212 if African-American)

The equation does not require weight because the results are reported normalized to 1.73 m^2 body surface area, which is an accepted average adult surface area. This equation should only be used for patients 18 years and older.

Reduce Rounding Errors
NKDEP recommends using serum creatinine values in mg/dL to two decimal places (e.g., 0.95 mg/dL) when calculating eGFR using the MDRD Study equation. This practice will reduce rounding errors that may contribute to imprecision in the eGFR value. Values in <u>μmol/L will need to be converted to mg/dL</u> for the purpose of this trial, and both values should be maintained in source documentation.

Source: <u>www.nkdep.nih.gov/professionals/gfr_calculators/orig_con.htm</u>

GUIDANCE FOR RADIOLOGISTS

Study-Related Imaging Studies: Standard-of-Care Imaging Per Institutional Norms and Complementary Imaging Per Protocol

A patient will be diagnosed with HCC on an imaging study obtained under a clinical indication (CT or MRI per institutional preference). If disease burden corresponds to a Stage II HCC and is within Milan criteria, the patient may become eligible for listing on liver transplant waitlist with HCC-exception MELD points. Patients may be eligible for ACRIN 6690 if they meet all other pertinent criteria detailed in the current UNOS/OPTN policy[1] (or appear to meet all criteria and the site follows Declaration of Intent to List procedures [see Section 5.3]).

Baseline Imaging

If the patient then is enrolled in the ACRIN 6690 study, **baseline** complementary imaging with the second imaging modality (MR or CT) is obtained under the study protocol. Ablative treatment must NOT occur before both **baseline** imaging exams are completed or between **serial** imaging scans at any time throughout the trial. Both **baseline** imaging exams must occur per protocol within a total time frame of 60 days (i.e., the last exam must be completed no later than 60 days after the first of the baseline exams) or both MR and CT will need to be completed per protocol after enrollment within 7 days of each other.

At times, imaging time points may overlap—an UNOS listing update may meet the same requirements as a post-ablation time point, for example—and multiple uses should be reported on the case report forms (i.e., baseline and the 90-day post-enrollment UNOS update may overlap, and should both be indicated on the forms).

Serial Imaging

Subsequently **serial** imaging with both modalities will occur within ~90-day intervals to obtain updated HCC-exception MELD points for the participant per UNOS/OPTN guidelines. Centers are welcome to schedule both modalities on the same day for participant convenience; CT is requested prior to MR if at all possible. On the ACRIN case report form used to report technical assessment and imaging findings to ECOG-ACRIN, the next **UNOS listing update time point** for HCC-exception points should be selected, e.g. the next **serial** imaging pair after baseline would be the 90-day time point, then 180-day time point, etc. Readers will be provided with a calendar that can be used to identify which **UNOS listing update time points** have already been obtained so they may select the appropriate time point label for the study at hand.

Post-Ablation Imaging

Under this protocol, **post-ablation** imaging with both CT and MRI is required within 28 to 60 days after completion of any local ablative therapy. If both TACE and thermal ablation are planned in a participant, imaging should occur 28 to 60 days after the last treatment step has been completed. On both, the CT and MRI ACRIN case report forms associated with this time point, **post-ablation** should be selected on the form to classify the imaging event appropriately.

Sets of **post-ablation** imaging studies that are less than 90 days old at the time of the next scheduled UNOS HCC-exception point update (**serial**) images do not have to (but may) be repeated at the time of exception point update and may count towards the serial imaging time point for the purpose of the trial. Should another round of treatment become necessary, the above rule for "dual purpose use" of the paired post-ablation imaging applies toward the immediate following serial imaging time point.

Reporting Lesions

All Class 5 lesions will be identified, reported on ACRIN case report forms, and defined as treated or untreated lesions (see details below). Readers also are asked to report the five (5) "most prominent" or "most concerning" Class 4 lesions. No other hard rules are dictated about how to make that determination; this is left up the best judgment of the (experienced) reader. The reader will not be required to fill out a detailed lesion reporting form for more than five (5) Class 4 lesions; rather, the ACRIN case report form requires the reader to document the fact that more than five (5) Class 4 lesions are present without an exact count.

Readers do not report benign nodules and benign liver lesions such as hemangiomas, focal nodular hyperplasia (FNH), adenomas, shunts, cysts, etc., on the ACRIN case report forms (i.e., no OPTN/UNOS Class 1 to 3 lesions are recorded for the purpose of this trial).

Reporting of "Untreated" and "Treated" Lesions

Untreated lesions are reported on a per-lesion basis on the ACRIN case report form for **untreated lesions**. Please answer all questions on the form and classify the lesions according to the scheme provided. Again, reporting of lesions should NOT include unequivocally benign lesions such as cysts, shunts, hemangiomas, etc. Based on the diagnostic criteria used on this trial lesions are either classified as cancer (Class 5) or non-cancer (Class 4) with the appropriate modifiers for growth, etc.

If a participant has undergone TACE, all lesions in the treatment field (right lobe, left lobe, or both) are considered **treated lesions** and are reported on the corresponding post-treatment ACRIN case report form. (Note that the lesion's original appearance and common features will have been previously reported on the **untreated lesions** form prior to treatment.)

Examples:
- Any Class 5 lesion that has been treated will be classified as **5T (T=treatment)** at that time and going forward.
- A previously-classified Class 4 lesion that shows no treatment effects may continue to be classified as Class 4.
- A previously-classified Class 4 lesion that shows necrosis/treatment effects (indicating that it may in fact have represented an HCC) may be classified as Class 5T after ablation.
- Any new, yet unrecognized HCC in a treated lobe, with or without signs of treatment effect shall be classified as a Class 5T lesion going forward.

If a participant has undergone **unilobar TACE** then all lesions in the treated lobe are to be reported as **treated lesions** on the appropriate form, and those in the **contralateral lobe** are considered **untreated lesions** and are to be reported on the appropriate form.

Depending on the kind of therapy that was administered, a single participant could require the reporting of **treated and untreated lesions**. If only local thermal ablative therapy (but no TACE) was administered, obviously only the treated nodule(s) would be considered treated and all others untreated.

Identifying and Numbering Liver Lesions on the Forms

Below is a snippet from the C2 Form, but most of the forms for imaging-based lesion reporting look the same. There are 3 different variables of importance: Lesion # (Record #) and Lesion ID (highest-# segment and running #). It is very important that the Lesion ID (highest-# segment, running #) be maintained across time. The Lesion # (Record #) is a computer accounting device and does not need to be maintained over time.

Assignment of variables:

1. **Lesion # (Record #)** – This variable is assigned sequentially as you write the information about the lesions on the form. The computer automatically assigns the record # so you must enter the lesions in lesion # order, so that the two fields agree.
2. **Lesion ID: Highest-# segment** – You chose this based on the location of the lesion. If the lesion can be seen in more than one segment, use the highest numbered segment for the Lesion ID. Even if the lesion grows over time, DO NOT CHANGE the ID.
3. **Lesion ID: Running #** – The running number will always be 1 if you only see one lesion per segment. If there are two lesions per segment then you assign the running number of 1 to the lesion most anterior-superior and 2 to the most posterior-inferior. After you have assigned a running number to a lesion, DO NOT CHANGE it across time points.

Please ensure that your colleague(s) in CT and MR follow the same logic. (Remember you are supposed to be blinded to what was found on the other modality.)

FOR EXAMPLE:
- Baseline imaging shows one lesion in segment 7, which is assigned a Lesion ID (highest-# segment, running #) of 7 (highest segment) and 1 (running number). If at a later UNOS update it has grown into segment 8, you would want to leave the name as 7 (highest segment) and 1 (running number) because a change to 8 (highest segment) would break the link with the earlier scans.
- Baseline imaging shows one lesion in segment 7, which is assigned a Lesion ID (highest-# segment, running #) of 7 (highest segment) and 1 (running number). If at a later UNOS update

you find another lesion in that segment, you would give it a Lesion ID of 7 (highest-# segment) and 2 (running number) regardless of its position in the segment. This is different from what you would do if the two lesions were found at the same time. If the two lesions were found at the same time then you would give running number 1 to the lesion more anterior/superior.

Overall, the fields that allow for ACRIN to track and follow lesions are the Lesion ID highest-# segment and running numbers. These are the fields that are pivotal. If you have previously reported lesions on case report forms, contact ACRIN Data Management if you are concerned that this clarification would necessitate a change to the data.

Naming of New Lesions (De Novo or Apparent After Treatment)
It is imperative that the systematic names of lesions are not changed during the trial except for when they go from untreated to treated, which, for instance, changes a Class 5A or 5B lesion to a Class 5**T** lesion, the only change being the "T" indicating prior treatment. The naming convention is based on the location of a lesion in a particular segment. Within the segment, multiple lesions are initially numbered sequentially beginning with the most anterior-superior and moving towards the most posterior-inferior. If lesions straddle two segments, the lesion should be named for the highest-numbered segment (not in the segment in which the lesion is predominantly located/the segment with the greatest tumor burden). If no dominant segment can be determined, then the highest-order segment should be used in the name, other involved segment(s) are indicated in the next data field.

New lesions may develop while the patient awaits transplant, either de-novo or unmasked by ablative therapy. Conceivably, the new lesion(s) could be located more anterior-superior in a segment that already had lesions described in it. In this situation, the reader MUST NOT change the name of existing lesions. Readers should name all new lesion(s) according to the anterior-superior to posterior-inferior rule, but **beginning with the next available higher numeral for that segment which had not been previously used.**

Class 5T should be applied to any new lesion that appears only after local ablative therapy that has not been identified previously (either representing a "zone of ablation" or a new cancer in a treated hepatic lobe where a lesion has not been previously identified on imaging). Furthermore, any previously-identified Class 4 lesion that becomes necrotic in response to treatment also should be classified as a Class 5T lesion from that point forward. The rationale for this naming convention is that regenerative nodules typically do not undergo necrosis in response to lobar treatment, but HCC does. Under the current policy, UNOS accepts an ablated lesion as an indication for HCC-exception points. Therefore, to maintain consistency with the policy, an ablated Class 4 lesion would qualify for HCC-exception points and will be named Class 5T after ablation for the remainder of the trial until transplantation. Importantly, the primary aim of the trial investigates the ability to correctly classify untreated liver lesions, therefore we intend to keep any lesion that remains or occurs in a treated hepatic lobe to either the non-cancerous categories (Class 4) or Class 5T which clearly indicates that the lesion has been subjected to the effects of treatment.

Defining Presence of HCC for a Given Lesion
Readers will indicate the presence of HCC by answering a binary yes/no question. In addition, readers will give the probability of the presence of HCC in a 100 point scale. A score of 100 indicates that the reader believes that cancer is definitely present and a score of 0 indicates that the reader believes that cancer is definitely not present. Phrased differently, the score is an expression of the certainty/confidence with which a reader believes HCC to be present, this certainly should be based on

all available information, including the imaging findings that explicitly power the lesion characterization of this trial and, particularly, information derived in MRI from imaging sequences such as T2 or diffusion weighted imaging, if performed. If reader confidence is particularly influenced positively or negatively by a specific sequence, readers are asked to specify that on the reporting form.

Defining Presence of Residual or Recurrent HCC for a Treated Lesion

In analogy, the trial asks readers to determine whether there is residual/recurrent cancer present immediately after local ablative therapy has been completed and on subsequent studies. When asked to define whether HCC is present or absent in the **post-ablation** patient, this question refers to the presence or absence of **residual or recurrent tumor** and does NOT ask whether HCC was present BEFORE treatment. Again, readers will indicate the presence of HCC in a binary yes/no question and a 100 point scale.

Questions about the imaging appearance of ablated lesions on the various post-contrast phases refer to the appearance of viable tumor associated with the ablated lesion, if in fact it is present. The two basic scenarios a reader is likely to encounter are:

1. The lesion is completely treated and the zone of ablation is "avascular" and no viable tumor is seen. There may be blood products present, but no contrast enhancement is perceptible. Such completely treated lesions may be hyper- or hypo-attenuating, or hyper- or hypo-intense compared with surrounding liver parenchyma, depending on their tissue composition and whether blood products are present. Importantly, hyperintensity/hyperattenuation would be seen even on pre-contrast phase but would not significantly change after contrast administration.

2. Visible tumor tissue is still present and associated with the zone of ablation (inside the zone of ablation, most commonly immediately outside and adjacent to the zone of ablation). In this instance, the appropriate descriptors on the post-ablation imaging form should be used to characterize the appearance of this residual or recurrent viable tumor tissue (rather than the complete zone of ablation).

Reference

1. OPTN Policy 3.6. v.December 13, 2012. Policy 3.6.4.4: Degree of Medical Urgency: Liver Transplant Candidates with Hepatocellular Carcinoma (HCC); available online via: http://optn.transplant.hrsa.gov/policiesAndBylaws/policies.asp. Accessed December 18, 2012.

ACRIN 6690

THE OPTIONAL ANCILLARY STUDY OF MULTIPLEXED BIOMARKERS

1.0   ABSTRACT

Amendment 3 to ACRIN 6690 introduces an ancillary study supported by the NCI's Early Detection Research Network (EDRN) to collect blood samples for multiplexed biomarker analysis that will be compared to results obtained from the main trial's imaging data and explant pathology. Centers may offer participation in this optional ancillary biomarker study to patients who have agreed to participate in ACRIN 6690. Those who choose to participate in the Ancillary Study of Multiplexed Biomarkers will agree to have two vials of blood collected at each time point for the ancillary study. Biological samples will be collected and analyzed at each imaging time point for the main ACRIN 6690 trial and at three (3) months post-transplantation. Biomarker results will be compared/correlated to imaging metrics indicative of progressive hepatocellular carcinoma (HCC) subsequently confirmed by gross pathology and immunohistochemical methods. This approach is significant because a number of probes have already been discovered that could assist HCC assessments and need to be validated. These biomarkers are currently the most promising probes for early detection of HCC: alpha-fetoprotein (AFP), Lectin-bound AFP (AFP L3%), Des-gamma Carboxyprothrombin (DCP), Golgi Protein 73 (GP-73), Hepatocyte Growth Factor (HGF), monocyte differentiation antigen (CD14), Ceruloplasmin (fucosylated CE), and hyaluronic acid (HA). The collection of blood (serum and plasma) in this study will allow biomarker analysis with imaging correlation and pathology validation to be performed, as well as establish a biorepository that can be correlated with results from ECOG-ACRIN's image archive and immunohistochemical results for future multiplexed biomarker testing.

2.0   BACKGROUND AND SIGNIFICANCE

2.1   Ancillary Study of Multiplexed Biomarkers

Used in conjunction with imaging acquisitions, multiplexed biomarker analysis has the potential to offer enhanced sensitivity and/or specificity, yielding improved HCC diagnosis and evaluative assessment criteria compared to imaging alone. First, multiplexed biomarker analysis, when seamlessly integrated into diagnostic imaging procedures as part of a standardized protocol, has the potential to decrease false positives and false negatives by improving the identification of normal, benign, and other non-HCC abnormalities in diseased livers from HCC. Second, imaging results and multiplexed molecular biomarker data can be used together to provide complementary information to determine: (i) successful application of ablative therapy, (ii) assess long-term treatment response, and (iii) detect recurrence. The goal of combining imaging features with multiplexed molecular biomarker information is to serially obtain non-invasive evaluative criteria equivalent to results derived from standard, clinical gross pathology and immunohistochemical methods.

Imaging is notoriously poor in predicting/measuring residual/recurrent disease following treatment(s) (i.e., hemorrhaging, fibrosis, inflammation, etc.). Therefore, biomarker(s) analysis in the context of liver disease has a potentially significant role in the subsequent decision-making required for prioritizing and performing successful liver transplantation, especially in light of a limited supply of available tissue for determination of eligibility. For example, a positive biomarker(s) correlation regarding tumor burden in the absence of imaging evidence for recurrent or residual disease may enhance current standards of clinical care by altering the frequency with which follow-up studies might be required in the future, or results may compel clinicians to consider additional treatment cycles until the disease yields

biomarker(s) results equivalent to remission with associated increased patient longevity and/or quality of life.

For the ancillary multiplexed biomarker study, blood (serum and plasma) will be collected and analyzed at each imaging time point for the main ACRIN 6690 trial and at three (3) months post-transplantation. Biomarker results will be compared/correlated to imaging metrics indicative of progressive HCC subsequently confirmed by gross pathology and immunohistochemical methods. This approach is significant because a number of biomarkers have already been discovered that could assist HCC assessments and need to be validated. However, a multiplexed panel of biomarkers still needs to be defined, clinically validated, and assessed within the context of imaging and immunohistochemical results.

The biomarkers to be studied in this protocol (see Abstract) are currently the most promising for the early detection of HCC.[1-11] Moreover, the collection of blood (serum and plasma) will allow multiplexed biomarker analysis with imaging correlation and pathology validation to be performed. Imaging assessments combined with biomarker analysis and used as additional evaluative criteria could yield complementary information to more accurately detect, stage, and assess HCC progression *in vivo.*

A rigorous correlation between imaging biomarkers with molecular biomarkers has not previously been attempted for HCC detection and characterization. This study affords the opportunity for detailed examination aimed at defining the specific role of multiplexed biomarker analysis subsequent to image feature analysis.

The biomarkers to be studied in this protocol are currently the most promising for the early detection of HCC.[1-6,9-38] Salient points and details from several recent reviews and publications are included below. The rationale for specific biomarkers chosen for initial analysis is as follows:

2.2   AFP, AFP-L3%, HA, and DCP

From the literature[2]:

> "AFP (alpha-fetoprotein) has been widely used as a diagnostic marker for HCC. However, AFP levels are sometimes elevated in patients with chronic hepatitis and cirrhosis who have no evidence of HCC. Therefore, the usefulness of AFP as a screening marker of HCC has been limited by its impaired specificity."

> "The fucosylated fraction of AFP (AFP-L3%) has been reported to be a specific marker for HCC. Moreover, its level predicts the malignant potential of HCC with subsequent unfavorable prognosis after treatment. However, measurement of AFP-L3% has not always been reliable for serum samples with low total AFP concentration determined by conventional lectin affinity electrophoresis or using a liquid-phase binding assay system (LiBASys).

> "Recently, a novel automated immunoassay for AFP-L3% using on-chip electrokinetic reaction and separation by affinity electrophoresis (micro-total analysis system; m-TAS) has been developed.

> "The diagnostic advantage of m-TAS AFP-L3% was observed in 432 patients (HCC, 112; BLD [Benign Liver Disease], 320) who had lower serum total AFP

concentrations (< 20 ng/ml). Serum AFP-L3% was measured using the m-TAS assay and the LiBASys assay in the lower serum AFP group. The sensitivity, specificity, accuracy, positive predictive value, and negative predictive value of serum AFP-L3% calculated using three cutoff values (1%, 7%, and 10%) to determine HCC in the lower serum AFP group are shown in [Table 1]. The sensitivity of m -TAS AFP-L3% was especially good (m-TAS/LiBASys: cutoff 1%, 67.9%/4.5%; cutoff 7%, 41.1%/3.6%; cutoff 10%, 21.4%/3.6%) in the subgroups with lower AFP concentrations (<20 ng/ml). The diagnostic accuracy of m-TAS AFP-L3% was superior to that of LiBASys AFP-L3%, and the cutoff value of 7% for m-TAS AFP-L3% had the most accurate diagnostic power (accuracy, 78.7%)."[2]

	Cutoff	Sensitivity	Specificity	Accuracy	PPV	NPV
(A)						
LiBASys AFP-L3	1%	50.8%	95.7	75.2	90.9	69.8
	7%	40.0	99.1	72.1	97.5	66.2
	10%	38.3	99.4	71.5	98.3	65.7
μ-TAS AFP-L3	1%	87.5	73.7	80.0	73.7	87.5
	7%	60.0	90.3	76.4	83.9	72.8
	10%	47.5	96.0	73.8	90.9	68.4
μ-TAS AFP	200 ng/ml	33.6	98.0	68.5	93.4	63.6
μ-TAS DCP	40 mAU/ml	55.8	95.3	76.9	91.1	71.5
(B)						
LiBASys AFP-L3	1%	4.5	99.4	74.8	71.4	74.8
	7%	3.6	100	75.0	100	74.8
	10%	3.6	100	75.0	100	74.8
μ-TAS AFP-L3	1%	67.9	80.6	77.3	55.1	87.8
	7%	41.1	91.9	78.7	63.9	81.7
	10%	21.4	96.9	77.3	70.6	77.9

Table 1. Sensitivity, specificity, accuracy, positive predictive values, and negative predictive values of μ-TAS AFP-L3%, LiBASys AFP-L3%, μ-TAS AFP, and μ-TAS DCP (*PPV* positive predictive value, *NPV* negative predictive value, *AFP* alpha-fetoprotein, *AFP-L3%* fucosylated fraction of AFP, *LiBASys* liquid-phase binding assay system, μ-TAS micro-total analysis system, *DCP* des-gamma-carboxy prothrombin).[2]

"The present study has demonstrated that the μ-TAS AFP-L3% value is more sensitive for discriminating HCC than is the conventional LiBASys AFP-L3%. This diagnostic sensitivity was especially good in subgroups with lower AFP concentrations, and improved the clinical utility of AFP-L3% for detection of early-stage HCC. In addition, to maximize the utility of this high sensitivity, we suggest that a cutoff value of 7% is most appropriate for discriminating HCC from BLD using this newly developed μ-TAS AFP-L3% assay."[2]

In addition to the AFP, AFP-L3%, HA, and DCP, HA (Hyaluronic Acid) will also be analyzed.[12-14]

> "Hyaluronic Acid is the best marker to date serially assessing liver cirrhosis. Serum concentration of HA is consistent with stage of fibrosis, and also decreases with a response to interferon therapy in patients with HCV chronic infection."[12]

According to the Wako Web site, Hyaluronic Acid LT is a commercially-available diagnostic test in China for the quantitative measurement of hyaluronic acid. The patient cohort described in this protocol will consist of patients with cirrhosis, and therefore the levels of HA will be valuable as a metric of liver cirrhosis, according to the manufacturer.

2.3   DCP

DCP (Des-gamma-carboxy prothrombin) has been used widely in Japan for HCC diagnosis and surveillance. DCP is an abnormal prothrombin molecule that is generated as a result of an acquired defect in the posttranslational carboxylation of the prothrombin precursor in malignant cells; this prothrombin defect in malignant cells is similar to the deficit in vitamin K deficiency and has been called *prothrombin induced by vitamin K absence*. Recent studies have examined the use of DCP to detect HCC in a cirrhotic patient population.[3]

Some results from Marrero et al,[3] are presented below.
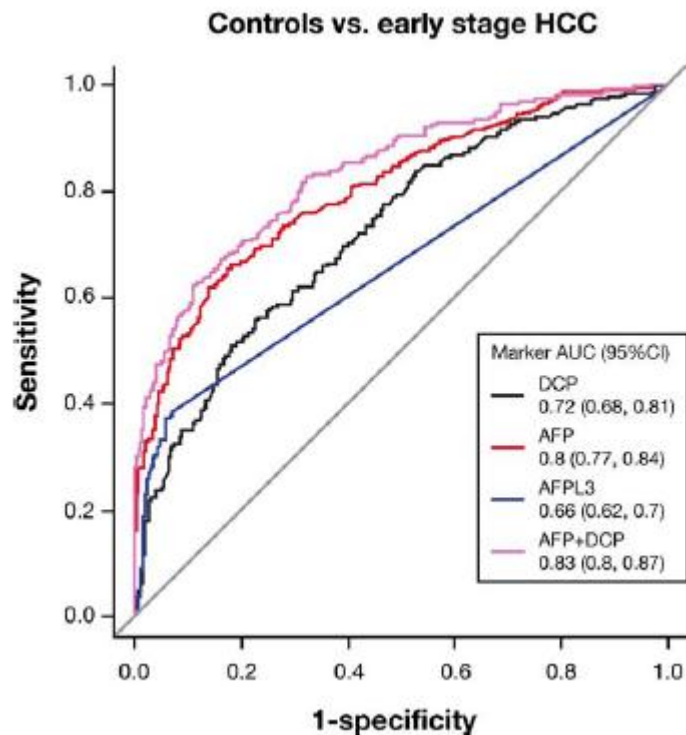
Figure 1. Receiver operating characteristics (ROC) curve evaluating those with early stage HCC (n = 208) and cirrhosis controls (n = 417). The area under the ROC curve is shown with its 95% confidence intervals. *DCP* is *black*, *AFP* is *red*, *AFP-L3%* is *blue*, and combination of *AFP* and *DCP* is *pink*.[3]

> "When all patients with HCC were evaluated, the area under the ROC (receiver operating characteristic) curve (AUC) for total AFP (0.83, 95% CI: 0.80–0.85) was similar to that for DCP (0.81, 95% CI: 0.78 – 0.84) but higher than AFP-

L3% (0.72, 95% CI: 0.69–0.75). However, when only early stage HCC (BCLC [Barcelona Center Liver Cancer] Stage 0 and BCLC Stage A) was compared with cirrhosis controls, AFP had the best AUC (0.80, 95% CI: 0.77–0.84) followed by DCP (0.72, 95% CI: 0.68 – 0.77) and then AFP-L3% (0.66, 95% CI: 0.62–0.70) (AFP *vs.* DCP: *P* = .006; DCP *vs.* AFP-L3%: *P* = .014; AFP *vs.* AFP-L3%: *P* < .0001) as shown in [Figure 1]. When intermediate-advanced stage HCC was compared with cirrhotic controls, DCP had the highest AUC (0.89, 95% CI: 0.86 – 0.92) compared with total AFP (0.84, 95% CI: 0.81–0.88) (*P* < .01), indicating that DCP was more predictive of late stage HCC than of early stage HCC."[3]

Marker	Cutoff	Sensitivity, % (95% CI)	Specificity, % (95% CI)
All HCC (n = 419)			
AFP	20	59 (55–64)	90 (86–93)
DCP	150	74 (70–79)	70 (65–74)
AFP-L3%	10	42 (37–47)	97 (93–100)
AFP + DCP	AFP = 20 or DCP = 150	86 (82–89)	63 (58–67)
Early stage HCC (n = 208)[a]			
AFP	20	53 (46–59)	90 (87–93)
DCP	150	61 (55–68)	70 (65–74)
AFP-L3%	10	28 (22–34)	97 (93–100)
AFP + DCP	AFP = 20 or DCP = 150	78 (72–83)	62 (58–67)

Table 2. The Sensitivity and Specificity of AFP, DCP, and AFP-L3% Using the Current Clinical Cutoffs (NOTE: DCP is des-gamma carboxyprothrombin in mAU/mL; AFP is α-fetoprotein in ng/mL; and AFP-L3% is percentage of AFP that is fucosylated. CI, confidence intervals.[3]
[a] Very early (BCLC stage 0) + early stage (BCLC stage A) HCC based on Barcelona staging classification.

"As shown in [Table 2], when using the currently recommended clinical cutoffs for AFP (20 ng/mL), DCP (150 mAU/mL), and AFP-L3% (10%), DCP had the best performance with a sensitivity of 61% for early stage HCC (BCLC stage 0 and BCLC stage A). However, as shown in [Table 3], when the cutoffs were determined for the point in the ROC curve that maximizes sensitivity + specificity, AFP (cutoff of 10.9 ng/mL) had the best performance for early stage HCC with a sensitivity of 66% and specificity of 82%. When AFP, AFP-L3%, and DCP were combined in a logistic regression model (after log10 transformation), AFP (odds ratio [OR], 4.2; 95% CI: 3.0–5.9) and DCP (OR, 3.0; 95% CI: 2.1–4.2) were independent markers of early HCC (BCLC Stage 0 and BCLC Stage A), whereas AFPL3% did not contribute significantly (OR, 1.1; 95% CI: 0.8–1.7); consequently, AFP-L3% was not included in further analysis of the combination of the markers. As shown in [Figure 1], the AUC for the combination of AFP and DCP (either marker elevated) mildly improved to 0.83 (95% CI: 0.79–0.86) from 0.80 for AFP alone and 0.72 for DCP alone. [Figure 2] shows a scatter plot of AFP and DCP for early stage HCC (BCLC Stage 0 and BCLC Stage A) and all cirrhosis controls; it is evident that the two markers do

not completely overlap, and, in cases of early stage HCC with low AFP levels, DCP can add to the diagnosis."[3]
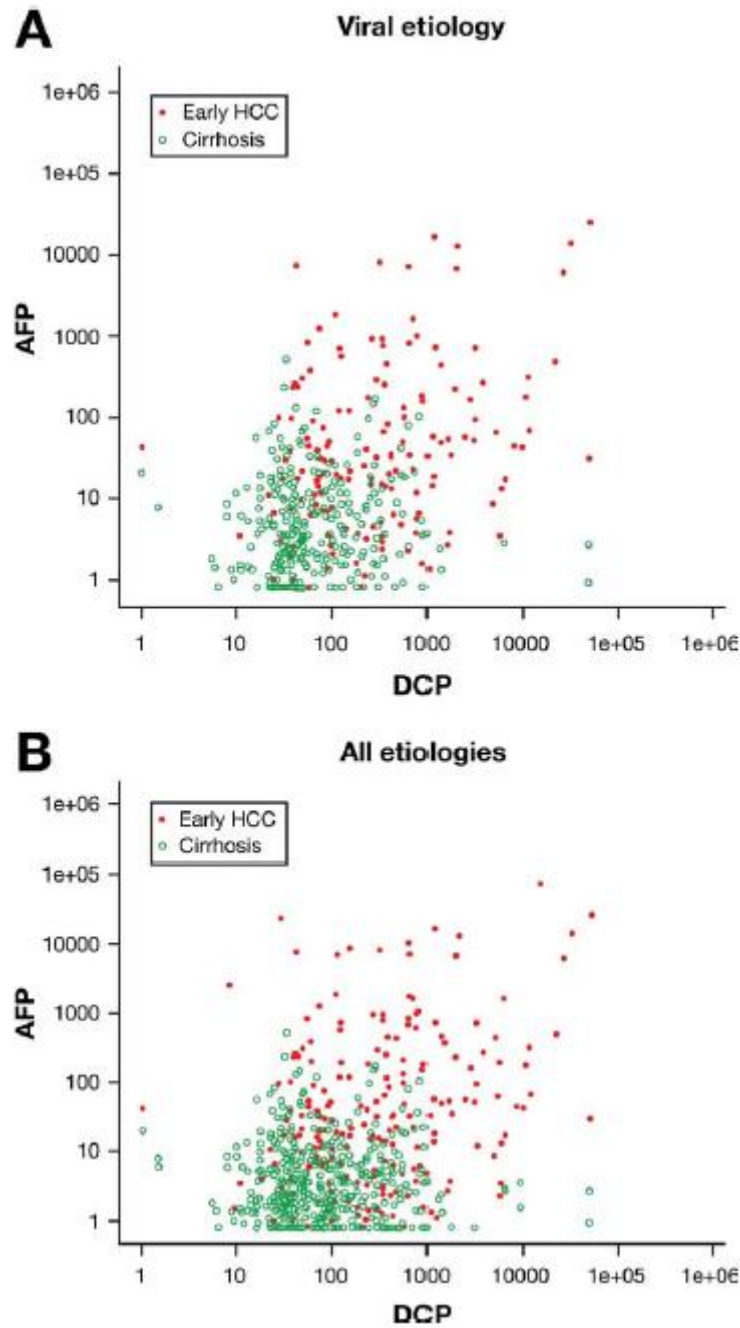
Figure 2. Scatter plot for α-fetoprotein (AFP) and des-gamma carboxyprothrombin (DCP) according to viral and nonviral etiology. *Red*, early stage HCC; *green*, cirrhosis controls.[3]

Table 3 shows that the combination of AFP and DCP improves the sensitivity for early stage HCC (BCLC Stage 0 and BCLC Stage A) to 70% when the cutoffs that maximize sensitivity + specificity were utilized but was not statistically significant to the single makers.[3]

Marker	Cutoff	Sensitivity, % (95% CI)	Specificity, % (95% CI)
All HCC (n = 419)			
AFP	10.3	70 (56–77)	82 (76–94)
DCP	298.5	66 (53–75)	82 (73–94)
AFP-L3%	1.7	50 (44–55)	94 (92–97)
AFP + DCP	AFP = 27 or DCP = 619	74 (68–81)	87 (80–92)
Early HCC (n = 208)[a]			
AFP	10.9	66 (56–77)	82 (71–90)
DCP	221.5	56 (47–87)	77 (46–86)
AFP-L3%	1.7	37 (31–45)	94 (91–96)
AFP + DCP	AFP = 11 or DCP = 598	70 (62–82)	80 (69–88)
BCLC stage A (n = 131)			
AFP	10.9	67 (58–75)	82 (78–85)
DCP	221.5	62 (53–69)	77 (73–81)
AFP-L3%	1.7	41 (32–49)	94 (92–96)
AFP + DCP	AFP = 11 or DCP = 598	77 (69–84)	76 (72–80)
BCLC stage 0 (n = 77)			
AFP	10.9	65 (55–75)	82 (78–85)
DCP	221.5	47 (36–57)	77 (72–81)
AFP-L3%	1.7	32 (20–43)	94 (92–96)
AFP + DCP	AFP = 11 or DCP = 598	65 (54–75)	76 (72–80)
Viral etiology and early HCC (n = 156)[a]			
AFP	14.1	67 (54–77)	79 (71–91)
DCP	69.5	79 (46–88)	60 (53–92)
AFP-L3%	1.7	35 (26–43)	93 (89–96)
AFP + DCP	AFP = 16 or DCP = 330	78 (64–86)	74 (67–88)
Nonviral etiology and early HCC (n = 52)[a]			
AFP	5.5	65 (48–81)	87 (78–99)
DCP	124	69 (33–90)	56 (37–91)
AFP-L3%	2.5	45 (30–59)	97 (93–99)
AFP + DCP	AFP = 6 or DCP = 877	67 (54–90)	83 (62–95)

Table 3. Cutoffs for AFP, DCP, and AFP-L3% at the Maximum Sensitivity + Specificity in the Receiver Operating Characteristic Curve (NOTE: DCP is des-gamma carboxyprothrombin in mAU/mL; AFP is α-fetoprotein in ng/mL; and AFP-L3% is percentage of AFP that is fucosylated.[3]

[a] A combination of very early (BCLC Stage 0) + early stage (BCLC Stage A) HCC based on Barcelona staging classification.

"As shown in [Figure 2] and [Table 3], the performance of DCP, but not that of AFP, is significantly affected by the etiology of liver disease for the diagnosis of early stage HCC. The sensitivity of DCP in patients with viral etiology is 79% for early stage HCC, whereas the combination of AFP and DCP in viral etiology was not better than each of the serum markers alone [Table 3]. For non-viral etiology, AFP performed better than the other markers alone, and adding other markers to it improved little. DCP appears to perform better in those with viral etiology."[3]

2.4   GP-73

GP-73, also called GOLM1 or GOLPH-2, is a resident Golgi glycoprotein, has recently been identified as a novel serum marker for the detection of HCC and its recurrence after surgery, with higher sensitivity and specificity than AFP.[15,16-26] Indeed, in one study, over 4000 patients were examined and recommended the clinical implementation of serum GP-73 measurement as a standard test for HCC.[5] Other studies, involving GP-73 have also shown correlation with outcome, following medical and surgical interventions for HCC.[5,6,18,26]
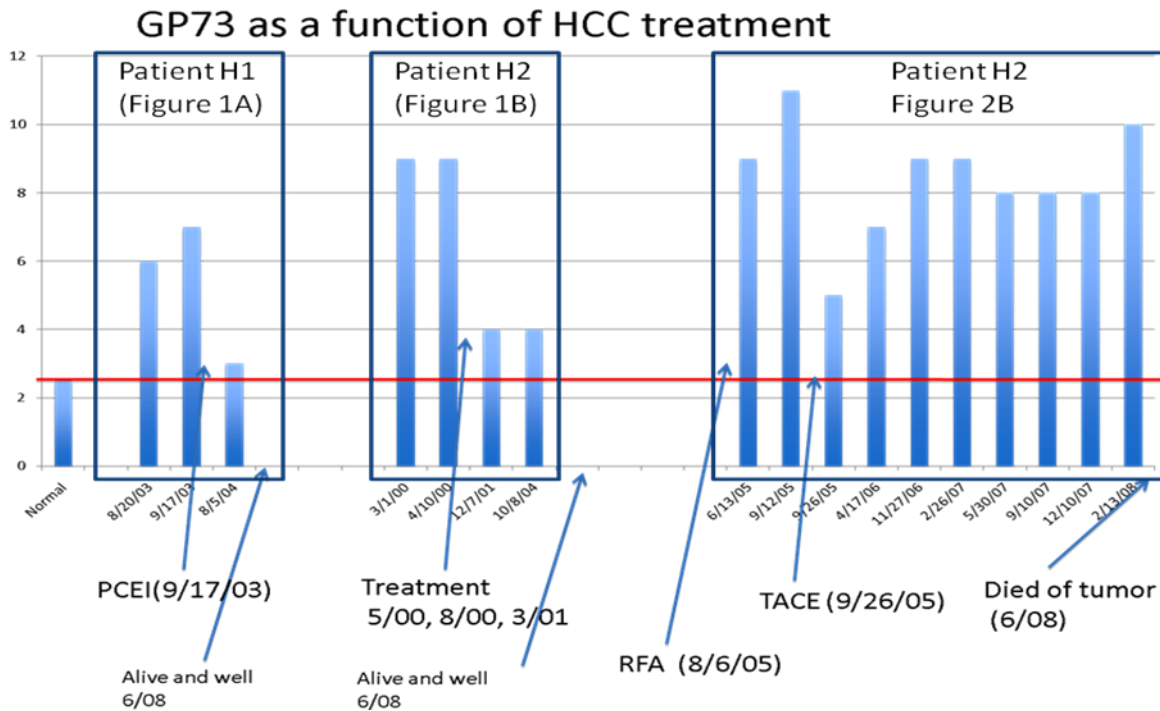
Figure 3. This figure highlights the key information presented by Hann H.W., *et al.* [6] in Figures 1-3 and is similar to several other reports [18, 28]. The figure represents three patients at various times after diagnosis of HCC and treatment. The treatments were PCEI (percutaneous ethanol injections), RFA (radiofrequency ablation), and TACE (transarterial chemoembolization). After treatment of the HCC, the levels of GP-73 declined in one patient and remained low. In the second patient, two treatments were necessary to decrease the levels of GP-73. In the third patient, the GP73 values did not decrease and this patient died of HCC. Normal GP-73 levels are 1 and the horizontal red line represents the average GP-73 levels of cirrhotic patients (approximately 2.2-fold above normal).

Since there is a correlation between elevated levels of alpha-fetoprotein (AFP) and the occurrence of HCC, determination of AFP levels is often included as a serum marker of disease. AFP as a sole indicator of HCC is of limited value, often being elevated in the absence of serious disease or not elevated when cancer is present or at an early stage.[34] Nevertheless, even the limited correlation between AFP and HCC underscores the potential of serum as a source of biomarkers of liver disease.

Changes in glycosylation and more importantly, fucosylation are known to occur with the development of cancer. To better exploit this characteristic, EDRN PIs have developed a targeted glycoproteomic methodology that allows for the identification of glycoprotein biomarkers in serum. This simple methodology first identifies changes in N-linked glycosylation that occur with the disease. This change acts as a "tag" so that we can extract out those specific proteins that contain that glycan structure. EDRN PIs initial work in an animal model led to the discovery of a protein, GP73, which is 3 times more sensitive at detecting HCC than the current marker, AFP. In the animal model of HCC, the change in glycosylation was an increase in core fucosylation.[29] This change was also observed in people who developed HCC.[9,35,36]

Receiver operator characteristic (ROC) curves were plotted to determine overall performance and to identify the sensitivity and specificity for each marker in differentiating HCC from cirrhosis.[29] A major goal of EDRNs biomarker discovery work has been the development of a more sensitive marker of early cancer. As shown in Table 4,[29] the specificity of each marker was determined at fixed points of sensitivity. As detailed in this table, in differentiating cirrhosis from stage 1 or 2 HCC, the AUROC curve for fucosylated kininogen was 0.79 with a specificity of 42% at a fixed sensitivity of 95%. Comparable results were obtained when comparing cirrhosis to all HCC stages. Similarly, Fc-AAT had an AUROC of 0.74 with a specificity of 28% at a fixed sensitivity of 95%.[29] Like fucosylated kininogen, results were similar when comparing cirrhosis to all HCC stages.

Table 4. Sensitivities and specificities of markers at the detection of stage I or II HCC

	FC-AAT[1]	FC-Kin[2]	GP73[3]	AFP[4]	GP73, AFP& Fc-kin
AUROC	0.74	0.79	0.89	0.83	**0.94**
SE	0.04	0.03	0.02	0.03	0.02
95% CI	0.67-0.81	0.73-0.85	0.85-0.93	0.77-0.88	**0.91-0.97**
p value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	<0.0001
%Specificity at 50% Sensitivity	81	84	97	98*	**99**
%Specificity at 75% Sensitivity	64	67	86	74	**95***
%Specificity at 90% Sensitivity	41	47	68	36	**77***
%Specificity at 95% Sensitivity	28	42	43	28	**70***
%Specificity at 100% Sensitivity	4	23	25	4	**36***

[1,2]Analysis of fucosylated alpha-1 anti-trypsin (FC-AAT) or fucosylated kininogen via lectin-FLISA; [3]GP73 was analyzed by immunoblot; [4]AFP was measured using a commercially available AFP ELISA kit. The best values for each category are given in bold. *Statistically different than the other values in the given group (p<0.5).[29]

"As shown in [Table 4], the marker GP73 had the best individual performance characteristics. GP73 demonstrates an AUROC of 0.89 with a specificity of 43% at a fixed sensitivity of 95%, in differentiating cirrhosis from stage I or II HCC. The addition of stage III or IV HCC patients did not alter the performance of GP73. For comparison, as Table 4 shows, AFP had a similar performance as GP73 with specificity of 28%, at a fixed sensitivity of 95%, and an AUROC of 0.83."[29]

"The performance of these markers when used in combination was also tested [Table 4]. This was done using either a combination of any two to four markers using logistic regression analysis. The combination of GP73, fucosylated kininogen and AFP gave the best overall results with an AUROC of 0.94 with a

specificity of 70% at a fixed sensitivity of 95%. This was much greater than any maker alone, as shown in [Table 4] (*P<0.05*). For all markers used in combination, performance was similar in both early tumors (stage 1 or 2) or with the analysis of all cases of HCC."[29]

The performance studies of GP-73 were designed to determine the ability of the markers to distinguish between people with cirrhosis without HCC and people with cirrhosis and HCC. This is because people with cirrhosis are in the highest risk group for HCC. However, ultimately we imagine the biomarkers will be used to stratify the individuals who are at risk for HCC, who have indeterminate imaging by CT or MR or negative screening by ultrasound. For example, if a person with cirrhosis has elevated levels of the biomarkers, they would be recommended for further follow-up investigations such as: (a) ultrasound liver imaging, if imaging wasn't done previously, or (b) more advanced imaging, such as MR or CT if an ultrasound was negative or indeterminate as well as defining timing of re-imaging if primary screening or surveillance tests were negative.

Therefore, these markers are intended to be used, ideally, in concert with imaging. We recognize that the imaging detection of HCC has greatly improved in the last decade and has been supplemented with the use of AFP levels to refine imaging interpretation. It is thus postulated that detection of HCC by imaging would be improved if the operator knew the individual had elevated levels of the biomarkers under investigation. This seems to be the case with AFP. The ultimate goal of these studies would be to determine if our biomarkers improve the performance of imaging interpretation, and how they could be used in screening for HCC.

2.5   HGF, CD14, and Fucosylated CE

Other glycoprotein biomarkers also show potential for detection and assessment of HCC. A recent study found hepatocyte growth factor (HGF), CD14, and fucosylated ceruloplasmin (CE) to be potential biomarkers for distinguishing early stage HCC from cirrhosis.[10]

A set of selected antibodies with results as summarized in the next section that showed distinctive potential markers to distinguish these two groups. The platform was chosen because the use of the lectin array optimized the lectins selected for these experiments.

"To confirm the protein variation detected by the Exactag labeling method, the AAL-overlay antibody array was used to achieve the expression analysis of a particular fucosylated glycoprotein from the original serum sample without depletion. Twenty-six (26) serum proteins were selected based on the proteins determined using Exactag labeling in the present study and also other cancer biomarker studies."

"A representative image of antibody arrays from one HCC serum and one cirrhosis serum is shown in [Figure 4a]. Fifty-four (54) arrays with 27 serum samples from HCC patients and 27 serum samples from cirrhosis patients were analyzed using the background subtracted mean intensity from each antibody [Figure 4a]. A linear regression analysis of histidine-rich glycoprotein response

to AAL was conducted from two independent antibody array experiments with the same sample set [Figure 4b]. The Pearson correlation coefficient was 0.76.

"The student's t-test was applied to analyze the variance of protein response to AAL in HCC and cirrhosis serum samples. The arrays showed that the Exactag labeling results, complement C3, CE, histidine-rich glycoprotein (HRG), CD14, and HGF showed significantly higher response in HCC sera than in the cirrhosis sera (p<0.05, [Figure 4b]). The ROC curves in Figure 4c were constructed for each of the five fucosylated proteins that showed differential expression to distinguish early HCC from cirrhosis. The AUC for complement C3, CE, HRG, CD14, and HGF was 0.737, 0.733, 0.750, 0.676, and 0.641."[10]
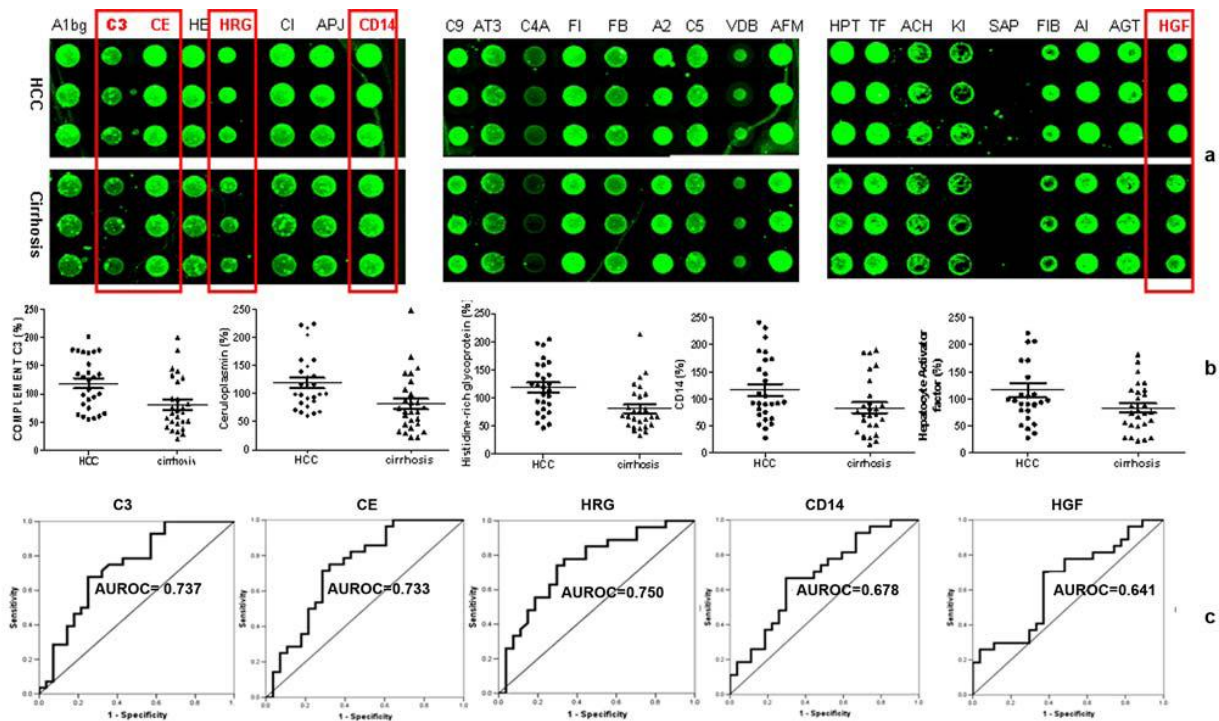
Figure 4. Fucosylated protein alteration confirmed by Antibody Microarray. (a) AAL-assisted antibody array of 26 selected proteins in the serum from HCC and cirrhosis patients. Proteins which showed significantly different response to AAL between HCC and cirrhosis were indicated by red rectangle ($p < 0.05$). (b) Comparison of response intensity of C3, CE, HRG, CD14, and HGF to AAL in HCC and cirrhosis. Each spot represents one serum sample, error bars indicate the standard deviation from 27 HCC and 27 cirrhosis patients. (c) ROC curves for C3, CE, HRG, CD14 and HGF.[10]

"The combination of the 5 proteins had an AUROC of 0.811, with specificity of 72% at a fixed sensitivity of 79% [Figure 5], while AFP has an AUROC of 0.661, with specificity of 35% at a fixed sensitivity of 79%.[10]

Figure 5. Combined ROC curve of C3, CE, HRG, CD14, and HGF.[10]

"Our results showed that C3, CE and HRG had an AUROC of 0.737, 0.733, and 0.750 in discriminating cirrhosis from HCC, which suggest the change of these proteins may be a common characteristic in cancer caused by the difference between tumor and normal cells. We have also found that HGF and CD14 were hyper-expressed in HCC serum. HGF has been reported to be a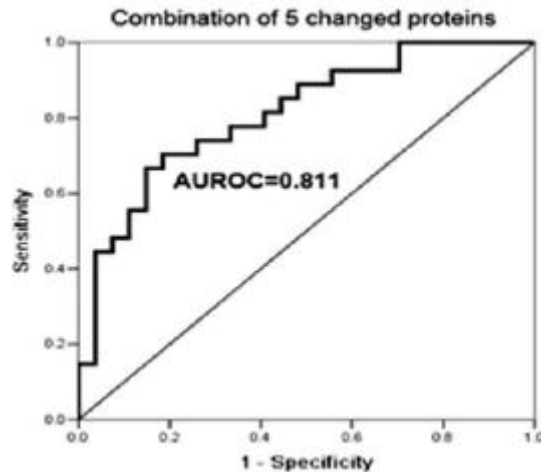 critical limiting step in invasive growth of tumor cells in HCC, while CD14 has been found to mediate the HGF activation via the CD14/TLR-2 pathway, where the increase of CD14 and HGF may relate to the development of HCC progression."[10]

The results suggest that C3, CE, HRG, CD14, and HGF could be used as biomarker candidates to supplement the current diagnostic criteria for HCC.

Additional biomarkers have been proposed by EDRN investigators for HCC analysis. The biomarkers herein listed are currently the most promising probes for early detection of HCC. The collection of blood (serum and plasma) will allow enhanced reference data sets to be established for future multiplexed biomarker analysis with imaging correlation and pathology validation.

A rigorous correlation between surrogate imaging biomarkers with molecular biomarkers has not previously been attempted for HCC detection and characterization. This study affords the opportunity for detailed examination aimed at defining the specific role of multiplexed biomarker analysis subsequent to imaging feature analysis. It is anticipated that the combination of non-invasive diagnostic imaging protocols will enhance the detection and characterization of early stage HCC.

2.6   Hypotheses for Ancillary Study of Multiplexed Biomarkers
1. Biomarkers (AFP, AFP-L3%, DCP, GP-73, HGF, CD14, CE, HA) can improve the sensitivity of CT and MRI for diagnosis of residual or recurrent viable HCC after focal ablative therapy in participants listed for liver transplant.

2. Biomarkers correlate with or aid CT and MRI for diagnosing and characterizing HCC burden in patients listed for liver transplant before and after ablative therapy or without ablative therapy.

3.0 STUDY OBJECTIVES/SPECIFIC AIMS
3.1 Primary Aim
3.1.1 To estimate the patient level sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of eight biomarkers, used alone, in combination, and in combination with CT/MRI for diagnosis of residual or recurrent viable HCC after focal ablative therapy in participants listed for liver transplant.

3.2 Secondary Aim
3.2.1 To estimate correlation in measuring patient level HCC burden between each biomarker alone or in combination and concurrent CT/MRI in patients before and after ablative therapy or without ablative therapy prior to transplant.

3.3 Exploratory Aim
3.3.1 To assess the sensitivity, specificity, PPV, and NPV of biomarkers on the basis of all available information and imaging sequences, and compare their performance.

4.0 ANCILLARY STUDY OF MULTIPLEXED BIOMARKERS OVERVIEW
Amendment 3 to ACRIN 6690 introduces an ancillary study supported by the NCI's Early Detection Research Network (EDRN) to collect blood samples for multiplexed biomarker analysis that will be compared to results obtained from the main trial's imaging and explant pathology histology. Centers with the appropriate equipment (see below) will ask participants consenting to the ACRIN 6690 main trial to consent to participate in this optional ancillary biomarker study.

All sites participating in ACRIN 6690 will be considered eligible for recruiting patients to the EDRN's Ancillary Study of Multiplexed Biomarkers if the site meets criteria outlined in Section 6.0.

Blood for EDRN's ancillary biomarker study will be collected at participating ACRIN 6690 institutions at the time of imaging or during routine blood collection proximal to the imaging time point (i.e., when blood is drawn prior to imaging as part of each patient's routine care or at another time point at the institution when, for example, AFP is being assessed). Two (2) separate tubes of blood, one for serum (a minimun of 8 mLs) and one for plasma (a minimum of 8 mLs) will be collected before each imaging scan, and at three (3) months after liver transplantation. Time points include:

- a) **BASELINE**: Before Initial CT or MR Images Acquired;
- b) Before Each Study-Related **SERIAL** Imaging Acquisition
    - CT and MR Acquired Every 90 Days per UNOS Listing Update Requirements
- c) Before Imaging Acquisition(s) for **POST-ABLATIVE** surgery; and,
- d) **AFTER TRANSPLANT:** Three (3) months (± 2 weeks) after liver transplantation.

An overview of EDRN's Ancillary Study of Multiplexed Biomarkers is outlined in Figure 6, which summarizes blood collection time point s in relation to ACRIN 6690 main trial imaging time points.

Following collection, tubes containing blood will be processed according to the Manual of Operations for the Collection of Serum and Plasma (available online at www.acrin.org/6690_imagingmaterials.org) for fractionating blood into serum and plasma components. After processing is complete, approximately 8 mL of serum and 8 mL of plasma will be transferred into 1 mL cryovials and stored at −70ºC or colder

until shipping to the Core Biomarker Testing Facility. Shipping materials and procedures also are discussed in the Manual of Operations for the Collection of Serum and Plasma.

The Core Biomarker Testing Facility will perform quality assurance testing on a subset of randomly sampled biospecimens and re-aliquot remaining specimens for shipment to either: (1) NCI Biorepository in Frederick, MD, to establish a biorepository for subsequent studies of revealed potential biomarkers or (2) participating laboratories for biomarker characterization. Results of the completed biomarker analysis will be sent to EDRN's DMCC for statistical analysis, to identify an optimal multiplexed panel of biomarkers using immunohistochemical methods. Multiplexed biomarker results will be correlated with CT and MRI image findings and evaluated for their potential to improve diagnosis and also for assessing HCC burden in patients listed for liver transplant before and after ablative therapy or without ablative therapy.

Figure 6. EDRN ANCILLARY STUDY OF MULTIPLEXED BIOMARKERS SCHEMA

OVERVIEW

Blood Draw Ancillary Study

Must be completed prior to imaging at all imaging time points and at three (3) months after transplantation surgery:

- before **baseline** complementary imaging;
- before each **serial** imaging series; and
- before **post-ablation** imaging;
- at three (3) months **after** transplantation.

Blood may be collected during routine blood draws at the participating institution or immediately prior to administration of imaging contrast agent.

HCC diagnosis by SOC **baseline** CT or MRI

PATIENT WAITLISTED WITH UNOS

Listing for liver transplantation with HCC-exception points

DECLARE INTENT TO LIST

Patient determined to be potential candidate for waitlisting and trial: Declaration of Intent to List source document completed by site

ENROLLMENT

Study-related (complementary) **baseline** imaging CT or MRI within 60 days after SOC **baseline** CT or MRI + **baseline** blood collection prior to complementary imaging

(if 60-day window cannot be accomplished because SOC baseline imaging is too old at time of enrollment, then both complementary and SOC baseline imaging + Eovist MRI need to be done within 14 days of each other)

Local ablative therapy
(consider pre-treatment biopsy if feasible)

Repeat **serial** imaging (main trial CT and MRI + blood collection prior to imaging) every 90 days per UNOS listing update requirements

Post-Ablation Imaging
(SOC + complementary imaging + blood collection prior to imaging, no less than 28 days and no more than 60 days after completion of ablation)

Transplant surgery

Blood collection at three (3) months after transplantation surgery

Explant radiology-pathology correlation and histopathology analysis

HCC = hepatocellular carcinoma; CT = computed tomography; MRI = magnetic resonance imaging; SOC = standard of care; UNOS, United Network for Organ Sharing.

Blood collection for the optional ancillary study will occur prior to each imaging time point and at three (3) months after liver transplantation, either during routine blood collection (e.g., for local AFP analysis) at the participating institution or prior to contrast administration using the IV placed for injection of contrast.

See the main trial protocol for the ACRIN 6690 schema without ancillary trial details.

5.0   PARTICIPANT SELECTION/ELIGIBILITY CRITERIA

Participants eligible for the main trial for ACRIN 6690 will be considered eligible for the ACRIN 6690 EDRN Ancillary Study of Multiplexed Biomarkers. No additional inclusion or exclusion criteria apply for patients willing to consent to the blood collection for the ancillary study.

6.0   SITE SELECTION

All sites participating in ACRIN 6690 will be considered eligible for recruiting patients to the EDRN's Ancillary Study of Multiplexed Biomarkers if the site meets the following criteria:

1. Personnel appropriate for drawing blood samples at all specified time points prior to imaging.
2. Facilities for processing blood samples into fractionates.
3. Personnel trained for processing serum and plasma specimens per ACRIN's Manual of Operations for the Collection of Serum and Plasma (available online at www.acrin.org/6690_imagingmaterials.aspx).
4. Available −70°C or colder storage with adequate capacity per the manual of operations.

7.0   DATA MANAGEMENT

Data collection and management for the Optional EDRN Ancillary Study of Multiplexed Biomarkers will be coordinated by the ECOG-ACRIN Diagnostic Imaging BDMC and EDRN DMCC. Briefly, all biologic specimens collected will be delivered to EDRN's Core Biomarker Testing Facility (EDRN's CBTF) for quality assurance testing. A subset of randomly selected specimens containing either serum or plasma will be sent to EDRN participating laboratories for analysis of eight (8) biomarkers listed in Section 10.0 of this appendix. Remaining samples will be used to establish a biorepository containing training and validation reference sets that will be used in subsequent research studies. Test results from the eight (8) biomarkers will be provided to EDRN's Data Management Coordinating Center (DMCC) for subsequent statistical analysis. Results from DMCC's biomarker analysis and biomarker-related data elements will then be provided to the ECOG-ACRIN BDMC for inclusion into the trial database and subsequent analysis of image features and biomarker findings correlated to pathology and immunohistochemical results. A transparent, open data exchange with accessibility to image and biomarker data will be ensured by policies and procedures adopted by ECOG-ACRIN and EDRN.

8.0   STUDY PROCEDURES

Details of the standard-operating-procedures (SOPs) used for blood collection in the ancillary study of multiplexed biomarkers are provided in the Manual of Operations for the Collection of Serum and Plasma (available online at www.acrin.org/6690_imagingmaterials.org).

For the optional EDRN Ancillary Study of Multiplexed Biomarkers, all main trial procedures should be completed as described in Section 8.0 of the main protocol. Only patients who consent to joining the EDRN Biomarker Ancillary Study will have blood drawn. The biomarker ancillary study introduces the addition of blood collection of two (2) vials of blood (for a minimum of 16 mL total) at each timing time point (once at **baseline**, once for each **serial** imaging series, and once after ablation has been completed for the **post-ablation** imaging) and at **three (3) months after liver transplantation**.

Blood must be collected at the participating institution and must be processed procedures provided in the Manual of Operations for the Collection of Serum and Plasma (available online at www.acrin.org/6690_imagingmaterials.org).

8.1   Blood Collection Time Points for the EDRN Ancillary Study of Multiplexed Biomarkers

Optimally, blood for the biomarker ancillary study will be collected at the time blood is drawn for routine care (AFP measurements, etc) or, if routine blood draws are performed off-site, then blood for the ancillary study should be collected at the participating institution prior to administration of contrast through the IV placed for the study. Two (2) vials (a minimum of 16 mL) of blood will be collected before each imaging study time point as dictated by the main ACRIN 6690 trial procedures, and at three (3) months after liver transplantation:

a) Before **baseline** study imaging;

b) Before <u>each</u> study-related **serial** imaging for UNOS listing updates (approximately every 90 days);

c) Before imaging **post-ablation**; and

d) **After transplantation:** Three (3) months (± 2 weeks) after liver transplantation.

8.2   STUDY PROCEDURES TABLE: EDRN ANCILLARY STUDY OF MULTIPLEXED BIOMARKERS ONLY

Study Procedure	BASELINE: Before Initial CT or MR Images Acquired	Before Each Study-Related SERIAL Imaging Acquisition (CT and MR Acquired Every 90 Days per UNOS Listing Update Requirements)	Before Imaging Acquisition(s) for POST-ABLATIVE Surgery	AFTER TRANSPLANT: Three (3) Months (± 2 Weeks) After Liver Transplantation
Informed Consent Form	X			
Draw Two (2) Tubes of Blood (at time of routine blood collection of AFP or other lab assessments **OR** immediately prior to administration of contrast agent *via* IV placed for contrast delivery)	X	X	X	X
Process Blood Samples for Serum and Plasma Within Four (4) Hours After Blood Draw	X	X	X	X
Submit Serum and Plasma Samples	X	X	X	X

9.0 ADVERSE EVENTS: EDRN ANCILLARY STUDY OF MULTIPLEXED BIOMARKERS, BLOOD DRAWS ONLY

Procedures for AE reporting and classification are outlined in the main trial protocol. The expected AEs related to blood collection at each imaging time point and at three (3) months after liver transplantation for the EDRN ancillary study are introduced here. Note that trial leadership encourages the sites to collect the blood at times of routine vein access.

9.1 Expected Adverse Events Associated With Blood Draws

- Hemorrhage (hematoma at the injection site);
- Phlebitis;
- Minor discomfort;
- Bleeding;
- Infection;
- Bruising.

10.0 STATISTICAL METHODOLOGY

In this study, biomarkers will be analyzed to determine if they can improve the sensitivity of CT and MRI for diagnosis of residual or recurrent HCC after focal ablative therapy. Biomarker results will be correlated with CT and MRI and evaluated for their potential to: a) improve diagnosis and b) assess HCC burden in patients listed for liver transplant, before and after ablative therapy or without ablative therapy. Biomarker analysis will first be performed on each biomarker individually. Second, biomarker combinations will be sequentially analyzed by including additional biomarkers one at a time to obtain an optimized biomarker panel. The end result might be that only one or two multiplexed biomarkers demonstrate the best correlation with immunohistochemical results. Biomarkers to be evaluated include:

- AFP   (Alpha-fetoprotein),
- AFP-L3% (Lectin-bound AFP),
- DCP   (Des-gamma Carboxyprothrombin),
- GP-73   (Golgi Protein-73),
- HGF   (Hepatocyte Growth Factor),
- CD14   (monocyte differentiation antigen),
- CE   (fucosylated Ceruloplasmin), and,
- HA   (Hyaluronic Acid)

10.1 Ancillary Study for Multiplexed Biomarker Endpoints

10.1.1 Primary Aim

10.1.1.1 *To estimate the patient level sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of eight biomarkers, used alone, in combination, and in combination with CT/MRI for diagnosis of residual or recurrent viable HCC after focal ablative therapy in participants listed for liver transplant.*

Though the data analyses for the Primary Aim will be performed for each time point of blood draw after ablative therapy and concurrent CT/MRI, the last time point prior to transplant will be used for *primary analysis*.

Pathology diagnosis of residual/recurrent HCC (YES/NO) at explant will be used as the "gold standard". Patients without pathology diagnosis at explant due to complete necrosis will be excluded (estimated proportion < 25%). ROC curves for each biomarker predicting pathology diagnosis will be plotted for each time point of blood draw after focal ablative therapy. Sensitivity and specificity of concurrent CT/MRI for predicting pathology diagnosis will be plotted on the same plot.

The 95% confidence intervals for sensitivity and total accuracy of the biomarker test will be calculated at the following points on ROC curve: a) optimal cutoff maximizing the sum of sensitivity and specificity as the *primary analyses*; b) cutoff corresponding to specificity of CT/MRI; and c) cutoff corresponding to sensitivity of CT/MRI.

As an exploratory analysis for the Primary Aim, we will use logistic regression to construct a biomarker panel using forward selection method. The score calculated from the final model will be treated as a composite biomarker and we will repeat the same analyses described above.

Since CT/MRI for has high specificity (above 85%) for patients who do not have residual/recurrent HCC but low sensitivity (~60%), as an exploratory analysis, we will examine the sensitivity and specificity of a simple rules combining concurrent CT/MRI report and biomarker panel prediction: predict residual/recurrence of HCC if either CT/MRI or biomarker panel is positive.

10.1.2 Secondary Aim

10.1.2.1 *To estimate correlation in measuring patient level HCC burden between each biomarker alone or in combination and concurrent CT/MRI in patients before and after ablative therapy or without ablative therapy prior to transplant.*

Pearson correlation will be calculated for each biomarker, natural log transform if appropriate, with the volume of the largest HCC tumor measured by CT/MRI. A panel will be developed using a multivariate linear regression model with CT/MRI tumor volume measurements as outcomes. Data from different time points will be combined and GEE (General Estimating Equations) method will be used to account for the correlations among outcomes in the same patient.

10.1.3 Exploratory Aim

10.1.3.1 *To assess the sensitivity, specificity, PPV, and NPV of biomarkers on the basis of all available information and imaging sequences, and, compare their performance after ablative therapy.*

For participants' assessment after ablative therapy, the trajectory of each biomarker will be plotted across all time points after ablative therapy. Patterns related to explant pathology diagnosis will be identified, e.g. no decrease for residual HCC, or a decrease followed by arise for recurrence. Simple decision rule will be constructed to predict residual/recurrence using the identified patterns and the sensitivity and specificity of the rule will be compared to that of CT/MRI.

10.2   Sample Size/Accrual Rate
The ancillary study will target at least 200 participants and will be open to all liver transplant centers participating in the main ACRIN 6690 trial. Details and the range of patient numbers needed for this study are provided below.

Participants agreeing to participate in the ACRIN 6690 main trial at sites with the technology required to conduct the ancillary biomarker study will be approached to consent. For the primary endpoint, we estimate that 50% to 75% of participants with HCC may have focal ablative therapy prior to transplantation, and up to 25% may not have explant pathology diagnosis due to complete necrosis. We further assume 10% dropout rate for various reasons. Therefore the estimated sample size for the primary aim is 440*.50(or .75)*.75*.9 = 148 to 222, averaged 185, depending on the proportion of focal ablative therapy. The estimated sample size for Aim 2 is 440*.5(or .25)*.9 = 99 to -198 as the low bound when at the time when all ablative therapy treated patients are excluded from data analysis, and 440*.9 = 396 as the upper bound when no patient has received focal ablative therapy.

10.3   Power Consideration/Stratification Factor
About 85% of patients with HCC treated with TACE have residual viable tumor at explant. Therefore, sensitivity of biomarker test for the primary aim is calculated from an estimated 185*.85 = 157 patients. This study does not have power to evaluate specificity alone due to small sample size (185*.15 = 28). The sensitivity for detecting residual/recurrence by CT/MRI is estimated as ~60%, with specificity ~90%. The total accuracy for CT/MRI is 65% (.85*.6+.15*.9). With total estimated 185 evaluable data from eligible participants and 157 from those with residual/recurring disease, the study will be able to estimate 95% CI with the precisions below.

Accuracy (%)	Half-length of the 95% CI	Sensitivity(%)	Half-length of the 95% CI
50	7.2	60	7.6
55	7.2	65	7.5
60	7.1	70	7.2
65	6.9	75	6.8
70	6.6	80	6.3
75	6.2	85	5.6

10.4   Tumor Burden / Tumor Volume Metrics
Inclusion criteria for the main ACRIN 6690 trial stipulate that a patient be enrolled on the UNOS transplant waitlist with HCC-exception MELD points. In order to qualify for these priority points, patients need to have a minimum stage of disease ("floor") but must not exceed a maximum permissible disease burden ("ceiling"). This is requirement by the UNOS policy, which currently regulates liver transplantation in the United States.

The criteria used in the current UNOS policy are derived from a publication of the so-called "Milan criteria".[37] HCC tumor volumes encountered in this study are expected to fit within these criteria.

A false negative imaging diagnosis of a Class 4 lesion (which in reality turns out to be an HCC) would result in not counting this lesion towards overall [imaging based] tumor volume analysis. It is therefore possible that imaging underestimates tumor stage and volume in a given patient. Conversely, it is possible that a false positive imaging diagnosis of a Class 5 lesion would lead to inclusion of that nodule

volume in the overall tumor volume measurement, which is inappropriate. It is therefore possible that imaging <u>over</u>estimates tumor stage and volume for a given patient.

Tumor volume calculations:
Patients are included in this study if they have at least one Stage 2 HCC (diameter > 2 cm). For the purpose of this study it is assumed that all HCC are spherical. Therefore, the volume is:

$$V = \frac{4}{3} \pi r^3$$

For a 2-cm diameter spherical nodule, the volume would therefore be 4.19 cm^3. The Milan criteria also specify the largest permissible disease burden acceptable for transplantation.

The Milan criteria state that a patient is selected for transplantation when he/she has:
- 1 lesion smaller than 5 cm;
  or
- up to 3 lesions smaller than 3 cm;
- no extrahepatic manifestations;
- no vascular invasion.

Therefore, the corresponding "ceiling" of tumor volume would be:
(a) 65.45 cm^3 for a 5 cm diameter spherical HCC
  plus
(b) 12.77 cm^3 for each 2.9 cm spherical HCC
  = total of 38.3 cm^3 for 3 x 2.9 cm spherical HCC

One might question why the maximum allowable tumor volume for a single lesion exceeds that of the allowable volume for multifocal disease. The purported rationale for this is that multifocal disease carries a more guarded prognosis than unifocal disease. The numbers used in the Milan criteria are based on a single center publication and are empirical. Subsequent proposed extended criteria seem to carry comparable prognostic power but are less restrictive in the allowable sizes, such as the UCSF (University of California, San Francisco) criteria.[38]

However, for the time being, transplant allocation in the U.S. and inclusion criteria in ACRIN 6690 use the widely accepted Milan criteria and will therefore be used for the purpose of analysis in this protocol.

10.5   Reporting Guidelines

Routine reports for this ancillary study will be included in the ECOG-ACRIN Biostatistics Center Mid-Year and Year End Updates and will be provided to oversight bodies, including DSMC for review during each of its twice-yearly meeting.

    Routine reports will include:
- Accrual and participant characteristics;
- Timeliness and completeness, eligibility and protocol compliance, and outcome data;
- All reported adverse events.

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